

COMPREHENSIVE
HANDBOOK OF

IODINE

NUTRITIONAL, BIOCHEMICAL,
PATHOLOGICAL AND
THERAPEUTIC ASPECTS

EDITED BY

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FOREWORD

This handbook is a comprehensive and valuable reference resource on all aspects of iodine – its chemistry, metabolism and the effects of iodine deficiency and excess.

Iodine is essential for the normal development of all animals and man. Its basic biological importance is indicated by the fact that the tadpole will not undergo metamorphosis into the frog without the function of the thyroid gland in secreting the iodine-containing thyroid hormones.

Iodine deficiency is now known to be responsible for effects on development of various organs, including particularly the brain, throughout the life cycle from fetus to old age. These effects are now designated by the term “Iodine Deficiency Disorders” (IDD), which refers to all the effects of iodine deficiency in a population which can be prevented by correction of the iodine deficiency [1].

Iodine deficiency is now recognized by WHO as the most common preventable cause of brain damage in the world today, with in excess of 2 billion at risk from 130 countries.

Recognition of this problem has led to the creation, in 1986, of the International Council for Control of Iodine Deficiency Disorders (ICCIDD) – an international non-governmental organization officially recognized by WHO, UNICEF and the UN System as the expert scientific group on all aspects of IDD [2].

The ICCIDD now comprises a network of 700 multidisciplinary professionals from more than 100 countries available to assist national programs. This has been carried out by a series of Regional Meetings held in collaboration with WHO and UNICEF. The ICCIDD has also provided expert advice at the global level to WHO and UNICEF, with a series of joint publications.

The virtual elimination of IDD was included in the list of 27 goals adopted by the UN World Summit for Children

in 1990. This summit declaration was signed by 71 Heads of State at the UN in New York and subsequently by 88 other governments. It has provided unprecedented and all important political support for iodization programs.

Since 1990, great progress has been made in the elimination of iodine deficiency with household coverage of iodized salt reaching 68% of the population at risk (1999) compared with less than 20% before 1990.

The ICCIDD publishes a quarterly IDD Newsletter, which is distributed free of charge in bulk by international agencies and by individual mailing.¹ The Newsletter also appears on the ICCIDD website as both text files and PDF. The Newsletter welcomes comments, new information and relevant manuscripts on all aspects of iodine nutrition as well as human interest stories on IDD elimination in countries.

There is a great opportunity for a major triumph in global public health with the global elimination of brain damage due to iodine deficiency disorders, comparable to the elimination of small pox and polio.

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¹For further details about the IDD Newsletter consult the ICCIDD website (www.iccidd.org) or contact Michael B Zimmermann MD, Editor IDD Newsletter, at the Human Nutrition Laboratory, Swiss Federal

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Section 1

Analytical Techniques

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1

Determination of Iodine in Seawater: Methods and Applications

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Abstract

The ocean is a huge reservoir of iodine. The transfer of iodine from the sea to the atmosphere, and subsequent deposition onto soils and incorporation into plants and animals, is one of the main pathways for iodine to enter into the human food chain. Kelp, being a good source of iodine, is taken as a food and nutritional supplement. Iodine is also extracted from seaweeds or seawater, and added to edible salt to supplement the intake of iodine for people who live in iodine-deficient areas. Iodine exists mainly as iodide and iodate, along with a small fraction of organic iodine compounds in seawater. The distribution of iodide and iodate in seawater varies with depth and geographical location. The determination of iodine in seawater is helpful in understanding the marine environment. This chapter provides an overview of the methods employed for the separation and determination of iodine in seawater, including capillary electrophoresis, ion chromatography, high-performance liquid chromatography, gas chromatography, spectrophotometry, ion-selective electrode, polarography, voltammetry, atomic emission spectrometry, and neutron activation analysis. The advantages and limitations of these methods are assessed and discussed.

Abbreviations

AES	Atomic emission spectrometry
CE	Capillary electrophoresis
GC	Gas chromatography
HPLC	High-performance liquid chromatography
IC	Ion chromatography
ICP-AES	Inductively coupled plasma atomic emission spectrometry
NAA	Neutron activation analysis
UV	Ultraviolet

Introduction

Iodine is an essential component of the thyroid hormones that play an important role in human development, growth, and metabolism, especially of the brain. Iodine deficiency in humans can cause several diseases or problems, which include spontaneous abortion, increased infant mortality, cretinism, goiter, and mental defects (Li *et al.*, 2001; Rong and Takeuchi, 2004). Seawater is a huge reservoir of iodine. One of the major pathways for the entry of iodine into the human food chain involves the transfer of iodine from the sea to the atmosphere, its subsequent deposition onto soil and incorporation into plants and animals (Chance *et al.*, 2007). Kelp, a type of macro algae in the ocean, is a rich source of iodine and is thus used as a food and nutritional supplement. Iodine in seaweeds is mainly iodide, with a very small fraction of iodine present as iodate and organoiodine in the form of monoiodotyrosine and diiodotyrosine (Martinelango *et al.*, 2006). Marine algae are also used as natural sources of iodine in the feeding of freshwater fish, which on consumption would improve the iodine intake of man (Schmid *et al.*, 2003). Generally, iodine is extracted from seaweeds or seawater, and added to edible salt to supplement the intake of iodine for people who live in iodine-deficient areas. The main pathways for the entry of iodine into the human food chain are shown in Figure 1.1. Iodine in seawater can also have an impact on the global biogeochemical cycle of iodine, affecting the supply of iodine to the atmosphere from the oceans. Iodine atoms are involved in atmospheric ozone depletion and aerosol formation reactions in the marine boundary layer, and hence have an influence on the earth's radiative balance and weather, which in turn may affect human health (Chance *et al.*, 2007).

Iodine is ubiquitous in seawater having a total dissolved concentration of about 400–500 nmol · l⁻¹ in most places,

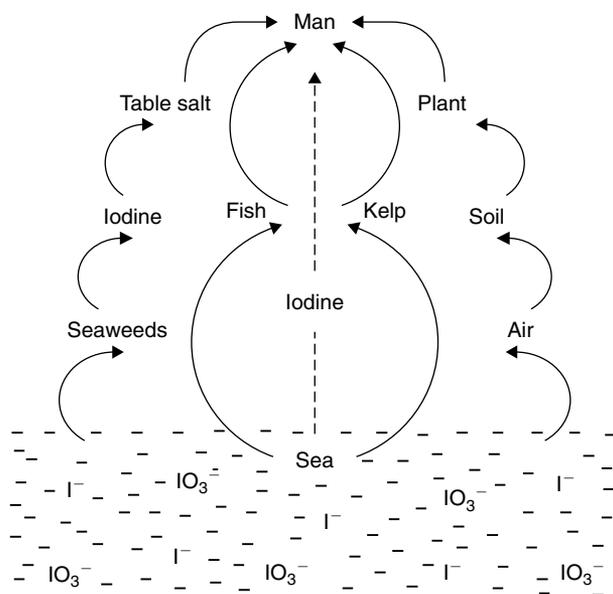


Figure 1.1 The main pathways for iodine present in the sea to enter into the human food chain. Seawater is a huge reservoir of iodine, and it may supply iodine to humans using several pathways. The main species of iodine in seawater are iodide (I^-) and iodate (IO_3^-).

where it exists mainly as iodide and iodate along with a small fraction of organic iodine compounds. The distribution of iodide and iodate in seawater varies with depth and geographical location. The thermodynamically stable form of iodine in seawater is iodate, which is the dominant form in most of the oceans. Iodide in the oceans is produced by biologically mediated reduction of iodate, also favorable under reducing conditions. Up to 50% of the dissolved inorganic iodine may be present as iodide in surface seawater. Organic iodine constitutes less than 5% of the dissolved iodine in the open ocean, but a large fraction (40–80%) of the dissolved iodine may be present in an organic form in estuarine and coastal waters (Chance *et al.*, 2007; Ito *et al.*, 2003).

The determination of iodine in seawater helps in understanding the marine environment. A variety of analytical methods have been proposed for the quantitative determination of iodine in seawater. This chapter discusses the methods employed for the separation and determination of iodine in seawater. These methods include capillary electrophoresis (CE), ion chromatography (IC), high-performance liquid chromatography (HPLC), gas chromatography (GC), spectrophotometry, ion-selective electrode, polarography, voltammetry, atomic emission spectrometry (AES), and neutron activation analysis (NAA). The advantages and limitations of these methods are also assessed and discussed. Since iodine is present in the ocean at trace levels and the matrices of seawater are complex, especially in estuarine and coastal waters, the methods developed for the

determination of iodine in seawater are usually of high sensitivity and selectivity (Chen *et al.*, 2007; Li *et al.*, 2001). Thus, these methods could also be used for the determination of iodine in other samples, such as food, blood, and urine samples, either directly or with minor modifications.

Capillary Electrophoresis

CE is based on the different mobilities of ions in an electric field, and is used for the analysis of charged species. It is a separation technique of high efficiency with low sample and solvent consumption (Li *et al.*, 2004). CE is a powerful tool for separation and quantification of inorganic ions. However, when the concentrations of target analytes are very low in samples, such as iodide and iodate in seawater, the method's performance turns out to be challenging because of the inherent limitations of concentration sensitivity. Incorporation of an online preconcentration technique helps address this challenge, and transient isotachopheresis appears to be one of the most viable options enabling high-sensitivity detection of trace-level inorganic analytes. The sample components can be enriched from diluted solutions by advanced concentration factors, i.e., up to 100 or more (Ito *et al.*, 2003). Thus, preconcentration techniques using transient isotachopheresis were widely adopted in a number of capillary electrophoretic methods for the determination of iodide and/or iodate in seawater.

Capillary electrophoresis was used for the determination of iodide in seawater, human urine and serum, and cooking salt (Pantuckova and Krivankova, 2004). The best separation results of iodide from other macro- and microcomponents in the tested matrices were obtained when host–guest interaction with alpha-cyclodextrin or ion-pairing with polyethylenimine was employed. Due to the relatively high cost of cyclodextrin, only the method using polyethylenimine was developed. The samples were injected into a fused-silica capillary coated with polyacrylamide and filled with the optimized background electrolyte composed of $20 \text{ mmol} \cdot \text{l}^{-1} \text{ KH}_2\text{PO}_4$ and 0.7% polyethylenimine. The detection limits at 230 nm were $0.17 \mu\text{mol} \cdot \text{l}^{-1}$ for seawater, $0.14 \mu\text{mol} \cdot \text{l}^{-1}$ for human urine, $0.17 \mu\text{mol} \cdot \text{l}^{-1}$ for human serum, and $89 \text{ nmol} \cdot \text{l}^{-1}$ for cooking salt, respectively. The relative standard deviations of the peak area and height in all matrices ranged between 0.93% and 4.19%.

Capillary zone electrophoresis with transient isotachopheresis as the online concentration procedure was developed for the determination of iodide in seawater (Yokota *et al.*, 2003). The effective mobility of iodide was decreased by the addition of $10 \text{ mmol} \cdot \text{l}^{-1}$ cetyltrimethylammonium chloride to an artificial seawater background electrolyte so that transient isotachopheresis functioned. The detection limit of iodide was $3.0 \mu\text{g} \cdot \text{l}^{-1}$. The relative standard deviations of peak area, peak height, and migration time

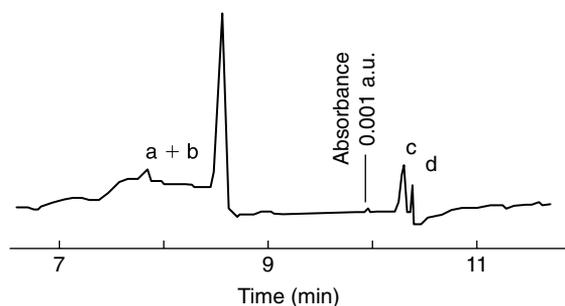


Figure 1.2 Electropherogram of iodide and iodate in surface seawater. The main speciation of iodine in seawater (iodide and iodate) could be determined simultaneously. Conditions: capillary, $375\mu\text{m}$ outer diameter \times $75\mu\text{m}$ inner diameter with a length of 122cm ; carrier electrolyte, artificial seawater containing $10\text{mmol}\cdot\text{l}^{-1}$ hexadecyltrimethylammonium chloride (pH 7.9); voltage = 8kV ; vacuum injection period = 20s ; terminating ion, $2\text{mol}\cdot\text{l}^{-1}$ phosphate; injection period = 4s ; detection = 221nm . Peaks: a = NO_2^- ; b = NO_3^- ; c = I^- ; d = IO_3^- . Reprinted from Yokota *et al.* (2004) with permission from Elsevier.

for iodide were 2.9, 2.1, and 0.6%, respectively. The proposed method was applied to the determination of iodide in seawater collected around the Osaka Bay. In addition, an improved transient isotachopheresis procedure was developed for the preconcentration of iodide from highly saline matrices (Hirokawa *et al.*, 2003). The procedure took advantage of introducing cetyltrimethylammonium chloride into the high sodium chloride background electrolyte, which was due to a specific interaction with iodide-amended placement of the analyte at a large distance from the matrix chloride. Computer simulation showed that 2-(*N*-morpholino)ethanesulfonate could be adopted as a suitable terminating ion to enable isotachopheretic focusing at the beginning of the capillary electrophoretic run. The sensitivity response of iodide was improved by a factor of 140 over normal capillary electrophoretic mode. This allowed direct ultraviolet (UV) detection of as low as $0.6\mu\text{g}\cdot\text{l}^{-1}$ iodide, and made capillary electrophoretic analysis of undiluted surface seawater samples feasible. The proposed method could be extended to the determination of other trace anions (e.g., iodate) in seawater. Furthermore, iodide and iodate in seawater could be determined simultaneously using capillary zone electrophoresis with transient isotachopheresis as an online concentration procedure (Yokota *et al.*, 2004). The effective mobility of iodide was decreased by an addition of $20\text{mmol}\cdot\text{l}^{-1}$ cetyltrimethylammonium chloride to an artificial seawater background electrolyte so that transient isotachopheresis functioned for both iodide and iodate. The detection limits of iodide and iodate were 4.0 and $5.0\mu\text{g}\cdot\text{l}^{-1}$ (as iodine), respectively. The relative standard deviations of the peak area, peak height, and migration times for iodide and iodate were 2.9, 1.3, 1.0, 2.3, 2.1, and 1.0%, respectively. The electropherogram of iodide and iodate in surface seawater is shown in Figure 1.2. A similar technique was developed by Huang *et al.* (2004b)

for simultaneous determination of iodide and iodate in seawater. The proposed method was based on the on-capillary preconcentration of iodide and iodate using the principle of transient isotachopheresis stacking, and direct UV detection of the separated species at 226 and 210nm , respectively. The preconcentration procedure took advantage of the electrokinetic introduction of the terminating ion 2-(*N*-morpholino)ethanesulfonate into the capillary, which enabled a longer transient isotachopheresis state. The valid calibration was demonstrated in the range of $3\text{--}60\mu\text{g}\cdot\text{l}^{-1}$ for iodide and $40\text{--}800\mu\text{g}\cdot\text{l}^{-1}$ for iodate. The detection limits were $0.23\mu\text{g}\cdot\text{l}^{-1}$ ($2\text{nmol}\cdot\text{l}^{-1}$) for iodide and $10\mu\text{g}\cdot\text{l}^{-1}$ ($57\text{nmol}\cdot\text{l}^{-1}$) for iodate. The method could be applied to direct speciation analysis of surface and seabed seawater. The comparison of capillary electrophoretic results with those of an IC proved that the method had acceptable accuracy.

CE following transient isotachopheretic preconcentration was used to determine iodide in seawater and compared with IC (Ito *et al.*, 2003). The carrier and terminating electrolytes were $0.5\text{mol}\cdot\text{l}^{-1}$ sodium chloride and $25\text{mmol}\cdot\text{l}^{-1}$ cetyltrimethylammonium chloride (pH 2.4), and $0.5\text{mol}\cdot\text{l}^{-1}$ 2-(*N*-morpholino) ethanesulfonate (pH 6.0), respectively. The calibration curve was linear in the range of $0\text{--}40\mu\text{g}\cdot\text{l}^{-1}$, and the detection limit was $0.2\mu\text{g}\cdot\text{l}^{-1}$. The relative standard deviations of migration time, peak height, and area of iodide in surface seawater were 0.6, 3.1, and 1.5%, respectively. The method was applied to seawater samples containing sub- and low-microgram per liter levels of iodide, and the results obtained agreed well with ion chromatographic data. A comparison of electropherogram and ion chromatogram of iodide in seawater samples taken at different depths is shown in Figure 1.3. In addition, capillary zone electrophoresis with isotachopheresis preconcentration could be used to determine total iodine in seawater (Huang *et al.*, 2004a). The method was based on the on-capillary reduction of iodine species to iodide by a reductant, introduced into the capillary before sample injection, and the preconcentration of iodide using isotachopheresis, followed by UV detection. The organoiodine compounds in the sample were treated with H_2O_2 and irradiation with UV light before analysis. The detection limit of the method was $0.4\mu\text{g}\cdot\text{l}^{-1}$. The relative standard deviations of migration time and peak area were 0.46 and 1.45% for total iodine of $19\mu\text{g}\cdot\text{l}^{-1}$, respectively. The correlation factor was 0.9991 ($n = 10$) for the concentration range of $12\text{--}115\mu\text{g}\cdot\text{l}^{-1}$. The electropherogram for the determination of total inorganic iodine of surface seawater and deep seawater is shown in Figure 1.4. The capillary electrophoretic results agreed with those of IC for the real seawater samples. Furthermore, a high-sensitivity capillary electrophoretic method was used to monitor vertical distributions of iodate, iodide, total inorganic iodine, dissolved organic iodine, and total iodine in the North Pacific Ocean

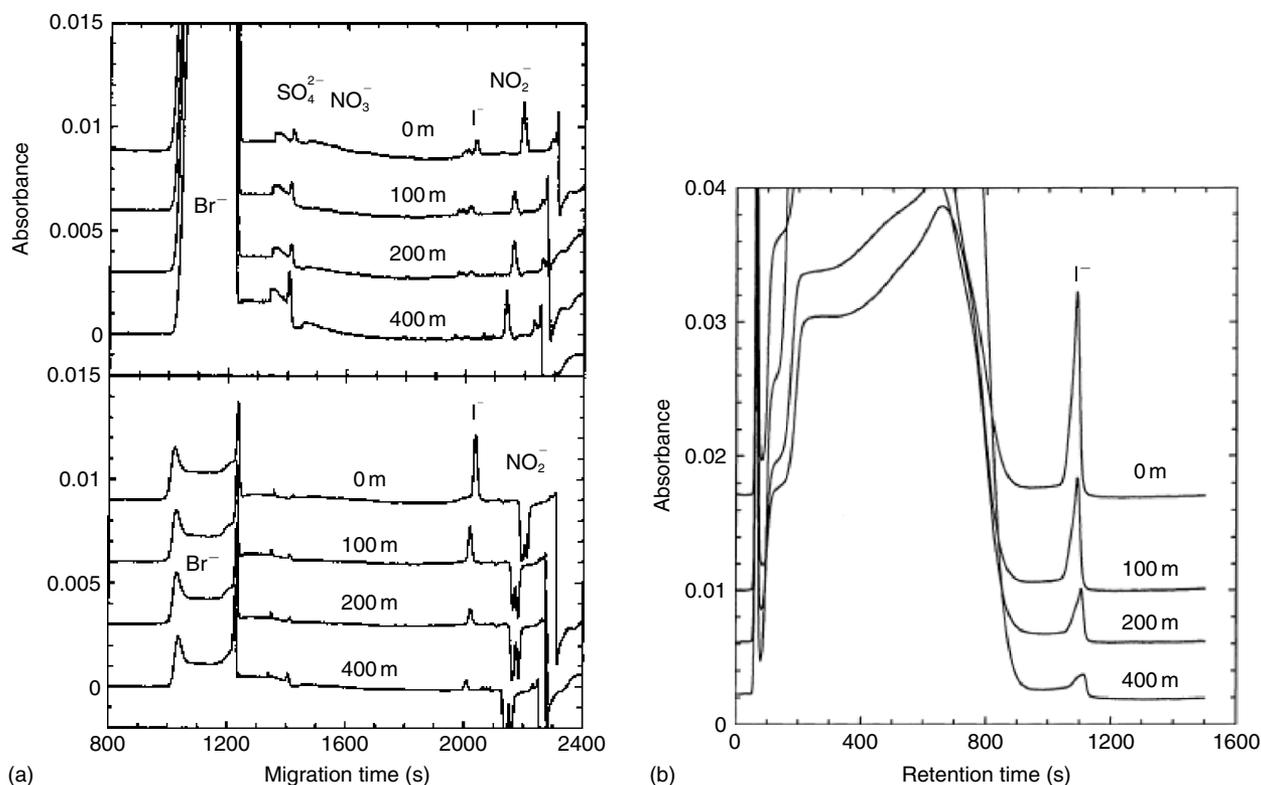


Figure 1.3 Comparison of (a) electropherogram and (b) ion chromatogram of iodide in seawater samples taken at different depths. Iodide in seawater was determined by CE or IC, and the results obtained by the two methods were consistent. The concentration of iodide in surface seawater was higher than that in deep seawater. In addition, the detection with (b) 226 nm was better (or more sensitive) than that with (a) 210 nm for the determination of iodide in (a). Conditions of CE (a): fused-silica capillaries, 375 μm outer diameter \times 75 μm inner diameter with length 100 cm; capillary temperature = 25 $^\circ\text{C}$; voltage = -8 kV ; carrier electrolyte, $0.5\text{ mol}\cdot\text{l}^{-1}$ NaCl and $25\text{ mmol}\cdot\text{l}^{-1}$ hexadecyltrimethylammonium chloride (pH 2.4); sample introduction time = 10 s; loading time of terminating electrolyte = 8 s; detection: (a) 210 nm; (b) 226 nm. Conditions of IC (b): a semimicrocolumn (100 \times 1.5 mm inner diameter) packed with TSKgel SAX (5 μm); mobile phase, $60\text{ mmol}\cdot\text{l}^{-1}$ NaClO₄, $0.7\text{ mol}\cdot\text{l}^{-1}$ NaCl and $5\text{ mmol}\cdot\text{l}^{-1}$ sodium phosphate buffer (pH 6.5); flow rate = $0.2\text{ ml}\cdot\text{min}^{-1}$; sample volume = 2 ml; detection: 226 nm. Reprinted from Ito *et al.*, (2003) with permission from Elsevier.

(0–5500 m) without any sample pretreatment other than UV irradiation before total iodine analysis (Huang *et al.*, 2005). The results suggested that the vertical distribution of iodine was associated with biological activities. The concentrations of all iodine species changed noticeably above 1000 m, but only minor latitudinal changes occurred below 1000 m, and only slight vertical alterations were observed below 2400 m.

Chromatography

Ion chromatography

Ion-exchange chromatography is regarded as one of the traditional high-performance liquid chromatographic techniques, and IC was developed from ion-exchange chromatography. When IC is used to separate and determine iodide in seawater, the large quantity of matrix ions (chloride, sulfate) saturate the active sites of the ion-exchange column, and thereby impede the separation of the target analytes. In addition, the

high ionic strength of the sample causes self-elution of the sample band during injection, leading to peak broadening and loss of separation efficiency. To overcome these difficulties, a matrix elimination ion chromatographic technique is often adopted, although some practical problems do persist, including the use of nonmetallic hardware to avoid corrosion (Hu *et al.*, 2002; Li *et al.*, 2001). In addition, an electrostatic ion chromatographic technique has been established which is also applicable for the direct determination of iodide in seawater, but which avoids the necessity for adding matrix ions at very high concentrations to the eluent. This technique was developed using an adsorbed layer of sulfobetaine-type zwitterionic surfactants as the stationary phase, and showed no affinity for sulfate and very low affinity for chloride (Hu *et al.*, 2002).

A matrix elimination IC with postcolumn reaction detection was developed for the determination of iodide in seawater (Brandao *et al.*, 1995). A Dionex IonPac AS11 anion-exchange column was used, and the mobile phase contained sodium chloride to remove interference of the

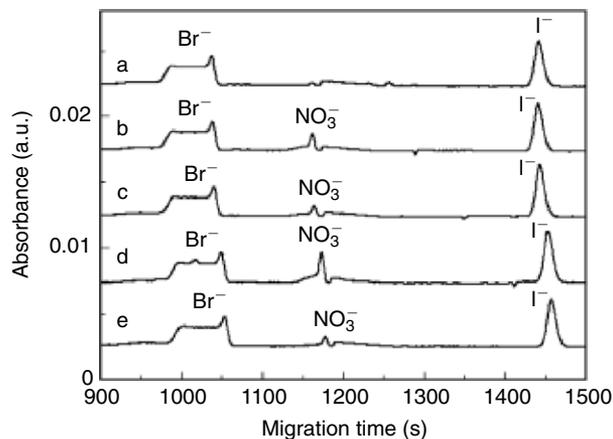


Figure 1.4 Electropherogram for the determination of total inorganic iodine in seawater. Samples: (a) surface seawater and (b) deep seawater at Kochi, (c) surface seawater and deep seawater at (d) 100 m and (e) 710 m at 41°N, 155°E in the Pacific Ocean. The change of total inorganic iodine with seawater depth was not very obvious. Conditions: capillary, fused-silica, 100 cm total and 87.7 cm to detector (75 μ m inner diameter); current = -195μ A; carrier electrolyte, $200 \text{ mmol} \cdot \text{l}^{-1}$ hydroxylamine hydrochloride and $300 \text{ mmol} \cdot \text{l}^{-1}$ NaCl; terminating electrolyte, $500 \text{ mmol} \cdot \text{l}^{-1}$ 2-morpholinoethanesulfonic acid monohydrate (pH 6.5); introduction time of reductant electrolyte = 7 s; sample introduction time = 4 s; introduction time of terminating electrolyte = 10 s; detection wavelength = 226 nm. Reprinted from Huang *et al.*, (2004a) with permission from Elsevier.

sample matrix in chromatographic separation and detection. This matrix elimination procedure was reinforced by a postcolumn reaction detection that was both selective and sensitive for iodide, and was based on the reaction of iodide with 4,4'-bis(dimethylamino)diphenylmethane in the presence of *N*-chlorosuccinimide. The wavelength of detection was 605 nm. The detection limit was $0.8 \text{ ng} \cdot \text{l}^{-1}$ for iodide in seawater, and the relative standard deviation was better than 4%. No interference from dissolved organic matter in seawater samples was observed. Bromide was a potential cause of interference, but was well separated from iodide. In addition, an ion chromatographic method with UV detection was developed for the determination of iodide in seawater (Ito, 1997). A high-capacity anion-exchange resin with a polystyrene-divinylbenzene matrix was used both for preconcentration and separation of iodide. Iodide in artificial seawater was trapped quantitatively ($98.8 \pm 0.6\%$) without peak broadening on a preconcentrator column, and was separated with $0.35 \text{ mol} \cdot \text{l}^{-1}$ NaClO_4 + $0.01 \text{ mol} \cdot \text{l}^{-1}$ phosphate buffer (pH 6.1). On the other hand, the major anions (chloride and sulfate) in seawater were partially trapped (5–20%) and did not interfere in the determination of iodide. The detection limit of the method was $0.2 \mu\text{g} \cdot \text{l}^{-1}$ for artificial seawater. The method was applied to the determination of iodide and total inorganic iodine in seawater samples taken near Japan. Later, a semi-micro ion chromatographic method was

developed by the same author for the determination of iodide in seawater (Ito, 1999). Large sample volume injections, including both on-column analyte focusing and on-column matrix elimination techniques, were examined for the determination of iodide in seawater. A semi-microcolumn ($35 \times 1 \text{ mm}$ I.D.) packed with styrene-divinylbenzene copolymer with high anion-exchange capacity and a mobile phase of $0.03 \text{ mol} \cdot \text{l}^{-1}$ NaClO_4 + $0.5 \text{ mol} \cdot \text{l}^{-1}$ NaCl + $5 \text{ mmol} \cdot \text{l}^{-1}$ sodium phosphate buffer (pH 6.0) was used. ClO_4^- ion in the mobile phase was effective for the elution of iodide and Cl^- ion both for the concentration of iodide with hydrophobicity and for the removal of interference by the major anions. Iodide in seawater was effectively concentrated on the column and eluted without peak broadening. The slope of calibration curve using the semi-microcolumn was about 2.8 times higher than that of a conventional column ($150 \times 4.6 \text{ mm}$ I.D.) with the same resin. The detection limit was $0.2 \mu\text{g} \cdot \text{l}^{-1}$ for artificial seawater. The method was successfully applied to seawater samples. The chromatogram of iodide in seawater sample by semi-micro IC is shown in Figure 1.5. The scale-down of ion chromatographic systems had several advantages over IC using conventional columns, which included increased efficiency in a shorter time, lower mobile phase consumption, and the use of smaller amounts of packing materials. In addition, IC was also applied to the determination of iodide and iodate concentrations in the subtropical waters along the continental shelf and slope waters of eastern Australia (Mctaggart *et al.*, 1994). The concentration of iodide in the shelf waters ranged from 0.07 to $0.18 \mu\text{mol} \cdot \text{l}^{-1}$ between 0 and 50 m, and ranged from 0.01 to $0.03 \mu\text{mol} \cdot \text{l}^{-1}$ below a depth of 100 m. However, iodate concentration showed the inverse trend to iodide, ranging from 0.32 to $0.39 \mu\text{mol} \cdot \text{l}^{-1}$ in the top 50 m, increasing to $0.46 \mu\text{mol} \cdot \text{l}^{-1}$ below a depth of 100 m. The results indicated that iodate was reduced to iodide in Coral Sea surface waters in the presence of low concentrations of both phosphate and nitrate.

An electrostatic ion chromatographic method was developed for the direct determination of iodide, bromide and nitrate in seawater (Hu *et al.*, 1999). An octadecylsilica column modified with a zwitterionic surfactant 3-(*N,N*-dimethylmyristylammonio)propane-sulfonate was used as the stationary phase, and an electrolytic solution was used as the eluent. The matrix species (such as chloride and sulfate) were retained weakly, and showed little or no interference. The method was applied to the determination of iodide, bromide, and nitrate in artificial seawater, giving detection limits of $0.8 \mu\text{g} \cdot \text{l}^{-1}$ for iodide, $0.75 \mu\text{g} \cdot \text{l}^{-1}$ for bromide, and $0.52 \mu\text{g} \cdot \text{l}^{-1}$ for nitrate, and relative standard deviations of $<1.2\%$. The real seawater samples were also analyzed successfully. Later, another electrostatic ion chromatographic method was developed for the determination of iodide in seawater by the same research group (Hu *et al.*, 2002). A reversed-phase ODS column was

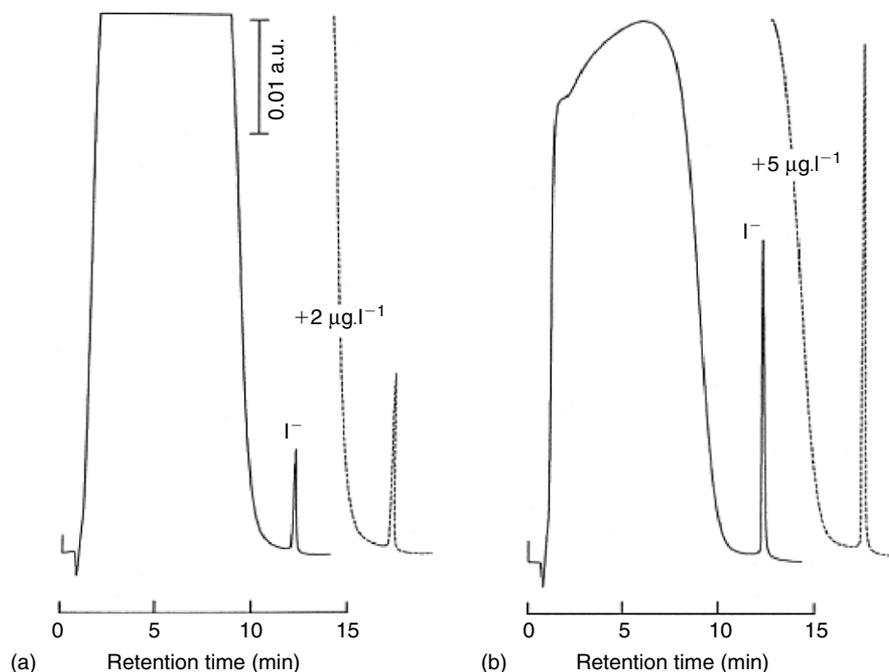


Figure 1.5 Chromatogram of iodide in seawater by semi-micro IC. The increase in peak height was very obvious after iodide (I^-) was added. In addition, the change in peak height with concentration of iodide (I^-) was also very obvious with the comparison of (a) with (b). Column, semi-microcolumn (TSKgel SAX resin, 35×1 mm inner diameter); mobile phase, $0.03 \text{ mol} \cdot \text{l}^{-1} \text{ NaClO}_4 + 0.5 \text{ mol} \cdot \text{l}^{-1} \text{ NaCl} + 5 \text{ mmol} \cdot \text{l}^{-1}$ sodium phosphate buffer (pH 6.0); flow rate = $0.3 \text{ ml} \cdot \text{min}^{-1}$; sample volume = 2 ml; detection, UV at 226 nm. The concentrations of iodide in seawater samples were 1.3 and $6.5 \text{ mg} \cdot \text{l}^{-1}$ in (a) and (b), respectively. Reprinted from Ito (1999) with permission from Elsevier.

modified by coating with Zwittergent-3-14 micelles, and an aqueous solution containing $0.2 \text{ mmol} \cdot \text{l}^{-1} \text{ NaClO}_4$ and $0.3 \text{ mmol} \cdot \text{l}^{-1}$ Zwittergent-3-14 was used as an eluent. The wavelength of detection was 210 nm. Nitrite, iodate, bromide, bromate, and nitrate showed very little or no retention, while iodide and thiocyanate were well separated. The detection limit was $0.011 \mu\text{mol} \cdot \text{l}^{-1}$, and the relative standard deviations for 0.1 and $0.3 \mu\text{mol} \cdot \text{l}^{-1}$ iodide in real seawater samples were 2.3 and 1.2%, respectively. The chromatogram of iodide in unspiked seawater and spiked seawater using electrostatic IC is shown in Figure 1.6.

An ion chromatographic method was reported for the determination of iodide in seawater and edible salt (Rong and Takeuchi, 2004). A laboratory-made C30 packed column was dynamically coated with poly(ethylene glycol), and the effects of eluent composition on retention behavior of inorganic anions were investigated. The detection limit was $9 \mu\text{g} \cdot \text{l}^{-1}$. The method was successfully applied to the rapid and direct determination of iodide in seawater and edible salt samples. However, the dynamically coated column could be used for only a short time (about 2 weeks) because the poly(ethylene glycol) modifier was gradually flushed away by the eluent during the experiment. In order to overcome this drawback, poly(ethylene glycol) groups were chemically bonded onto C30-bonded

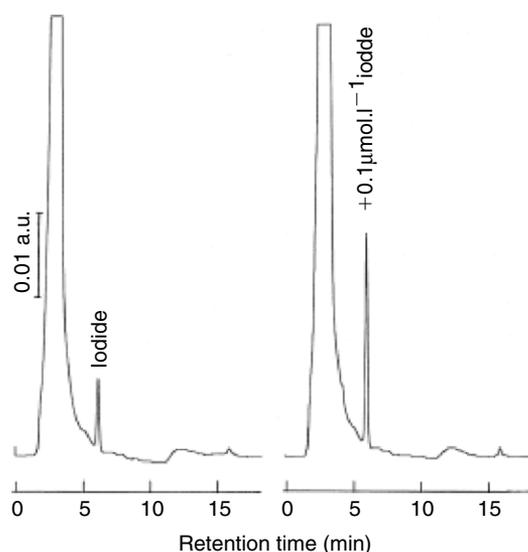


Figure 1.6 Chromatogram of iodide in seawater using electrostatic IC. Left trace: unspiked seawater. Right trace: seawater spiked with $0.1 \mu\text{mol} \cdot \text{l}^{-1}$ iodide. The increase in peak height was very obvious after iodide was added. Column: ODS-packed column (250×4.6 mm inner diameter) coated with Zwittergent-3-14; mobile phase, $0.2 \text{ mmol} \cdot \text{l}^{-1} \text{ NaClO}_4$ and $0.3 \text{ mmol} \cdot \text{l}^{-1}$ Zwittergent-3-14; flow rate = $1.0 \text{ ml} \cdot \text{min}^{-1}$; sample injection volume = $400 \mu\text{l}$; detection: UV at 210 nm. Reprinted from Hu *et al.*, (2002) with permission from Elsevier.

silica gel via diol groups (Rong *et al.*, 2006). Poly(ethylene glycol)-bonded C30 binary phases allowed determination of iodide in seawater samples without any interference. The detection limit for iodide was $13 \mu\text{g} \cdot \text{l}^{-1}$, while the limit of quantitation was $43 \mu\text{g} \cdot \text{l}^{-1}$. The proposed method was successfully applied to the determination of iodide in seawater with long-term durability.

An anion-exchange chromatography with spectrophotometric detection was developed for the determination of iodide, iodate, and organic iodine in freshwater and seawater (Schwehr and Santschi, 2003). Iodide was determined directly by this method, and iodate and total of organic iodine species were determined as iodide with minimal sample preparation. The method was applied to determine iodide, iodate as the difference of total inorganic iodide and iodide after reduction of the sample by NaHSO_3 , and organic iodine as the difference of total iodide (after organic decomposition by dehydrohalogenation and reduction by NaHSO_3) and total inorganic iodide. The detection limit was about $1 \text{ nmol} \cdot \text{l}^{-1}$ ($0.2 \mu\text{g} \cdot \text{l}^{-1}$), and the relative standard deviation was less than 3%. The method was applied to determine the concentrations of iodide species in rain, surface- and groundwater, estuarine, and seawater samples.

Although UV detection is still the most widely used method, electrochemical detection is employed for improved specificity and sensitivity. Disposable and conventional silver working electrodes were compared for the determination of iodide, using high-performance anion-exchange chromatography with pulsed amperometric detection (Liang *et al.*, 2005). The results showed that the disposable working electrode manifested results equal to or better than those of the conventional working electrode, and could be used for the determination of iodide. Also, the disposable electrode could work consecutively for about 44 h with no degradation. The disposable electrode was applied to the determination of iodide in seawater and soil samples. The detection limit was $0.5 \mu\text{g} \cdot \text{l}^{-1}$, and the recoveries ranged from 96% to 104%.

Inductively coupled plasma mass spectrometry has several inherent advantages over conventional detection techniques, including its sensitivity and element specificity. However, IC with inductively coupled plasma mass spectrometry was not used for the analysis of iodide and iodate in seawater, except in a very recent report (Chen *et al.*, 2007). This could be because the high concentration of matrices in seawater led to loss of sensitivity from the build-up of salts on the sampler and skimmer cones of the mass spectrometer. In addition, chloride interfered with eluting very near to iodate because the column was overloaded when the chloride concentration was very high in seawater. Recently, a nonsuppressed IC with inductively coupled plasma mass spectrometry was developed for simultaneous determination of iodate and iodide in seawater (Chen *et al.*, 2007). An anion-exchange column

Agilent G3154A/101 was used for the separation of iodate and iodide. In order to minimize salt deposition on the sampler and skimmer cones of the mass spectrometer, NH_4NO_3 solution was used as the mobile phase, and a low volume of $10 \mu\text{l}$ with diluted sample was directly injected into the ion chromatographic system. The linear range was $5.0\text{--}500 \mu\text{g} \cdot \text{l}^{-1}$. The detection limits were $1.5 \mu\text{g} \cdot \text{l}^{-1}$ for iodate and $2.0 \mu\text{g} \cdot \text{l}^{-1}$ for iodide. The proposed method was used to determine iodate and iodide in seawaters without sample pretreatment, except dilution. The chromatogram of iodate and iodide in seawater by nonsuppressed IC with inductively coupled plasma mass spectrometric detection is shown in Figure 1.7.

High-performance liquid chromatography

Recently, HPLC with complexes has become more significant in the separation of inorganic ions, but only a few papers deal with the determination of anions (Xu *et al.*, 2004a, b). A novel method for the determination of iodide was established using size exclusion chromatography with iodine–starch complex (Li *et al.*, 2001). Iodide was converted into iodine, sequestered with starch, and then separated from the matrix using a Shim-pack DIOL-150 size exclusion column with methanol– $0.01 \text{ mol} \cdot \text{l}^{-1}$ aqueous phosphoric acid as the mobile phase and UV detection at 224 nm. The calibration graph was linear from 1.0 to $100.0 \text{ ng} \cdot \text{ml}^{-1}$ for iodide with a correlation coefficient of 0.9992. The detection limit was $0.2 \text{ ng} \cdot \text{ml}^{-1}$. The method was successfully applied to the determination of iodide in seawater and urine. The recovery was from 92% to 103%, and the relative standard deviation was in the range of 1.5–3.7%. The chromatogram of iodide in seawater by size exclusion chromatography with UV detection is shown in Figure 1.8.

Gas chromatography

Another method developed for the determination of iodide was GC–mass spectrometry (Mishra *et al.*, 2000). Iodide was oxidated to iodine with 2-iodosobenzoate, and then converted into 4-iodo-*N,N*-dimethylaniline in the presence of *N,N*-dimethylaniline. The derivative was extracted into cyclohexane and determined by GC–mass spectrometry. The method could also be used to determine iodine by derivatization in the absence of 2-iodosobenzoate, and iodate by its reduction with ascorbic acid to iodide and subsequent derivatization. The calibration graph was linear from 0.02 to $50 \mu\text{g} \cdot \text{l}^{-1}$ of iodide with a correlation coefficient of 0.9998. The limit of detection was $8 \text{ ng} \cdot \text{l}^{-1}$ of iodide. The proposed method was applied to the determination of iodate in iodized table salt and free iodide and total iodine in seawater. The recovery was in the range of 96.8–104.3%, and the relative standard deviations were from 1.9% to 3.6%. A sample clean-up by solid-phase extraction with a LiChrolut EN cartridge was

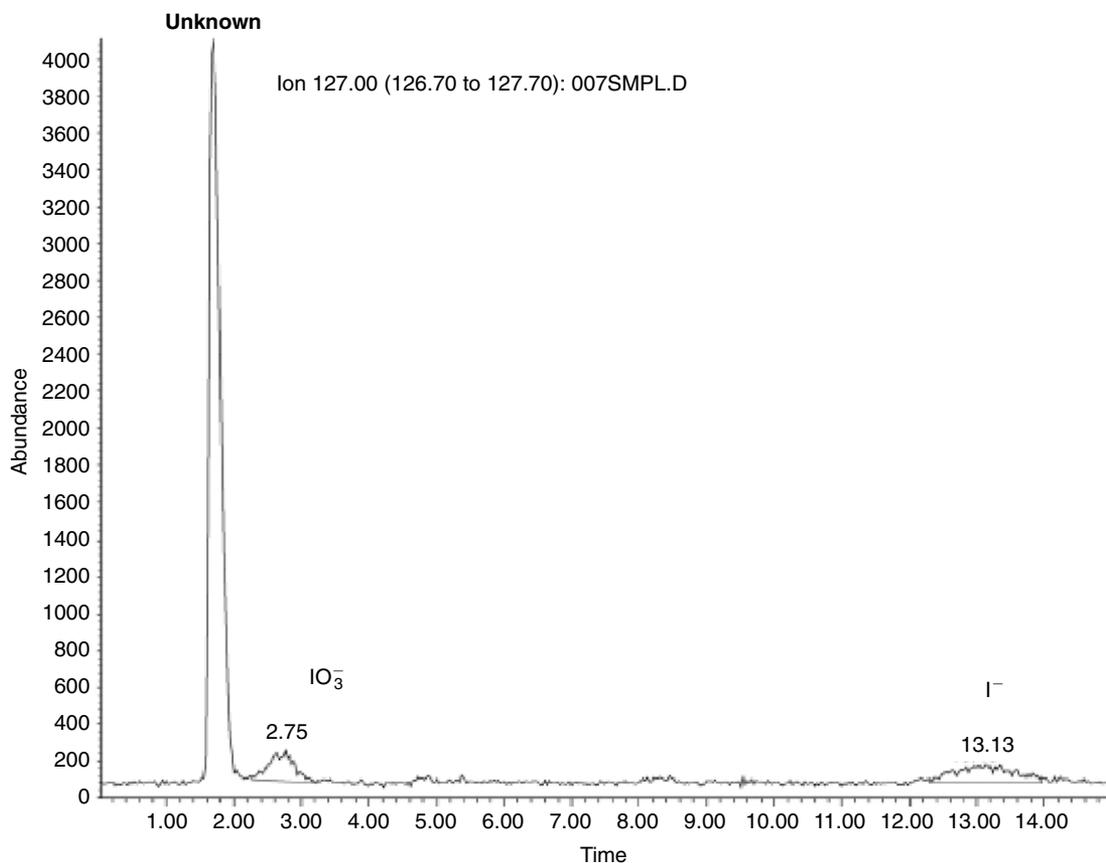


Figure 1.7 Chromatogram of iodate and iodide in seawater by nonsuppressed IC with inductively coupled plasma mass spectrometric detection. The main speciation of iodine in seawater, iodate (IO_3^-) and iodide (I^-), could be determined simultaneously. Conditions: column, Agilent G3154A/101 (150 \times 4.6 mm inner diameter); column temperature = 20°C; injection volume = 10 μl ; mobile phase, 20.0 $\text{mmol} \cdot \text{l}^{-1}$ of NH_4NO_3 at pH 5.6; flow rate = 1.0 $\text{ml} \cdot \text{min}^{-1}$. The ICP-MS conditions: flow rate of plasma gas (Ar) = 15 $\text{l} \cdot \text{min}^{-1}$; flow rate of auxiliary gas (Ar) = 1.0 $\text{l} \cdot \text{min}^{-1}$; flow rate of carrier gas (Ar) = 1.15 $\text{l} \cdot \text{min}^{-1}$; sampling depth = 7.5 mm; integration time = 1 s; dwell time = 0.5 s. The ^{127}I was selected for detection by single-ion monitoring mode. Reprinted from [Chen *et al.*, \(2007\)](#) with permission from Elsevier.

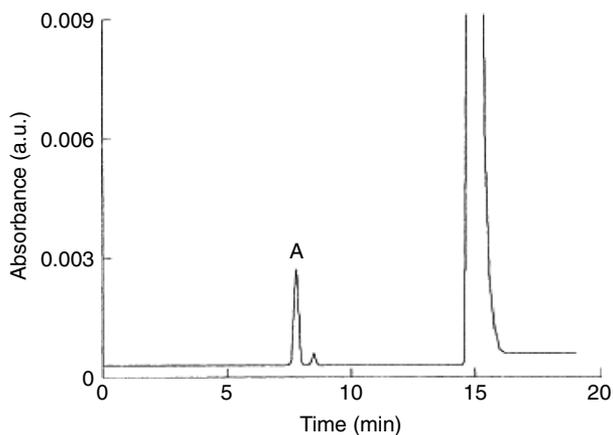


Figure 1.8 Chromatogram of iodide in seawater by size exclusion chromatography with UV detection. Iodide in seawater could be determined indirectly after it was converted to iodine–starch complex. Conditions: column, Shim-pack DIOL-150; mobile phase, methanol–0.01 $\text{mol} \cdot \text{l}^{-1}$ aqueous phosphoric acid (10:90); flow rate = 1.2 $\text{ml} \cdot \text{min}^{-1}$; detection = 224 nm; injection volume = 20 μl . Peak A = iodine–starch complex. The concentration of iodide in seawater was 9.2 $\text{ng} \cdot \text{ml}^{-1}$. Reprinted from [Li *et al.*, \(2001\)](#) with permission from Elsevier.

also proposed. In addition, the concentrations of volatile organoiodine in seawater could be determined by GC with electron capture detection ([Schall and Heumann, 1993](#)). A purge and trap technique was used to isolate the organoiodine compounds from the seawater samples. The iodinated compounds CH_3I , CH_2I_2 , CH_2ClI , $\text{CH}_3\text{CH}_2\text{CH}_2\text{I}$, and $\text{CH}_3\text{CHICH}_3$ were determined in Arctic seawater with mean concentrations in the range of 0.3–6.2 $\text{ng} \cdot \text{l}^{-1}$.

Other Methods

CE and chromatography are the most frequently employed methods for the determination of iodine in seawater. Other methods used, such as spectrophotometry, ion-selective electrode, polarography, voltammetry, AEC, and NAA will be discussed briefly in the following sections.

Spectrophotometry

Spectrophotometry is a conventional and inexpensive technique. However, it also has several limitations,

including low sensitivity and selectivity. Spectrophotometric determination of iodate in seawater involved the reaction of IO_3^- with excess I^- under acid conditions to form I_2 . It was known that when the acidification was by mineral acid, nitrite would interfere with the method by oxidizing I^- to I_2 in a way analogous to IO_3^- . Although some researchers had used sulfamic acid for acidification, because it was reported to destroy nitrite in solution, the results obtained by Chapman and Liss (1977) showed that for a seawater sample containing $0.31 \mu\text{mol} \cdot \text{l}^{-1}$ ($39 \mu\text{g} \cdot \text{l}^{-1}$) IO_3^- , the presence of $0.5 \mu\text{mol} \cdot \text{l}^{-1}$ NO_2^- would lead to an error of 25% in the iodate determined colorimetrically using sulfuric acid, and 15% when sulfamic acid was used.

Ion-selective electrode

Tetrakis(4-*N,N*-dimethylaminobenzene)porphyrinato-manganese(III) acetate was used as a novel carrier for a selective iodide ion electrode (Farhadi *et al.*, 2004). The sensor exhibited not only excellent selectivity to iodide ion compared to Cl^- and lipophilic anions such as ClO_4^- and salicylate, but also a Nernstian response for iodide ion over a wide concentration range from 1.0×10^{-2} to $7.5 \times 10^{-6} \text{mol} \cdot \text{l}^{-1}$. The potentiometric response was independent of the pH of the solution in the pH range 2–8. The electrode could be used for at least 2 months without any considerable divergence in the potential. The electrode was applied to the determination of iodide in seawater samples and drug formulations.

Polarography

Iodate in seawater could be determined directly by differential pulse polarography with a precision of 2.5% (Herring and Liss, 1974). The same technique might be used to determine total iodine following oxidation of iodide to iodate by UV irradiation. The iodide concentration was obtained from the difference between the total iodine and the iodate values. The method was applied to the determination of IO_3^- and I^- in the Santa Barbara Basin of Southern California. Furthermore, the effect of pH value on the determination of iodate using differential pulse polarography was studied in detail (Lin, 1999). Sensitivity for the determination of iodate decreased rapidly below pH 7 or above pH 9. The decrease below pH 7 might be caused by conversion of iodate into iodide, followed by the successive formation of I_2 and I_3^- . The decrease above pH 9 was probably because of the combination of iodate and hydroxide. Theoretical calculations indicated that the optimum pH for the determination of iodate was about pH 7.4. In addition, sulfite could be used to facilitate the removal of oxygen in the samples before iodate in seawater was analyzed by differential pulse polarography (Wong and Zhang, 1992a). The reaction between sulfite and dissolved oxygen was almost

instantaneous. In comparison to the removal of oxygen by bubbling the sample with an inert gas alone, the time needed for the analysis of a sample for iodate might be reduced to about 15 min.

Voltammetry

Total inorganic iodine in seawater could be determined by cathodic stripping square wave voltammetry (Wong and Zhang, 1992b). The pH value of the sample solution was adjusted to about 1–2, and iodate was reduced to iodide with sodium sulfite. The pH value of the sample solution was then raised to 8–9, and the total inorganic iodine as iodide was determined by cathodic stripping square wave voltammetry. Later, a similar method was developed and applied to the storage study of seawater samples for preserving iodine species (Campos (1997). Iodate was promptly reduced to iodide by ascorbic acid at pH less than or equal to 2.7, and total reducible iodine as iodide was determined using cathodic stripping voltammetry. The detection limits of iodide and total iodine were 0.1 and $0.2 \text{nmol} \cdot \text{l}^{-1}$, respectively. The method was used to determine the dissolved iodine speciation in a variety of seawater samples stored in diverse ways. The iodine speciation was preserved in samples stored for at least 2 months at -16°C , 4°C , and room temperature. However, iodide concentration increased up to three times the original value after longer periods of storage (up to 1 year) at 4°C and room temperature, while total iodine concentrations remained unchanged. The frozen method might be adopted for preserving iodine speciation when long-term storage of the samples is required. In addition, molecular iodine in seawater could also be determined using adsorptive stripping voltammetry by a derivatization to iodoform with alcoholic KOH solution (Moller *et al.*, 1995). The presence of molecular iodine in concentrations of about $10^{-9} \text{mol} \cdot \text{l}^{-1}$ was detected in some surface samples from the Adriatic Sea (Moller *et al.*, 1996). It was proposed that molecular iodine was formed by sun irradiation, and most probably by the reaction of ozone with iodide.

Atomic emission spectrometry

Iodide could be determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) using iodine vapor generation (Anderson *et al.*, 1996). Iodide was oxidized *in situ* to I_2 with potassium nitrite in sulfuric acid in a simplified continuous-flow manifold, which was quantitated by ICP-AES at 206.16 nm. The detection limit for iodide was $0.04 \text{mg} \cdot \text{l}^{-1}$, and the recoveries from seawater, saltwater, and freshwater standard reference materials ranged from 86.5% to 118.6%, averaging 98.2%. Molecular iodine was analyzed by ICP-AES without iodine vapor generation reagents. For the samples containing both molecular iodine and iodide, total iodine was determined with oxidizing reagents, molecular iodine alone was

determined without oxidizing reagents, and iodide was calculated from the difference. Furthermore, iodide, iodine, and iodate could be determined by ICP-AES using *in situ* chemical manipulation (Anderson and Markowski, 2000). Molecular iodine was determined directly, iodide was oxidized *in situ* to iodine by the addition of sodium nitrite in sulfuric acid, and iodate was determined by prereduction before analysis, as well as an *in situ* oxidation ICP-AES procedure. For the samples containing iodine and iodide, total iodine was determined by *in situ* oxidation, molecular iodine was determined without the use of oxidizing reagents, and iodide was calculated from the difference. For the samples containing iodine, iodide and iodate, prereduction was used and the iodine and iodide concentrations were subtracted for the quantitation of iodate.

Neutron activation analysis

A method for the determination of iodide, iodate, organic iodine, and total iodine in seawater was developed by radiochemical NAA combined with ion-exchange pre-separation (Hou *et al.*, 1999). The filtered seawater was passed through an anion-exchange column. The column was washed with deionized water, and the effluent and washings were collected for the determination of the sum of iodate and organic iodine. The column was washed with $0.5 \text{ mol} \cdot \text{l}^{-1} \text{ KNO}_3$ solution, and the washing was discarded. Iodide retained on the column was then eluted using $2.0 \text{ mol} \cdot \text{l}^{-1} \text{ KNO}_3$ solution. Another aliquot of seawater was first acidified to pH 5–6 with $1.0 \text{ mol} \cdot \text{l}^{-1} \text{ HNO}_3$, and $0.3 \text{ mol} \cdot \text{l}^{-1} \text{ KHSO}_3$ solution was added to convert iodate into iodide. The solution was then passed through an anion-exchange column, and the organic iodine was obtained by collection of the effluent and washings of deionized water. The washing and elution of the ion-exchange column were performed using the same method as that for the separation of iodide, and the eluate was collected for the determination of total inorganic iodine (iodide and iodate). The iodine content was determined by NAA with post-irradiation separation using CCl_4 extraction. The detection limit for iodine was $0.2 \mu\text{g} \cdot \text{l}^{-1}$. The method was applied to analysis of iodine speciation in seawater collected from Roskilde Fjord, Denmark. In addition, NAA was used to determine iodine-129 in seawater, seaweed, lake water, lake sediment, and grass collected from the Baltic Sea area (Hou *et al.*, 2002). NAA is expensive, not readily accessible to all laboratories, and has a potential interference problem from bromide.

Summary Points

1. The ocean is a huge reservoir of iodine.
2. Iodine in seawater has a strong relationship with human health.

3. Iodine present in oceans is supplied to the human body in different ways, directly or indirectly.
4. Iodine exists mainly as iodate and iodide, along with a small fraction of organic iodine compounds in seawater.
5. The distribution of iodine speciation in seawater varies with depth and geographical location.
6. Determination of iodine and iodine speciation in seawater is helpful in understanding the marine environment.
7. A number of methods, including CE, IC, HPLC, GC, spectrophotometry, ion-selective electrode, polarography, voltammetry, AE, and NAA, have been developed for the determination of iodine in seawater.
8. IC and CE are the most frequently employed methods for the analysis of iodine in seawater.
9. The methods developed for the determination of iodine in seawater can also be used for the determination of iodine in other samples, such as food, blood, and urine samples, directly or with minor modifications.
10. In the future, more attention should be paid to improve the sensitivity, selectivity, and automatization of the method used for the determination of iodine in seawater.

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2

Assay of Iodine in Foodstuffs: Methods and Applications

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Abstract

Dietary assessment methods, total diet studies, and the duplicate portion method are evaluated with regard to their adequacy for obtaining iodine intake data. The advantages and shortcomings of catalytic spectrophotometric methods, nuclear analytical methods, spectrometric, electrochemical, and other techniques, which are used for the determination of iodine in foodstuffs and related materials, are reviewed.

Abbreviations

AAS	Atomic absorption spectrometry
AOAC	The Association of Official Analytical Chemists
CSC	Compton suppression counting
CSV	Cathodic stripping voltammetry
EINAA	Epithermal instrumental neutron activation analysis
ENAA	Epithermal neutron activation analysis
FAO	Food and Agriculture Organization
FAQ	Food amount questionnaire
FFQ	Food frequency questionnaire
GC	Gas chromatography
HPGe	High-purity germanium
HPLC	High-performance liquid chromatography
IAEA	The International Atomic Energy Agency
ICP-IDMS	Inductively coupled plasma-isotope dilution mass spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectrometry

IDD	Iodine deficiency disorders
INAA	Instrumental neutron activation analysis
INFOODS	International food composition database
LC	Liquid chromatography
NAA	Neutron activation analysis
PS-NAA	Pre-separation-neutron activation analysis
QCM	Quartz crystal microbalance
RDI	Recommended daily intake
RNAA	Radiochemical neutron activation analysis
TXRF	Total reflection X-ray fluorescence
UNICEF	United Nations Children's Fund
WHO	World Health Organization

Introduction

A major amount of iodine enters the human organism via the food chain. Hence, knowledge of iodine levels in foodstuffs and diets is essential for the assessment of iodine intake by man to ascertain whether the recommended daily level (RDI) (WHO, 2001) is met.

Lack of iodine leads to iodine deficiency disorders (IDD), while excessive iodine dietary intake can result in pathological problems, namely goitrogenic effect (Underwood, 1971; WHO, 1996). Low iodine intake resulting in IDD is recognized as a global concern, while excessive iodine intake is not so frequent. For most people it is unlikely that they will exceed the upper level of iodine intake, which is given in Table 2.1 (FAO/WHO, 2006), from normal foods and supplements.

In general, iodine content in foodstuffs is low, usually in the range of 10–200 $\mu\text{g} \cdot \text{kg}^{-1}$ fresh mass (Koutras *et al.*, 1985), except for in seafood. Therefore, analytical methods with a sufficiently low detection limit are required for the iodine assay of foodstuffs. The choice of an adequate

Table 2.1 Tolerable daily intake upper levels (UL) for iodine, µg

Age (years)	UL for EU (EC/SCF, 2002)	Age (years)	UL for USA/ Canada (IOM, 2001)
Children 1–3	200	Children 1–3	200
Children 4–6	250	Children 4–8	300
Children 7–10	300	Children 9–13	600
Children 11–14	450		
Adolescents 15–17	500	Adolescents 14–18	900
Adults (above 18)	600	Adults (above 19)	1100

methodology for the assessment of iodine intake is also important. In many countries a significant portion of the daily iodine intake is achieved by using various supplements, namely iodized salt. This portion may not necessarily be equally taken into account by all methods of iodine intake assessment. In this chapter, approaches to assessment of iodine intake from foodstuffs and diets are evaluated, analytical methods for iodine determination in the above matrices are reviewed, and iodine content in selected foodstuffs is listed.

Obtaining Iodine Intake Data

The main purpose of iodine assay in foodstuffs is to obtain data on intake by an individual or a population. Commonly used approaches to evaluate intake of various diet components, such as nutrients, vitamins, minerals and trace elements, contaminants, and so on, involve dietary assessment methods, total diet studies, and the duplicate portion method. In general, their ease of execution and the validity of the results obtained differs for all diet components, and this particularly applies to iodine. The use of biomarkers to assess iodine intake, specifically iodine excretion in urine, is also mentioned.

Dietary assessment methods

The dietary assessment methods are based on taking a dietary history, such as diet recall (usually 24-h recall, but sometimes for longer periods), diet history (an interview about “typical” or “usual” food intake), and food frequency and/or amount questionnaires (food frequency questionnaire, FFQ and/or food amount questionnaire, FAQ, respectively). More details about these methods are discussed by Margetts and Nelson (1997). Intake of iodine from the data obtained is calculated using various national or international food composition databases, such as INFOODS (Scrimshaw, 1997; Schlotke and Møller, 2000; Braithwaite *et al.*, 2006). There are currently over 150 food composition tables in use around

the world (Heintze *et al.*, 1988; Rand, 1991). An older bibliography of food composition tables was published by the FAO (1975). An inventory of European composition databases was prepared by West (1990). The regularly updated International Food Composition Tables Directory can be seen on the FAO home page (http://www.fao.org/infoods/directory_en.stm).

Tables of food composition include information about the average nutrient content of the most commonly consumed foods for most nutrients. They are less comprehensive with regard to food contaminants and nonnutritive constituents that frequently occur in foods and are suspected or known to have biological activity. This is especially true for iodine. For instance, a review of minerals and trace elements in 7 major reference and 17 user databases revealed that iodine values were not included in food items in 14 databases, while the percentage of food items for which a value for iodine was reported varied from 2% to 100% (Braithwaite *et al.*, 2006). This suggests that more analyses of foods for iodine content are needed. Moreover, a prominent problem with all dietary assessment methods is the high prevalence of under-reporting, estimated to range up to 70% in certain groups (Macdiarmid and Blundell, 1998). Clearly, errors in the measurement of food intake add to errors arising from differences between the composition of the food consumed and the values recorded in the database. The quality of the dietary components intake tables depends on the quality of the database, the accuracy with which foods can be identified, the quality of the food consumption data, and the accuracy with which the food composition database and the programs (or calculations) are prepared (Greenfield and Southgate, 2003). A special problem of iodine intake data is associated with iodized salt, which is nowadays in use in most developed and many developing countries. It follows from a review of the IDD global situation (WHO, 2001) that, of the 130 countries with IDD, 98 (75%) have legislation on salt iodization in place and a further 12 have it in draft form. If food composition tables with values for cooked foods are not available, the iodine intake from iodized salt may not be fully taken into account by dietary assessment methods.

Total diet studies

A total diet study consists of purchasing foods commonly consumed, processing them as for consumption (table-ready), combining the foods into food composites or aggregates, homogenizing them, and analyzing them for analytes of interest. The analytical results are combined with food intake information for different population groups, and the dietary intake of the food components by the groups is estimated. Intake through drinking water and water used in cooking are also included in the total diet study assessment. The total diet study, also known as the Market Basket Survey/Study, is recommended by the

WHO for accurate estimates of dietary intakes of various dietary components.

The total diet studies differ from other surveillance programs because:

- they focus on components in the diet, not individual foods,
- the foods are processed as for consumption in the home; thus, they take into consideration the impact of home cooking on the decomposition of less stable chemicals, and the formation of new ones, and
- assessment of background, rather than regulatory, concentrations of the analytes in the foods is sought.

The accuracy of the total diet study depends on two fundamental data components: (i) the quantity of each prepared food consumed by individuals, usually obtained in a separate study by the dietary assessment method and (ii) the background concentration of analytes of interest in the foods as ready for consumption. In order not to overestimate dietary intakes, the analytical methods used to measure analytes of interest should have appropriately low detection limits.

The total diet study is considered well-suited for assessment of iodine intake, especially because it takes into account all factors, which may influence iodine levels in foods prepared as ready for consumption, including iodized salt.

Duplicate portion method

The most accurate way to assess the nutrient intake of a person is to analyze an exact duplicate of the foods eaten over the survey period. This approach is seldom used because of obvious practical problems, in addition to the cost and the time involved in the analyses. Nevertheless, the duplicate portion method is the method to which all other methods should be compared (Margetts and Nelson, 1997). An example of such a comparison has recently been given by Lightowler and Davies (2002). They compared iodine intakes estimated from weighed dietary records with those obtained by direct analysis of duplicate diets in a group of vegans. A mean daily iodine intake in males was significantly lower when estimated from dietary records (42 µg) compared with that determined from duplicate diets (137 µg). Conversely, in females the mean daily iodine intake from dietary records (1448 µg) was significantly higher than that from duplicate diets (216 µg). Variation in the iodine intake determined by the two different methods may be attributed to the absence of iodine content of some foods, in particular foods suitable for vegan consumption, in food composition tables and the variability in iodine content of seaweed. This example again demonstrates that more reliable information on iodine content of foods, incorporating the variation within

Table 2.2 Methods for assessment of iodine intake from diet

<i>Method</i>	<i>Ease of performance</i>	<i>Adequacy and data quality</i>
Dietary assessment methods	Relatively easy	Frequently not sufficient
Total diet studies	More difficult	Good
Duplicate portion method	Most difficult	Best

foods, is needed, and that the method of duplicate portion is the best choice for obtaining iodine intake data.

Table 2.2 gives an evaluation of the above methods concerning applicability and adequacy of the assessment of iodine intake.

Biomarkers of iodine intake

There are various well-established biomarkers of intake and/or nutritional status of numerous food components (Margetts and Nelson, 1997; Willett, 1998). In the case of iodine, a good measure of iodine intake is urinary excretion, because most (more than 90%) of iodine ingested is excreted in urine. Thus, the urinary iodine concentration, even in casual urine samples, is a good marker of iodine nutrition. Urinary iodine concentration varies with fluid intake, so these values have limited use for casual samples from an individual, but they are well-suited for assessing a population group, because individual variations tend to average out.

Iodine intake and urinary excretion were compared among adults in the Netherlands (Brussaard *et al.*, 1997). Food consumption was measured by 3-day food records, and 24 h urine was sampled twice. On average, iodine intake (mean of 3 days) in men was in the recommended range of 150–300 µg · day⁻¹, but average intake in women was not. A mean 24 h urinary excretion sample confirmed this observation. In a British study, iodine intake and iodine deficiency in vegans were assessed by the duplicate portion method and urinary iodine excretion (Lightowler and Davies, 1998). The iodine intake was estimated using chemical analysis of 4-day weighed duplicate diet collections, while the probability of IDD was judged from the measurement of urinary excretion in 24 h urine specimens during the 4 days. A wide variation of iodine intake was found. The mean iodine intake in men was lower than the RDI, and the mean intake in females was above the RDI. The probability of IDD in the group investigated was moderate to severe. The findings highlighted that vegans are an “at risk” group for iodine deficiency, and also raised the question of adequate iodine intake in groups where cows’ milk is not consumed.

The examples demonstrate the importance of the evaluation of iodine intake by measurement of urinary

excretion. Therefore, analytical methods for iodine determination in urine are also mentioned in this chapter.

Analytical Methods for Iodine Determination in Foodstuffs and Related Materials

Reliable data on iodine content in foods can only be obtained by the careful performance of appropriate, accurate analytical methods carried out by trained analysts. The choice of the appropriate methods performed in the state of statistical control and under other quality control measures is the second crucial prerequisite to ensuring the quality of the results obtained. Obtaining a sufficiently homogeneous and representative sample is the third basic prerequisite for arriving at valid and meaningful analytical results.

The determination of iodine in food has been a difficult analytical problem for many years, and inconsistent results have been obtained in interlaboratory studies (Heckmann, 1979), although a variety of analytical methods capable of iodine determination at various levels in foodstuffs have been developed. The main difficulty is the volatility of iodine when present in the elementary form or in the forms of its volatile compounds. The procedures for iodine determination differ in decomposition methods, analytical principles, detection limits, specificity, accuracy and precision, robustness, and sensitivity to interference. From the practical point of view, they also differ in the ease of performance, equipment needed, and the time and costs involved.

Sample decomposition

Most analytical techniques require sample decomposition, which is a delicate problem in iodine determination, because elementary iodine and some of its compounds, such as HI and CH_3Cl , are highly volatile. This problem can be circumvented in two ways. First is the conversion of all iodine species into elementary iodine (I_2) by distillation or combustion, with subsequent trapping of the analyte for further processing. Secondly, on the contrary, is the conversion of all volatile species to nonvolatile ones, such as iodide or iodate.

Sample decomposition procedures of the first type involve distillation (AOAC, 1984), Schöniger combustion in an oxygen atmosphere in a closed flask (Schöniger, 1955, 1956), combustion in a stream of oxygen flowing through a heated tube (Muramatsu *et al.*, 1988; Gu *et al.*, 1997; Gelinas *et al.*, 1998b; Norman and Iyengar, 1998), or pyrohydrolysis-combustion in a wet oxygen flow (Schentger and Muramatsu, 1996). The second type of decomposition procedure comprises dry ashing (fusion) with alkaline ashing aids, such as with NaOH, NaOH + NaNO_3 , Na_2CO_3 , and oxidative fusion with Na_2O_2 in a Parr bomb (Merz and

Pfab, 1969). Recently, a quick (~ 3 min) alkaline-oxidative fusion in a mixture of Na_2O_2 + NaOH at 850–900°C has been developed, which prevents volatilization losses of halogens and many other elements (Kučera and Krausová, 2007). Wet ashing using oxidizing acids or acid mixtures can also be used, provided that the oxidation potential is high enough to oxidize iodine to the nonvolatile iodate. This can be achieved by applying mixtures of H_2SO_4 -chromate (Spitzky *et al.*, 1958), H_2SO_4 - HNO_3 - HClO_4 (Gochman, 1966), or HClO_3 - HNO_3 (Knapp and Spitzky, 1970). No losses of iodine were found in wet digestion with HNO_3 at temperatures up to 280°C using several pressure- and temperature-controlled closed devices (Knapp *et al.*, 1998). Low temperature ($\sim 90^\circ\text{C}$) extraction with tetramethylammonium hydroxide (TMHA) also yielded no measurable losses of iodine from biological samples of various origins (Fecher *et al.*, 1998; Knapp *et al.*, 1998; Rädlinger and Heumann, 1998).

Catalytic spectrophotometric methods

Since the development of a catalytic spectrophotometry method for iodine assay by Sandell and Kolthoff (1934, 1937) based on the catalytic effect of iodide on the discoloration of yellow Ce(IV) by reduction with As(III) in $\text{H}_2\text{SO}_4/\text{HCl}$ medium, this technique was for a long time the “state of the art” for the determination of iodine in biological materials, including foodstuffs and urine. Among the other possible decomposition methods, alkaline ashing is the most commonly used prior to the final determination of iodine by the Sandell-Kolthoff reaction (Aumont and Tressol, 1986). This method of organic matter destruction is involved in the official procedure of the AOAC (1980). Other digestion procedures were also employed to guarantee that all iodine species are converted to iodide and that substances, such as nitrates, thiocyanate, or ferrous ions, that might interfere by reducing or oxidizing the ceric or arsenite reactants, are removed (Zack *et al.*, 1952; Aumont and Tressol, 1986; IDD Newsletter, 1993; Pino *et al.*, 1996; May *et al.*, 1997; Gültepe *et al.*, 2003; Gamallo-Lorenzo *et al.*, 2005). For iodine determination in urine, chloric acid digestion was reported to be highly effective, among other techniques (Zack *et al.*, 1952). A mixture of chloric acid and sodium chromate or a mixture of perchloric acid and nitric acid under reflux, and potassium carbonate fusion were also employed, and proved to provide comparable results (IDD Newsletter, 1993; Dunn *et al.*, 1993). In order to eliminate the use of irritating strong acids, a digestion method using ammonium persulfate was developed (Pino *et al.*, 1996). To speed up the initial analytical step, microwave-assisted alkaline digestion with TMHA combined with microwave-assisted distillation was employed for the determination of iodide and total iodine in edible seaweed (Gamallo-Lorenzo *et al.*, 2005). Automated modes of the Sandell-Kolthoff reaction

for measurement of urinary iodine using Technicon AutoAnalyzer II (AAII) systems, with either dialysis or acid digestion, were compared with a method employing manual alkaline ashing. The automated modes yielded higher results, due to interfering thiocyanates which participated in the catalytic reaction (May *et al.*, 1990). However, a comparison of five methods, with both manual and improved automatic digestion, as well as with both manual and automated reading of the Sandell–Kolthoff reaction, yielded agreement among the methods tested, and also with an inductively coupled plasma mass spectrometry method (May *et al.*, 1997). Another comparison of an autoanalyzer method (AAII), where the mineralization takes place in a continuous flow manner with a manual mineralization and discoloration in a 96-well microtiter plate read by a PC-controlled photometer, was performed by Wuethrich *et al.* (2000). Both implementations of the Sandell–Kolthoff method applied for iodine assay in urine showed no obvious discrepancy. A detection limit of $0.1 \mu\text{mol} \cdot \text{l}^{-1}$ ($12.7 \mu\text{g} \cdot \text{l}^{-1}$) was achieved for the latter method. Other modifications of the Sandell–Kolthoff reaction include the use of a brucine solution (Matthes *et al.*, 1973; Aumont and Tressol, 1986) or diphenylamine-4-sulfonic acid (Trokhimenko and Zaitsev, 2004) to stop the As–Ce–I reaction after a selected time. It is not intended to list all the modifications and improvements of the procedure originally developed by Sandell and Kolthoff (1934, 1937) here, but to show that this old principle of iodine determination in biological materials, such as foodstuffs and urine, is still viable, largely applied, and continuously undergoing improvements. Two other kinetic-photometric methods for the determination of total iodine in biological materials were also developed. One is a flow injection method based on the catalytic action of iodide on the color-fading reaction of the $\text{Fe}(\text{SCN})_2^{2+}$ complex. It was used by Arda *et al.* (1998) for the determination of iodine in milk. When organic matter was destroyed by alkaline dry ashing or, alternatively, Schöniger combustion, a detection limit of $0.99 \mu\text{g}$ was obtained. The second method is based on the catalytic effect of iodine on the oxidation of chlorpromazine by hydrogen peroxide. Tomiyasu *et al.* (2004) demonstrated, by the determination of iodine in urine and foodstuffs, that a detection limit of 1.6 ng can

be achieved. The main advantage of catalytic spectrophotometric methods is the low cost of the equipment needed. On the other hand, these methods may suffer from interference and are therefore rarely used for the determination of the lower range of iodine levels occurring in foodstuffs and related materials.

With the development of analytical techniques, new methods for iodine determination in biological materials and foodstuffs became available. These newer techniques mostly involve nuclear analytical methods and various types of spectrometric, chromatographic, and electrochemical techniques.

Neutron activation analysis and other nuclear analytical techniques

Neutron activation analysis (NAA) is, nowadays, the second most widely-reported analytical technique for the determination of iodine in foodstuffs and other types of biological materials. The main advantage of this technique is that iodine can be determined nondestructively, using so-called instrumental neutron activation analysis (INAA). By irradiation with thermal and epithermal neutrons (energies of 0.025 eV and $>0.5 \text{ eV}$ – 10 keV , respectively) in a nuclear reactor, the stable iodine ^{127}I is transformed to the ^{128}I radioisotope, which has a half life of 24.99 min . Irradiation of stable ^{127}I with fast neutrons (energy $>0.1 \text{ MeV}$) yields the radioisotope ^{126}I , which has a half life of 13.03 days . The β^- decays of both ^{128}I and ^{126}I are associated with emission of well-measurable γ -rays provided that a semiconductor high-purity germanium (HPGe) detector is used for counting, which is nowadays the most common practice. The nuclear reactions involved, and parameters of the radionuclides formed on neutron activation of stable ^{127}I , are listed in Table 2.3.

There are several approaches to the use of NAA for iodine determination in foodstuffs and other biological materials. Of the two nuclear reactions of stable ^{127}I , the reaction of $^{127}\text{I}(n,\gamma)^{128}\text{I}$ with thermal and epithermal neutrons is almost exclusively used, because favorable nuclear parameters (high activation cross-section and resonance integral for thermal and epithermal neutrons, respectively, cf. Table 2.3) provide much lower detection limits compared

Table 2.3 Nuclear reactions and parameters of iodine radioisotopes used in neutron activation analysis

Radioisotope	Nuclear reaction	Cross-section (10^{-24} cm^2)	Resonance integral (10^{-24} cm^2)	Half life	Main γ -ray energy, keV (absolute intensity)
^{128}I	$^{127}\text{I}(n,\gamma)$ $^{128}\text{I} \rightarrow ^{128}\text{Xe}$	6.2 ± 0.2	147 ± 6	24.99 min	442.9 (16.9), 526.6 (1.6)
^{126}I	$^{127}\text{I}(n,p)$ $^{126}\text{I} \rightarrow ^{126}\text{Xe}$	0.0009 ± 0.0001	–	13.03 days	388.6 (34.2), 666.4 (33.2)

with the reaction of $^{127}\text{I}(\text{n,p})^{126}\text{I}$ with fast neutrons. However, irradiation of biological materials with thermal and epithermal neutrons also results in high background activities from ^{24}Na , ^{42}K , ^{38}Cl , and $^{80,82}\text{Br}$ activation products originating from the elements Na, K, Cl, and Br, which are present in large amounts in this type of sample, including foodstuffs. Thus, the detection limit of INAA is usually not sufficiently low to enable iodine determination in most types of foodstuff samples (cf. Table 2.4). The detection limit of the INAA mode can be moderately improved using cyclic INAA (Elson *et al.*, 1983).

Significant improvement of the detection limit is achieved by taking advantage of a high resonance integral for the reaction of $^{127}\text{I}(\text{n},\gamma)^{128}\text{I}$ (cf. Table 2.3) on irradiation with epithermal neutrons, in so-called epithermal neutron activation analysis (ENAA), nowadays more often called epithermal instrumental neutron activation analysis (EINAA). Activation with epithermal neutrons is achieved by shielding off thermal neutrons from the reactor pile neutron spectrum by irradiation of samples behind filters made of cadmium or boron (Kučera, 1979). In such conditions, iodine is activated more selectively (together with other nuclides with high resonance integrals), because the high background activities from the elements Na, K, and Cl whose radioisotopes are formed according to the “ $1/v$ ” law (v – velocity of neutrons) are suppressed. For this reason, EINAA has been used extensively for iodine determination in foodstuffs since the beginning of the

eighties until now (Fardy and Mcorist, 1984; Stroube and Lutz, 1985; Stroube *et al.*, 1987; Rao *et al.*, 1995; Hou *et al.*, 1997a, b; Nichols *et al.*, 1998; Kucera *et al.*, 2004; Akhter *et al.*, 2004; El-Ghawi and Al-Sadeq, 2006). Further improvement to the detection limit of EINAA was achieved using Compton suppression counting (CSC, sometimes also called anticoincidence counting), which selectively enhances the signal-to-background ratio of the ^{128}I radioisotope by suppressing the detection of the background activities. Two groups of authors recently applied EINAA with CSC for iodine determination in food samples and/or food reference materials, and achieved detection limits in the range of 11–20 ng · g⁻¹ (Serfor-Armah *et al.*, 2003; Yonezawa *et al.*, 2003). Noteworthy, in one laboratory only a relatively low epithermal neutron fluence rate of $2 \times 10^{11} \text{ cm}^{-2} \cdot \text{s}^{-1}$ in the SLOWPOKE reactor was used (Serfor-Armah *et al.*, 2003).

An ultimately low detection limit can be achieved using radiochemical neutron activation analysis (RNAA), which consists of sample destruction and selective separation of the radioisotope ^{128}I . Several RNAA procedures have been developed and applied for iodine determination in foodstuffs. Samples are usually decomposed using Schöniger combustion (Dermelj *et al.*, 1990) or alkaline-oxidative fusion (Kučera and Krausová, 2007) in the presence of an iodine inactive carrier. However, alkaline and acidic dissolution of cereal grains was also used (Shinonaga *et al.*, 2000). Elementary iodine is liberated employing a redox reaction with Na_2SO_3 and NaNO_2 in dilute H_2SO_4 or HNO_3 . The liberated iodine is extracted with chloroform or tetrachlormethane (Dermelj *et al.*, 1990; Kučera *et al.*, 2004). The chemical yield of separation, mostly in the range of 85–95%, is determined spectrophotometrically or with the aid of the ^{131}I radiotracer added prior to sample decomposition. Separation of iodine using bismuth sulfide coprecipitation followed by radiochemical purification with palladium iodide and radiochemical isolation by bismuth sulfide coprecipitation was also developed (Rao and Chatt, 1993). For iodine determination in urine, a simple separation procedure consisting of the use of iodinated exchange-resin proved to yield results comparable with the procedure based on iodine extraction (Dermelj *et al.*, 1992). The RNAA procedures developed were employed for the determination of iodine in daily diet samples in Slovenia (Pokorn *et al.*, 1999), Poland (Kunachowicz *et al.*, 2000), in cereal grains in Austria (Shinonaga *et al.*, 2000), in fish samples in Libya (Arafa *et al.*, 2000), in Asian diet samples (Kučera *et al.*, 2004; Akhter *et al.*, 2004), and in Polish infant formulae (Osterc *et al.*, 2006), just to give a few recent examples. An alternative way to eliminate the radionuclides ^{24}Na , ^{42}K , ^{38}Cl , and $^{80,82}\text{Br}$ in iodine determination by NAA is selective iodine separation prior to irradiation in so-called pre-separation NAA (PS-NAA). Rao and Chatt (1991) developed a PS-NAA procedure based

Table 2.4 Comparison of iodine detection limits, m_D , using various modes of NAA in a nuclear reactor^a

NAA mode	Nuclear reaction	m_D^b (ng · g ⁻¹)	Experimental conditions ^c
INAA	$^{127}\text{I}(\text{n},\gamma)$ $^{128}\text{I} \rightarrow ^{128}\text{Xe}$	200–300	$t_i = 1 \text{ min}$, $t_d = 10 \text{ min}$, $t_c = 20 \text{ min}$
ENAA	$^{127}\text{I}(\text{n},\gamma)$ $^{128}\text{I} \rightarrow ^{128}\text{Xe}$	10–40	$t_i = 0.5 \text{ min}$, $t_d = 10 \text{ min}$, $t_c = 20 \text{ min}$
RNAA	$^{127}\text{I}(\text{n},\gamma)$ $^{128}\text{I} \rightarrow ^{128}\text{Xe}$	0.5	$t_i = 2 \text{ min}$, $t_d = 15\text{--}20 \text{ min}$, $t_c = 20 \text{ min}$
RNAA	$^{127}\text{I}(\text{n,p})$ $^{126}\text{I} \rightarrow ^{126}\text{Xe}$	400 ^d	$t_i = 20 \text{ h}$, $t_d = 10 \text{ d}$, $t_c = 5 \text{ h}$

^aThermal, epithermal, and fast neutron fluence rates of $5 \times 10^{13} \text{ cm}^{-2} \cdot \text{s}^{-1}$; $1.5 \times 10^{13} \text{ cm}^{-2} \cdot \text{s}^{-1}$; and $3 \times 10^{13} \text{ cm}^{-2} \cdot \text{s}^{-1}$; respectively.

^b3 σ criterion.

^c t_i = irradiation time; t_d = decay time; t_c = counting time; counting with a coaxial HPGe detector having relative efficiency 23%; FWHM resolution 1.8 keV; and the peak-to-Compton ratio 51 for the 1332.4 keV photons of ^{60}Co .

^dCounting with a well-type HPGe detector; active volume 150 cm³; FWHM resolution 2.3 keV for the 1332.4 keV photons of ^{60}Co .

on microwave acid digestion in closed Teflon bombs and iodine separation by coprecipitation of iodide with bis-muth sulfide, while Norman and Iyengar (1998) used sample combustion followed by trapping the liberated iodine on charcoal. Both procedures were successfully used for iodine determination in various diet samples and biological and environmental reference materials. Another PS-NAA method capable of determination of iodide and iodate in seawater, urine, and milk was developed by Hou *et al.* (2000), whereas Bhagat *et al.* (2007) elaborated a PS-NAA procedure for the iodine speciation in milk. Advantages of PS-NAA procedures are that the radiation burden of personnel is minimized compared with RNAA, and iodine speciation can be performed. Although iodine is not so ubiquitous an element in the environment, appropriate measures are taken to prevent contamination of samples during their processing prior to irradiation.

The low cross-section of the reaction of $^{127}\text{I}(\text{n,p})^{126}\text{I}$ with fast neutrons (cf. Table 2.3) and a low abundance of neutrons with energies higher than $\sim 9\text{ MeV}$, which are needed for this reaction, in the neutron spectrum of a nuclear reactor result in a detection limit which is not sufficient for iodine determination in most types of foodstuffs, even if an RNAA procedure is applied. However, this reaction, which is completely independent in relation to the reaction of $^{127}\text{I}(\text{n},\gamma)^{128}\text{I}$ with thermal and epithermal neutrons may be useful for cross-checking results in analysis of foodstuff samples with higher iodine contents, using the so-called self-verification principle in NAA (Byrne and Kučera, 1997). Detection limits of various NAA modes, which were achieved in the author's laboratory are compared in Table 2.4.

Concerning other nuclear analytical techniques, isotope dilution analysis proved to be capable of iodine determination in milk with a detection limit of $5\ \mu\text{g} \cdot \text{l}^{-1}$ (Ünak *et al.*, 2004), while a detection limit of $0.1\ \mu\text{g} \cdot \text{g}^{-1}$ (dry mass) was obtained by radioisotope X-ray fluorescence on analysis of spiked milk samples (Crecelius, 1975). A similar detection limit of $0.5\ \mu\text{g} \cdot \text{g}^{-1}$ was reported for freeze-dried milk using energy-dispersive X-ray fluorescence analysis, the procedure being applicable also for iodine determination in potable water and egg yolks (Holynska *et al.*, 1993). Total reflection X-ray fluorescence (TXRF) analysis was found to be suitable for iodine determination in iodine-enriched mineral water containing $94\text{--}100\ \text{mg} \cdot \text{l}^{-1}$ of iodine, in seaweed samples with iodine levels of $450\text{--}4520\ \text{mg} \cdot \text{kg}^{-1}$, and in dietary supplement tablets containing $26\text{--}205\ \text{mg} \cdot \text{kg}^{-1}$ of iodine (Varga, 2007).

Of the nuclear analytical techniques, NAA is the most important for iodine determination in foodstuffs. The main advantage of NAA is the ultimately low detection limit, which can be achieved using RNAA or PS-NAA, nondestructive performance of INAA and EINAA, freedom from interference and matrix effects of all modes of NAA for the determination of total iodine content,

and a low uncertainty of results achievable, especially by RNAA. For these reasons, and because NAA has recently been recognized as primary method of analysis, NAA is frequently used for the certification of iodine content in food-related reference materials, and for checking accuracy of other methods. Disadvantages of NAA are that access to a nuclear reactor – the most intensive source of neutrons – is needed, and this type of analysis is associated with handling radioactive samples, which requires specially equipped laboratories and adherence to the radiation safety regulations.

Spectrometric techniques

Mass Spectrometry Various spectrometric techniques can be used for iodine determination in biological materials, including foodstuffs. Until now, mass spectrometry has been applied most extensively for this purpose. One of the first reports concerned the use of isotope dilution laser resonance ionization mass spectrometry to determine iodine in oyster tissue using the long-life ^{129}I radioisotope to spike the samples. A detection limit of $100\ \text{ng}$ of iodine was achieved using the procedure developed (Fasset and Murphy, 1990). Since the introduction of inductively coupled plasma-mass spectrometry (ICP-MS) this method became a powerful tool for iodine determination in various foodstuffs and related materials because of the favorable detection limits and method selectivity. Applications of ICP-MS were reported for iodine determination in fresh milk and milk powders (Baumann, 1990; Vanhoe *et al.*, 1993; Sturup and Buchert, 1996), urine (Allain *et al.*, 1990), milk, plants and tissues (Schramel and Hasse, 1994), food-related certified reference materials and/or candidate reference materials (Kerl *et al.*, 1996; Larsen and Ludwigsen, 1997; Knapp *et al.*, 1998; Gelinas *et al.*, 1998a; Haldimann *et al.*, 2000; Andrey *et al.*, 2001; Resano *et al.*, 2005; Santamaria-Fernandez *et al.*, 2006), seafood (Julshamn *et al.*, 2001), and total diet samples (Haldimann *et al.*, 2000). In these procedures, various sample decomposition and/or treatment methods were employed. For iodine determination in urine the sample preparation involved only 10-fold dilution with a diluent containing europium as an internal standard, followed by direct nebulization in the plasma (Allain *et al.*, 1990). For the determination of iodine in milk and milk powder by flow injection ICP-MS, a simple sample preparation was used based on the dilution of the sample by an alkaline solution containing KOH and TMAH (Sturup and Buchert, 1996). However, incomplete extraction of iodine with TMAH was observed for certain sample types (Fecher *et al.*, 1998; Gelinas *et al.*, 1998a). Low recoveries were explained by the presence of insoluble components, e.g., covalent bond forms of iodine (Haldimann *et al.*, 2000). In such cases total sample mineralization is required, such as combustion in an oxygen

stream (Gelinas, 1998a), Schöniger combustion (Knapp *et al.*, 1998), high pressure, usually microwave-assisted, wet ashing in closed vessels using HNO_3 (Knapp *et al.*, 1998) or $\text{HNO}_3 + \text{HClO}_4$ (Larsen and Ludwigsen, 1997; Knapp *et al.*, 1998). However, if iodine was present as iodide and nitric acid was used in the wet ashing system, the observed signal in ICP-MS was not stable (Julshamn *et al.*, 2001; Vanhoe *et al.*, 1993). For the correction of nonspectroscopic matrix effects ICP-IDMS was developed using a spike with the ^{129}I radioisotope (Rädlinger and Heumann, 1998; Haldimann *et al.*, 2000; Santamaria-Fernandez *et al.*, 2006). To avoid problems with sample decomposition, a procedure of solid sampling – electrothermal vaporization ICP-MS – was developed and applied to iodine determination in nutritional, as well as soil, reference materials (Resano *et al.*, 2005). For the determination of iodine species, mostly iodide and iodate, in aqueous solutions ion chromatographic systems were coupled with ICP-MS (Heumann *et al.*, 1994; Stärk *et al.*, 1997). Accurate results can be obtained, especially if ion chromatography is coupled with inductively coupled plasma-isotope dilution mass spectrometry (ICP-IDMS) (Heumann *et al.*, 1994). The reported range of detection limits for total iodine is down to a few $\text{ng} \cdot \text{ml}^{-1}$ for milk (Baumann, 1990) and $6\text{--}10 \text{ ng} \cdot \text{g}^{-1}$ for other nutritional samples (Gelinas *et al.*, 1998b; Rädlinger and Heumann, 1998; Resano *et al.*, 2005; Schentger and Muramatsu, 1996). It can be concluded that ICP-MS proved to be very useful to determine iodine in nutritional samples; however, the full exploitation of this technique requires careful optimization of the analytical parameters.

Optical Emission Spectrometry Inductively coupled plasma-optical emission spectrometry (ICP-OES) has not been used for iodine determination in foodstuffs very often. The reason is that the most intensive emission line of iodine of 178.218 nm is interfered by the phosphorus spectral line of 178.222 nm. In green algae *Chlorella* enriched with iodine, the element determination with ICP-OES after sample solubilization with TMAH using an interference-free iodine line of 182.980 nm appeared feasible (Niedobová *et al.*, 2005a). The detection limit obtained was $3 \mu\text{g} \cdot \text{g}^{-1}$. The phosphorus interference was eliminated in procedures in which iodine was separated by precipitation of AgI (Naozuka *et al.*, 2003) or liberation of elementary iodine using vapor generation ICP-OES (Niedobová *et al.*, 2005b). Both procedures were applied for iodine determination in milk samples, and the respective detection limits of $40 \mu\text{g} \cdot \text{g}^{-1}$ (dry mass) or $20 \mu\text{g} \cdot \text{l}^{-1}$ were achieved.

Atomic Absorption Spectrometry This technique also offers the possibility to determine iodine in aqueous solutions, seaweed, and foodstuffs samples, but its utilization has been limited so far. The reasons are that atomic absorption spectrometry (AAS) equipment is not intended

to make measurements in the vacuum UV range, therefore they require modifications, and due to the lack of a commercially available iodine lamp the radiation source has to be produced individually (Bermero-Barrera *et al.*, 1999).

Among spectroscopic techniques, ICP-MS has a prominent position in iodine determination in foodstuffs, due to a low detection limit, high selectivity, and the ease of coupling with chromatographic separation procedures. The highest accuracy is obtained in ICP-IDMS, which has been recognized as the primary analytical method. Since iodine is a monoisotopic element, the only possibility to perform isotope dilution is to use a spike with the ^{129}I radionuclide. Thus, similar regulations are required in ICP-IDMS for work with radioactivity as for NAA procedures. Another disadvantage of ICP-MS is that it requires the most expensive equipment of all techniques developed for iodine determination (if we do not consider the cost of a nuclear reactor, which usually serves many other purposes than irradiation for NAA).

Electrochemical techniques

Total iodine in fresh milk was measured using an iodide-selective electrode after addition of KCl to increase the electrical conductivity by Crecelius (1975). The iodide electrode method is simple, fast, and inexpensive, providing a detection limit of $50 \mu\text{g} \cdot \text{l}^{-1}$, but it may give erroneous results due to insufficient specificity if, for instance, formaldehyde is used for milk storage. A cathodic stripping voltammetry (CSV) method was developed for iodine determination in table salt and egg samples by Yang *et al.* (1991). The method involves sample treatment by Schöniger combustion and the determination of iodide by CSV of the solid phase formed with the quarternary ammonium salt Zephiramine. For the determination of iodide in table salt, another CSV method was also reported. Iodide was preconcentrated on a carbon-paste electrode via an ion-pairing reaction followed by oxidation to iodine (He *et al.*, 2003). Electrochemical detection of iodine was also used in conjunction with chromatographic methods, which are reported below.

Electrochemical techniques found many fewer applications for the determination of total iodine in foodstuffs compared with other techniques. They are inexpensive, but their limited specificity requires careful validation of the procedures developed. On the other hand, electrochemical detection of iodine species separated by chromatographic methods seems to be well-established.

Chromatographic methods

Urinary iodide was measured using paired-ion reversed-phase high-performance liquid chromatography (HPLC) with electrochemical detection employing a silver working electrode (Rendl *et al.*, 1994). A detection limit of $5 \mu\text{g} \cdot \text{l}^{-1}$ was achieved.

A head-space flow injection method for online determination of iodide in urine, with chemiluminescence detection with a detection limit of $10 \mu\text{g} \cdot \text{l}^{-1}$, was also developed (Burguera *et al.*, 1996). Reversed-phase ion-pair liquid chromatography (LC) with electrochemical detection was successfully used in a collaborative study on the determination of iodine in liquid milk and dry milk powder (Serti and Malone, 1993). Speciation analysis of iodine in milk was performed using size-exclusion chromatography with ICP-MS detection (Sanchez and Szpunar, 1999). For the determination of iodine in nutritional reference materials gas chromatographic (GC) measurement of the 2-iodopentan-3-one derivative with a ^{63}Ni electron-capture detector was used following either combustion of samples in a stream of oxygen or oxidation in a basic solution of peroxodisulfate (Gu *et al.*, 1997). The combination of a miniaturized alkaline ashing step and column-switching HPLC has been shown to be a powerful approach for the determination of total iodine in urine and other types of biological materials, with a limit of quantification of $70 \text{ ng} \cdot \text{g}^{-1}$ for solid biological materials (Andersson and Forsman, 1997). Recently, a method based on anion-exchange chromatographic separation coupled with amperometric detection at a modified platinum electrode was developed by Cataldi *et al.* (2005). The method was successfully applied to determine iodide content in milk, common vegetables, and waste waters.

Chromatographic methods are especially useful for iodine speciation when coupled with ICP-MS, electrochemical detection, or chemiluminescence detection. The total iodine can be determined following various ashing procedures with a moderately low detection limit.

Other methods

A fluorescence-based method was successfully tested for iodine determination in iodine-supplemented food, such as table salt and milk powder. It is based on the fluorescent quenching capability of iodine/triiodide using a highly fluorescent compound consisting of a synthetic metal ion receptor coupled with a signaling element. The detection limit for triiodide down to a concentration of 10^{-8} mol was reported (Zhao *et al.*, 2003).

A quartz crystal microbalance (QCM) method for the determination of iodine in foodstuffs was proposed by Yao *et al.* (1999). The method is based on sensitive response to mass change at electrodes of piezoelectric quartz crystal. After sample decomposition, iodide in the sample solution is transformed to the elementary iodine in an acidic environment. The free iodine is then adsorbed at gold electrodes of QCM, and the iodine content is estimated through a decrease in QCM frequency. A detection limit of $0.5 \mu\text{g} \cdot \text{l}^{-1}$ in aqueous solutions was reported. This method is also applicable for the determination of iodine in urine and other biological samples.

Choice of an Analytical Method Versus the Iodine Level in Foodstuffs and Diets

Various analytical techniques for the determination of different levels of total iodine or iodine species in foodstuffs and related materials are presently available. They differ in principles, equipment needed, detection limits, reliability, i.e., accuracy and precision of results, the ease of performance, sample throughput, and analysis cost. The choice of the most appropriate method largely depends on the purpose of the analysis, e.g., whether it concerns routine monitoring and/or screening or whether delicate certification of a foodstuff reference material is to be carried out. Obviously, one of the decisive parameters is whether the method's detection limit is sufficiently low for the given purpose. For this reason, it appears useful to give the typical iodine levels in various foods to facilitate the choice of the appropriate method(s). Table 2.5 lists the average iodine content of foods (fresh and dry basis), which was adapted from the data reported by Koutras *et al.* (1985).

The iodine content of food varies with geographical location, because there is a large variation in the iodine content of various environmental areas. In plants, the iodine levels depend on the iodine content of the soil in which they are grown. This factor influences the iodine content of the local food chain, and consequently locally produced foodstuffs, as demonstrated in Table 2.6, which compares the iodine levels of several foods in ready-to-eat form from two regions of Greece. One is the area of Thessalia, with endemic goiter shown to be due to iodine deficiency, while the other is the region of Athens, which is goiter-free, but where food additives are not used regularly (Koutras *et al.*, 1970).

Table 2.5 Average iodine content of foods ($\mu\text{g} \cdot \text{kg}^{-1}$) and urine ($\mu\text{g} \cdot \text{l}^{-1}$)

Food	Fresh basis		Dry basis	
	Mean	Range	Mean	Range
Fresh water fish	30	17–40	116	68–194
Marine fish	832	163–3180	3715	471–4591
Shellfish	798	308–1300	3866	1292–4987
Meat	50	27–97	179	96–346
Milk	47	35–56	450 ^a	335–540 ^a
Eggs	93		395 ^a	
Cereal grains	47	22–72	65	34–92
Fruits	18	10–29	154	62–277
Legumes	30	23–36	234	223–245
Vegetables	29	12–201	385	204–1636
Urine ^b	130	20–300		

^aRecalculated from the data given by Koutras *et al.* (1985) using a dry/fresh mass ratio determined in the author's laboratory as 10.4 and 23.4% for medium-fat milk and eggs, respectively.

^bEstimated from the data given in WHO (2001).

Table 2.6 Iodine content of water, milk, and food items in areas with and without endemic iodine-deficiency goiter (Koutras *et al.*, 1970)

Food item	Athens			Endemic area		
	n	Mean	Range	n	Mean	Range
Drinking water, $\mu\text{g} \cdot 100\text{ml}^{-1}$	12	0.47	0.35–0.77	163		
Cows' milk, $\mu\text{g} \cdot 100\text{ml}^{-1}$	12	4.15	7.50–12.60			
Sheep's milk, $\mu\text{g} \cdot 100\text{ml}^{-1}$	–	–	–	59	9.4	1.5–35.3
Goats' milk, $\mu\text{g} \cdot 100\text{ml}^{-1}$	–	–	–	56	2.2	ND–15.7
Egg, $\mu\text{g}/\text{egg}$	15	13.4	1.8–48.8	19	1.9	0.5–6.0
Chicken dishes, $\mu\text{g}/\text{portion};$ average mass 240 g	16	125.5	2.7–597.0	16	23.8	ND–151.0
Meat dishes, $\mu\text{g}/\text{portion};$ average mass 250 g	10	6.5	ND–18.0	16	3.0	ND–12.3
Fish dishes, $\mu\text{g}/\text{portion};$ average mass 222 g	9	63.9	2.4–158.0	–	–	–
Legume dishes, $\mu\text{g}/\text{portion};$ average mass 300 g	14	3.0	ND–7.6	16	2.0	ND–14.3
Greek soft cheese, $\mu\text{g} \cdot 100\text{g}^{-1}$	15	15.1	6.7–33.0	15	8.5	3.6–17.5
Bread, $\mu\text{g} \cdot 100\text{g}^{-1}$	12	1.56	ND–14.5	21	0.54	ND–3.7

Note: ND, not detectable.

The iodine content present in the upper crust of the earth is leached by glaciation and repeated flooding, and is carried to the sea. Therefore, seafood is the richest source of iodine. A great variation in iodine levels occurs in seafood due to an inherent biological capacity of the individual species to accumulate iodine from the sea (Koutras *et al.*, 1985). Thus, due to biological and geographical variability the data shown in Table 2.5 cannot be used universally, and cannot replace more detailed food composition databases. An excerpt from a recent Norwegian food composition table is given for illustration in Table 2.7.

Table 2.8 presents another example of marked differences of iodine levels in daily diet samples in different countries, using the results achieved within a WHO and IAEA coordinated research project on dietary intake of several important minor and trace elements in diets consumed in a number of developed and developing countries (Dermelj *et al.*, 1990).

To review the present status of usage of various analytical techniques for the iodine determination in foodstuffs,

Table 2.7 Iodine content of Norwegian foods ($\mu\text{g} \cdot \text{kg}^{-1}$, fresh basis) according to The Norwegian Food Composition Table (2006)

Milk and milk products	
Milk and milk-based beverages	
Milk	170–220
Yoghurt	160–240
Cream, sour cream	120
Cheese	
Cheese, full fat	190–1400
Cheese, whey, goat's milk	3050
Cheese, reduced fat	180–660
Cheese, low fat	200–1410
Cheese, whey, cow's and goat's milk, fat reduced	2000
Egg	390–490
Egg white	30
Egg yolk	1200
Poultry and meat, raw	
Chicken breast and thigh, meat and skin	0 ^a
Lamb, beef, pork	200
Liver of lamb, beef, pork	20–40
Dishes with poultry or meat	
Hamburger, double, with bread, cheese, lettuce, dressing, etc., fast food restaurant	20
Hamburger, extra large, with bread, cheese, etc., fast food restaurant	40
Chicken burger, breaded, fried, with bread, lettuce, dressing, etc., fast food restaurant	10
Lasagne, with minced meat, frozen, industry made	50
Pasta dish, with turkey and cheese sauce, frozen, industry made	160
Fish and shellfish	
Fatty fish, raw	330–500
Lean fish, raw	500
Shellfish, fish offal	100
Fish products, sandwich fish	100–2340
Cereals	
Flour	0 ^a –10
Crisp bread, crackers, etc.	10
Cookies, sweet biscuits, rusks	10–220
Potatoes, vegetables, fruits, and berries	
Potatoes, storage, raw	0 ^a
Vegetables, raw and frozen	0 ^a –50
Fruits and berries, raw/flesh	0 ^a
Margarine, butter, oil, etc.	
	60
Other dishes, products, and ingredients	
Pizza, with meat balls and ham, frozen, industry made	50
Quiche, with ham and cheese, frozen, industry made	180

^aDetermined with insufficient detection limit, which is not reported.

it may be stated that the catalytic spectrophotometric methods, namely those based on the Sandell–Kolthoff reaction, ICP-MS, and NAA methods are most widely used. ICP-IDMS and NAA procedures, being the primary analytical methods, have the highest metrological value

Table 2.8 Values for iodine in IAEA daily diet samples from different countries ($\text{mg} \cdot \text{kg}^{-1}$, dry mass) according to Dermelj *et al.* (1990)

Country	Number of diet samples	Range	Median
Brazil	9	0.282–0.633	0.49
China	11	0.045–6.33	0.16
Italy	19	0.062–0.456	0.18
Japan	5	0.088–7.65	0.55
Spain	20	0.150–1.96	0.46
Sudan	5	0.084–0.540	0.16
Thailand	8	0.057–0.187	0.11
Turkey	6	0.065–0.337	0.16

and are therefore most frequently employed for certification of iodine levels in food-related reference materials and for checking accuracy of other methods. Hyphenated methods are usually needed for iodine speciation.

Conclusions

- Total diet studies and the duplicate portion method provide the most adequate data for the assessment of iodine intake from diet.
- A low-cost assay of iodine in foods and urine can be performed using catalytic spectrophotometric methods, namely those based on the Sandell–Kolthoff reaction. These methods, however, are not optimal for determination of the lower range of iodine levels occurring in foodstuffs, because of possible interference.
- NAA provides a possibility of nondestructive performance of reliable determination of total iodine in foodstuffs and diets at very low levels. The lowest detection limit of all analytical techniques can be achieved if RNAA or PS-NAA is used. PS-NAA is also suitable for iodine speciation analysis.
- Of the spectrometric techniques, ICP-MS is well-established and very frequently used for reliable determination of total iodine at very low levels, especially if ICP-IDMS is employed. Coupling of ICP-MS with chromatographic separation procedures is especially useful for the determination of iodine speciation in foodstuffs.
- Electrochemical techniques are inexpensive, but their limited specificity requires elimination of possible interference in the determination of even moderately low iodine levels in foodstuffs. They are well-suited for the detection of iodine species separated by chromatographic techniques.
- Chromatographic techniques are a well-established tool for iodine speciation when coupled with other detection methods.
- Results with the highest metrological value and the lowest uncertainty can be obtained using NAA and ICP-IDMS

procedures. They are therefore best suited for certification of iodine content in food-related reference materials, and for checking the accuracy of less expensive methods of iodine determination.

- The choice of analytical method(s) should take account of a wide range of iodine levels in foodstuffs and diets, the best criterion being fitness for purpose. One method may not be optimal and/or capable for iodine determination at all iodine levels occurring in foodstuffs and diets.

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3

Determination of Iodine *In Vivo* and *In Vitro* by X-Ray Fluorescence Analysis: Methodology and Applications

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Abstract

The thyroid gland has sophisticated mechanisms for regulating the iodine uptake, storage, and excretion that are necessary for maintaining a normal body function. Iodine uptake and excretion can be assessed by ^{131}I measurements or urinary iodine, whereas information on the amount of iodine stored in the thyroid, in the iodine pool, is not as commonly obtained. Information regarding the iodine pool and its relationship to the clinical state of patients can, however, increase our knowledge of the thyroid and help us understand the background of several thyroid diseases. The iodine pool can be estimated by noninvasive X-ray fluorescence (XRF) analysis. Depending on the choice of equipment, the iodine estimation can be made quantitatively or by imaging the iodine distribution. The technique involves a radioactive source or an X-ray tube for irradiation of the stable iodine in the thyroid, together with a detector that registers the subsequent emission of characteristic X-ray photons. So far, the method has been useful in confirming mechanisms that previously were known only from indirect evidence. Because of the very low radiation dose, the method is applicable even for children and pregnant women, and the technique can thus be used for monitoring iodination programs. Important information can also be gained regarding changes in the iodine pool over time in patients exposed to excess iodine after, for example, amiodarone treatment or X-ray contrast containing iodine. The other groups of special interest are patients with thyroiditis, hyperthyroidism, and thyroid cancer. In this review, the XRF technique, with emphasis on the physical background and clinical applications, is described. Necessary equipment, together with the methodological limitations and benefits, is discussed and a brief guide before starting XRF measurements is also provided.

Abbreviations

Bq	Becquerel
HPGe	High-purity germanium
MDC	Minimum detectable concentration
MC	Monte Carlo
NIS	Sodium/iodide symporter
Si(Li)	Silicon lithium
Sv	Sievert
T_4	Thyroxine
T_3	Triiodothyronine
Tg	Thyroglobulin
XRF	X-ray fluorescence

Introduction

The thyroid gland is dependent on iodine for production of the iodine-containing hormones thyroxine (T_4) and triiodothyronine (T_3). Since iodine is a trace element found only in low concentrations in the environment, the thyroid has, during evolution, developed a complex mechanism to enrich and store iodine within the thyroid follicles. Iodine is taken up from the circulation into the thyrocytes and incorporated into thyroglobulin (Tg) by means of the sodium/iodide symporter (NIS). Tg functions both as a substrate in T_4 and T_3 hormone production, and as a storage molecule for excessive iodine in the follicle. Most of the thyroid iodine is presumed to be organic, but non-organic iodine may also be present in smaller amounts.

The availability of iodine varies geographically, and therefore, the size of the total iodine pool, as well as the concentration of iodine in the thyroid gland, can vary significantly depending on the access to iodine and on the thyroid size. Factors such as sex, age, and diet (that may

contain substances impairing iodine uptake, goitrogens) also affect iodine concentration.

Determination of the thyroid iodine content by X-ray fluorescence (XRF) investigation offers a unique possibility to study the intrathyroidal iodine pool. Apart from clinical applications in subjects with thyroid disease, it is of utmost interest to apply the method in situations of iodine deficiency or iodine overload. This review gives special attention to the XRF technique, and also describes the application of XRF *in vivo* and *in vitro*.

X-Ray Fluorescence Analysis

XRF is not a new method; since the first measurements of stable iodine in the thyroid by Hoffer *et al.* (1968), the use of XRF has spread to include several other elements in medical applications, as well as applications in occupational and environmental surveillance. Today, XRF is primarily used as a nondestructive method for investigation of metals, minerals, environmental samples, food constituents, and body fluids. Examples of *in vivo* XRF elemental analysis are measurements of lead in bone (Ahlgren and Mattsson, 1979; Somervaille *et al.*, 1985; Todd and Chettle, 1994) and studies on cadmium, mercury, gold, and platinum (Ahlgren and Mattsson, 1981; Börjesson *et al.*, 1993, 1995), but the method is not, to our knowledge, used clinically as a tool in the routine assessment of thyroid function. Some *in vivo* applications of the method are listed in Table 3.1.

Basic principle

XRF is based on the principle that stable atoms can emit characteristic X-rays when exposed to ionizing radiation, such as gamma- or X-ray photons from a radioactive source or an X-ray tube. When a photon impinges on a sample, it can either be absorbed in the sample or scattered through the material. In the event that a photon is absorbed, the photon energy is transferred to an atomic electron, which is subsequently ejected from its shell. This process of photoelectric absorption will leave the atom in an ionized state, with a vacancy in the shell originally containing the ejected electron (Einstein, 1905). In this state the atom is unstable, and to restore the stability, the vacancy is filled by an electron from an outer shell. The released energy, which equals the difference in binding energies for the two shells, may appear as a characteristic X-ray photon emitted from the atom (Figure 3.1). Since each element has a unique set of electron binding energies, it emits X-rays with energies specific for that element.

The difference in electron binding energies between the elements can be understood by studying the forces that hold the atoms together. Each element in the periodic table has a specific number of protons in its atomic nucleus and hence, a definite charge. Due to the electrostatic attraction between the atomic nucleus and the electrons, the binding

Table 3.1 Examples of elements measured with *in vivo* XRF

Element	Z	Organ	References
Natural iodine	53	Thyroid	Hoffer <i>et al.</i> , (1968)
Lead	82	Bone	Ahlgren and Mattsson (1979)
Iodine	53	Human tissue from contrast media for urography	Grönberg <i>et al.</i> , (1983)
Iron	26	Eye, skin	
Copper	29	Eye, skin	
Zinc	30	Eye, skin	
Strontium	38	Bone	
Cadmium	48	Kidneys, liver	
Iodine	53	Thyroid, blood	
Xenon	54	Brain	
Barium	56	Lungs	
Platinum	78	Kidneys, liver, tumors	
Gold	79	Kidneys, liver, bone joints	
Mercury	80	Kidneys	
Lead	82	Bone	
Thorium	90	Liver, spleen	
Uranium	92	Bone, lung	

Notes: *In vivo* XRF has been utilized for analysis of a number of elements in different applications. Some of these elements are shown in the table, along with the organs and tissues in which the elements have been analyzed. Data from Börjesson *et al.*, (2003), unless otherwise stated (Ahlgren and Mattsson, 1979; Grönberg *et al.*, 1983; Hoffer *et al.*, 1968). Note that most elements have rather high atomic numbers, Z.

energy of the electrons will depend on the charge of the atomic nucleus. The electron shell structure appears because nature has put restrictions on the spatial distribution of the electrons around the nucleus. For a given element the electrons in the innermost (K) shell will be more tightly bound than those in other shells, such as the L-shell, due to increasing distance from the nucleus. In order to specify the atomic transitions and hence, their energies, the characteristic X-rays (and their peaks in the spectrum) are labeled as K_{α} – the capital letters K, L, and so on mean that the electron was emitted from the K- or L-shell, and the Greek letters α , β , and γ designate the shell that the vacancy-filling electron originated from (in this example, K_{α} , the L-shell) (Figure 3.2). A typical X-ray spectrum from an irradiated sample contains a number of peaks. The energy of the peaks identifies the elements that the sample is composed of, and the peak intensity is thus proportional to the abundance of each element in the sample. For iodine, the K_{α} peak in a spectrum is found at the energy 28.6 keV (Figure 3.3).

XRF for analysis of iodine in the thyroid

Iodine (having atomic number 53) is present in different forms in the thyroid gland. Photoelectric absorption,

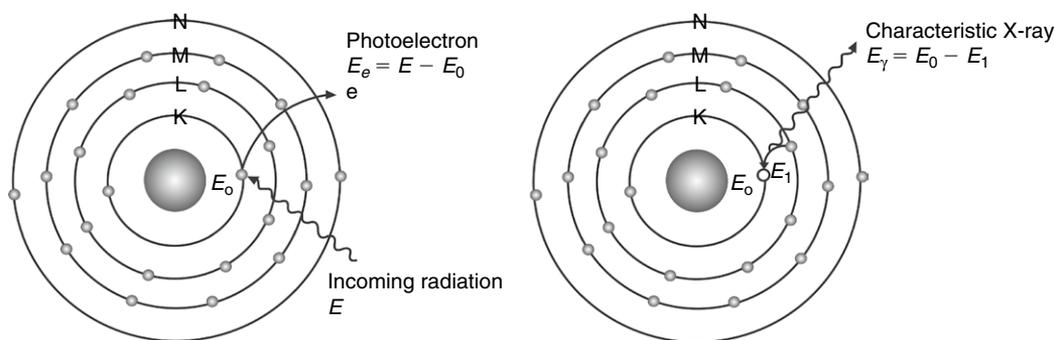


Figure 3.1 A schematic description of photoelectric absorption and the following emission of a characteristic X-ray photon. If the energy, E , of the incoming photon is higher than the binding energy, E_0 , of an atomic electron, that electron can be ejected with kinetic energy $E_e = E - E_0$. The emitted characteristic X-ray photon will have an energy, E_γ , equal to the difference in binding energy of the shell that the electron originates from and the shell that contains the vacancy, respectively ($E_\gamma = E_1 - E_0$).

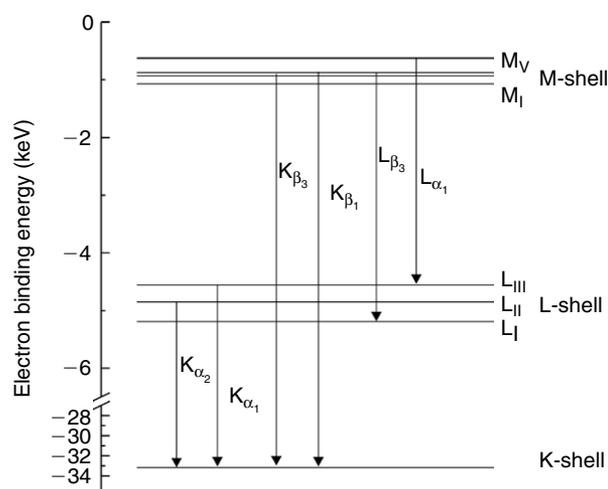


Figure 3.2 Electron binding energies for iodine. The figure also shows the Siegbahn notation for electronic transitions resulting in the emission of characteristic X-rays. The splitting of the L- and M-shells in different energy levels (so-called subshells) cannot be explained by increasing distance from the nucleus. The effect is instead due to the interaction between the individual electrons in the shells, and is appropriately described by the theory of quantum mechanics. In the measurements the detector system is seldom capable of resolving the energy difference between K_{α_1} and K_{α_2} , which are therefore grouped as K_α in **Figure 3.3**.

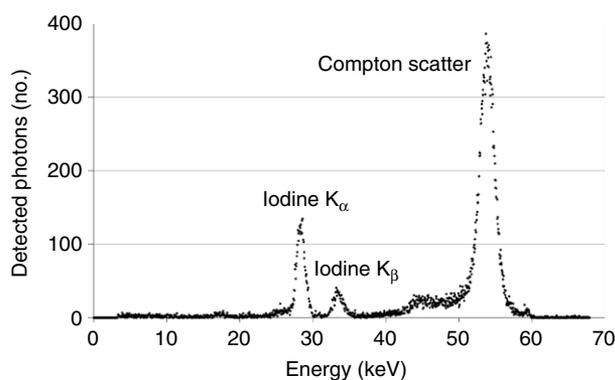


Figure 3.3 A typical spectrum obtained from a measurement of an iodine solution with ^{241}Am as irradiation source. The characteristic iodine peaks are considerably smaller than the peak of Compton-scattered source photons. In the Compton scattering process, which involves the outer electrons, the incoming photon transfers a part of its energy to an atomic electron, which is then knocked out from the atom. The direction of the continuing photon is changed in the process, and the amount of energy loss is determined by the scattering angle.

though, only involves the innermost electrons and gives a characteristic X-ray signal that is unaffected by the chemical or oxidation state of iodine. This property means that all iodine can be measured, but the result does not give any information about the amount that is, for example, Tg-bound. To measure the iodine, the criteria for photoelectric absorption, that is the incoming photon radiation must have energy higher than the binding energy of an electron in one of the innermost atomic shells, must be fulfilled. For XRF analysis of iodine the limit is 33.2 keV (which is the binding energy of K-shell electrons). In contrast, if the

incoming photons have too high energy, the detected signal will become weaker due to the decrease in interaction probability above the binding energy (the K-absorption edge), with increasing energy of the incoming radiation (**Figure 3.4**). Therefore, a suitable excitation source for iodine emits photons with energy closely above 33.2 keV.

If considering the cross-section together with the considerable amount of scattering, the optimum photon energy would be approximately 45 keV (Grönberg and Mattsson, 1981). The other qualities associated with a good radioactive source in this application are long half-life and high specific

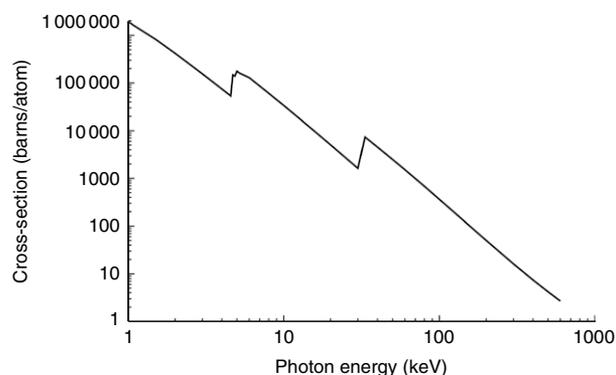


Figure 3.4 Interaction probability (cross-section) for photoelectric absorption in iodine (1 barn equals 10^{-28}m^2). Starting from the high-energy end of the figure the cross-section increases steadily until the binding energy for the electrons in the K-shell is reached (the K-edge). After a sudden drop the curve then continues to increase until the binding energy for the L-electrons is reached. The splitting of the L-shell (Figure 3.2) is seen in the curve.

activity. A comparison of different radionuclides showed that ^{241}Am (half-life 432.7 years), emitting photons of 59.54 keV, has the best overall qualities for XRF analysis of iodine (Aubert and Fragu, 1983).

Assuming a suitable source energy and also an accurately preserved iodine pool (by preserving *in vitro* and measuring *in vivo*) the measurement should be performed in a way that provides a representative result. Because of the low iodine concentration, even in the thyroid gland ($\sim 0.4 \text{mg/ml}$), and the high amount of Compton scattering in the overlying tissue, as well as in the gland, the major challenge (as in all XRF measurements of low-concentration substances) is to obtain a characteristic X-ray signal that is strong enough. Compton scattering is thus a process that affects the detected iodine signal the most and it is of great importance to correct for this.

Preservation of *In Vitro* Samples

When measuring *in vitro*, the iodine content should be preserved, a condition which is automatically satisfied in *in vivo* measurements. There are situations when there is no possibility to analyze the samples immediately after excision, and a suitable preservation method is then crucial. Although methods such as homogenization (Tadros *et al.*, 1981), formalin fixation (Tang Fui and Maisey, 1983) and lyophilization (Zaichick and Zaichick, 1997) have been applied, there is no standard procedure for XRF. Which method to apply depends to some extent on the sample size and has been investigated by Hansson *et al.*, (2008). When obtaining small samples from surgery, the more attractive alternative of keeping the samples frozen until measurement is a better choice than homogenization. If, however, the whole gland is available and the mean concentration is the primary

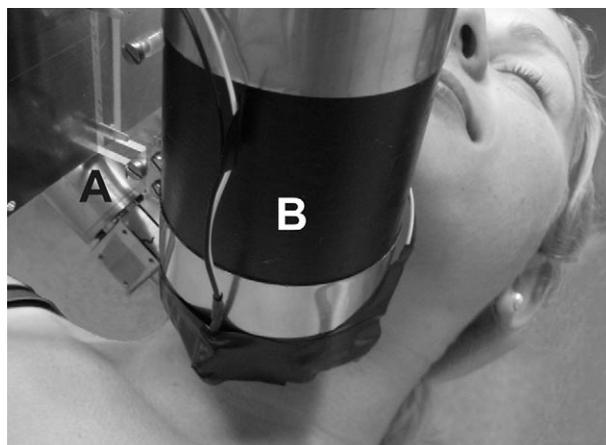


Figure 3.5 An example of XRF measurement geometry. A basic measurement geometry where an 11.1 GBq ^{241}Am source (A) and an HPGe detector (B) are assembled in fixed positions relative to each other and focused onto a point. Both source and detector are shielded and collimated with lead. The other equipment options may involve multiple sources or an X-ray tube and another detector type.

question, methods involving homogenization (even though sectioning of the thyroid has been proved to lead to iodine losses) might be considered.

System characteristics

As mentioned before, Compton scattering contributes to the background in the iodine peak. This unwanted addition can, however, be minimized by a suitable measurement geometry (Hansson *et al.*, 2004). When using polarized X-rays as a radiation source, the influence of Compton photons is the lowest with a 90° angle between the source and the detector. Considering the patient neck geometry though, it might be more convenient to use a smaller source–detector angle such as 60° (Tang Fui and Maisey, 1983). Moreover, a smaller angle is favorable because of the possibility of involving a lesser amount of overlying tissue, an advantage which means less scattering of the incoming radiation, as well as of the emitted K_α photons.

The choice of detector and electronics also affects the detected signal intensity. XRF for iodine analysis involves low-energy radiation and suitable detectors typically have high energy resolution, and also high efficiency in the energy range of interest. Examples of applicable detectors are high-purity germanium (HPGe) or silicon lithium Si(Li), both fulfilling the previously stated requirements. The disadvantages are the size of the detectors and the common need for liquid nitrogen cooling, even though there are systems with electrical cooling available. A typical source–detector equipment set-up is shown in Figure 3.5 where an HPGe detector is positioned with an angle of 56° to the source, which in this case is ^{241}Am shielded in lead.

Besides the previously mentioned equipment requirements, there are three main issues that have to be specified for all applications of quantitative *in vivo* and *in vitro* XRF measurements: choice of analyzed volume, accurate positioning, and correct calibration.

Choice of Analyzed Volume

The part of the irradiated volume that the detected signal originates from can be defined as analyzed volume. If the iodine is not homogeneously distributed, the best estimate of the intrathyroidal iodine pool would be a measurement of the whole thyroid. For such a measurement the analyzed volume must be large enough to totally enclose the thyroid gland, and the detected spectra could be distorted by scattering of the incoming radiation, as well as of the emitted characteristic X-rays. Total attenuation (due to scattering and absorption) in normal tissue is approximately 2% per mm for the exciting photons and approximately 3% per mm for the emitted fluorescent radiation, and thus is not irrelevant. Even if the process becomes more complex, those events could be corrected for by calibrations and mathematical corrections of the signal.

Given a homogeneous iodine distribution in the thyroid, the simplest choice of analyzed volume from a calibration point of view would be a volume well-fitted within one of the thyroid lobes. This alternative has the advantage of not giving an unnecessary addition in radiation dose, but the drawback is that a small volume demands either a high activity source or a long measurement time to give a sufficiently strong detected signal and thereby a low minimum detectable concentration (MDC).

A third alternative is a fusion of the two options previously mentioned. The resulting analyzed volume will then be partly inside and partly outside the thyroid lobe, including different volumes of thyroid tissue depending on the thyroid size. Even though this alternative is very flexible, an undesired consequence is that differently sized lobes with the same concentration might give different signals. This is partly because of a difference in irradiated or detected volumes and partly because of a signal addition coming from photons scattered in thyroid tissue that is not directly irradiated.

An investigation using Monte Carlo (MC) simulations of various analyzed volumes showed that larger source beam divergence (but with constant number of photons per unit area) and wider detector collimator opening not only increased the number of K_{α} (Figure 3.6), but also the number of Compton registrations (Hansson *et al.*, 2007).

For *in vitro* studies, one does not have to consider the radiation dose. Even though the dose is relatively low, it is important for all *in vivo* investigations and should always be a cause for optimization with the aim of keeping the dose as low as possible (while still gaining the necessary information).

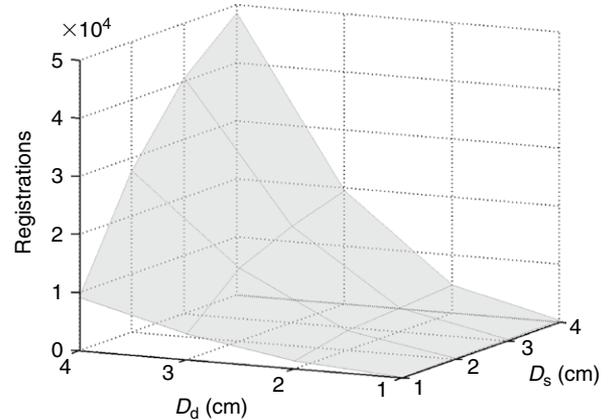


Figure 3.6 Changes in detected characteristic X-rays for different sizes of analyzed volumes. Differently sized analyzed volumes were created by varying the extents of the source beam (D_s) and the detector view (D_d) at the thyroid lobe center (in a neck phantom). For source beam diameter 1.0 cm, 10^9 primary photons were simulated and the same photon fluence (photons/cm²) was maintained for the other values of D_s . For an increasing source beam divergence and detector view at the simulated lobe center (D_s and D_d) more iodine K_{α} (shown at the vertical axis) and Compton photons were detected.

Moreover, compared to *in vivo* measurements, *in vitro* set-ups are generally easier to control regarding both measurements and calibrations. This being so, an analyzed volume enabling irradiation and detection of the whole sample may be preferable. The alternative to be used, however, should be determined considering the measurement situation and available equipment.

Positioning

Inaccurate positioning is a major source of error in *in vivo* XRF measurements of the thyroid gland. Thyroid localization can be done in several ways, with palpation and ultrasound being the most commonly applied methods. These methods may, however, be insufficient in some cases, especially when the thyroid is small and positioned deep into the neck. An MC simulation of the effects of missing the thyroid with a maximum of 5 mm showed that the highest detector signal was obtained for correct positioning with a decreasing number of detected counts for an incorrect estimation of the thyroid location (either more superficial or deeper). Moreover, it was worse to position the source-detector focus incorrectly deeper into the neck compared with more superficial positioning relative to the thyroid. The underestimation in iodine content could be as much as 30% while focusing 5 mm deeper into the neck. In these estimations it was assumed that the measurement set-up was such that, even if mispositioned, some part of the thyroid was still irradiated and detected (Hansson and Isaksson, 2007).

Positioning is thus an important issue in XRF and should be performed carefully with the aid of ultrasound.

Reiners *et al.* (1996, 1998) have improved the positioning by using ultrasound in combination with an electromagnetic positioning system. Their electromagnetic positioning system consists of a sensor registering the thyroid space coordinates relative to a transmitter. The sensor can then be mounted upon the XRF equipment, which is maneuvered into the exact location.

Calibration

Correct calibration is important in quantitative XRF measurements. Since the body mostly consists of low- Z elements there is a significant amount of scattering. Every individual has specific features, and, between subjects, there are geometrical differences such as source–lobe–detector distance and gland depth. Depending on the position of the gland in the neck the detected signal may thus be affected to a highly variable amount.

Calibrations are commonly performed with phantoms simulating the neck. Due to the mentioned parameters a neck phantom alone may be inadequate as a means of calibration. For individual calibrations, additional mathematical corrections are always required and, even then, there will be uncertainties in the results due to the nature of radiation interaction, which makes it difficult to completely correct for all scattering and absorption events.

A great aid in the standard calibration could be MC simulations (Hansson and Isaksson, 2007; O'Meara *et al.*, 1998). The physical processes involved in photon interactions are probabilistic and can be described statistically. MC methods use random number generation and probability statistics to investigate problems, and are thus applicable for understanding the XRF process and can also function as an aid in the calibrations. Most programs allow flexible geometry definitions and the result can be made to almost perfectly resemble any measurement situation.

Radiation Dose

The radiation burden associated with XRF measurements is generally very low. The dose depends on, for example, the source, source activity, measurement time, collimation, and type of investigation. To obtain a dose as low as possible these parameters and their effects on the dose should be carefully considered and weighed against each other. Changing one parameter leads to an alteration in the others. For example, increasing the source activity enables a shorter measurement time or harder collimation, while still giving the same radiation dose.

Examples of local radiation doses (equivalent doses) to the thyroid are 0.06 mSv with a stationary system (^{241}Am source activity 11.1 GBq) (Milakovic *et al.*, 2006; Reiners *et al.*, 1996), and 0.17 mSv with a scanning system (Jonckheer and Deconinck, 1982). Investigations with

scanning systems generally give higher doses, but may also provide more information. To put these doses into perspective they can be compared to other investigations like a gamma camera examination of the thyroid or a dental X-ray giving approximately 2 mSv (150 MBq $^{99\text{m}}\text{Tc}$ administered activity) and 0.1 mSv (ICRP60), respectively, weighted to the whole body (effective dose). To make a fair comparison though, the doses listed above as local to the thyroid have to be weighed against the thyroid radio sensitivity, and would thus decrease to 3 μSv and 8.5 μSv (effective dose), which is approximately 0.4% of the dose from a $^{99\text{m}}\text{Tc}$ scan.

As has been pointed out in this section, there are a number of parameters to consider before starting XRF measurements; some of the most important ones are briefly summarized in Table 3.2. It must however, be emphasized that even though there are some issues regarding an XRF set-up, the investigation itself and the analysis of the results are quite simple and straightforward.

Table 3.2 What to consider before starting XRF measurements

Equipment	
XRF system	Scanning or stationary
Geometry	Angle between source and detector, collimation, distances
Source	X-ray tube or Am-241
Detector	HPGe or Si(Li)
<i>In vitro</i> analysis	
Sample state	Homogenized or intact
Sample preparation	Fixation or freezing
Analyzed volume	Depending on several factors such as sample size
Calibration	Measurement geometry
Measurement time	Signal strength
<i>In vivo</i> analysis	
Positioning	Palpation, ultra-sonography, and/or electromagnetic-guided
Analyzed volume	Radiation dose, iodine homogeneity, and signal strength
Calibration	Neck phantom, mathematical corrections, and/or computer simulations
Measurement time	Signal strength, radiation dose

Notes: A short list of things to consider regarding equipment and application while using X-ray fluorescence for iodine analysis. Depending on the application (*in vitro* or/and *in vivo*) the demands on system features might vary. Scanning systems give an image of the thyroid iodine content, whereas a stationary system only gives a single output value referring to a selected volume. Parameters like source–detector angle, relative distances, collimation and radiation source have to be specified. Moreover, *in vitro* studies sometimes call for sample preparation, and in comparison to *in vivo* measurements the radiation dose, which is closely related to measurement time and source strength, has little importance. Note that permission from national radiation protection authorities may be needed for using the irradiation source.

Applications

Knowledge of the whole iodine cycle from absorption in the small intestine all the way to the hormone action on the cells is important for understanding iodine homeostasis in health, and the mechanisms involved in thyroid disease. Among those using XRF the general opinion seems to be that this method can give additional information and increase understanding regarding these issues.

After the first introduction by Hoffer *et al.* (1968), there were a number of groups who started using XRF. The applications and equipment varied, but the common purpose was to investigate iodine content and distribution within the gland with this new technique. At that time there were two types of XRF facilities available – nonimaging stationary systems and imaging (scanning) set-ups. These systems had different demands primarily on the source, but also on the detector. For a scanning system, which maps the iodine distribution within the gland, the measurement time in a single measurement point had to be considerably shorter than the time involved in an analysis with a stationary, exclusively quantitative system. Moreover, in order to keep the spatial resolution high, the collimation (often with multi-hole collimators) was tight and a high source activity was thus required. There are a number of references where scanning systems were applied *in vivo* for both quantification and iodine mapping (Aubert *et al.*, 1981; Gillin *et al.*, 1977; Hoffer and Gottschalk, 1971; Johnson *et al.*, 1979; Patton *et al.*, 1976). These scanning systems used single or multiple high-activity sources with some of the most powerful systems employing as much as twenty 37 GBq ^{241}Am sources. Nowadays, it may be difficult to get approval for these systems in some countries, and the stationary systems with the advantage of lower radiation dose and a more compact design may be more attractive.

Independent of the employed system, it should be remembered that XRF alone cannot give a complete description of the thyroid status. Moreover, the method gives information of the amount of iodine, but does not say anything about its chemical state. The one thing that can be provided though is important information about the iodine store momentarily and also the changes over time. While other methods, such as radioiodine scans and urinary iodine investigations, show the uptake and excretion of iodine, XRF shows the result of the uptake and excretion function, which tells how much iodine is presently found and stored in the gland. XRF can thus be considered as complementary to radioiodine scans, which explore the rapid iodine pool turnover. With the introduction of this new method, several questions that had been difficult to investigate earlier became burning issues. The clinical usefulness of the method, however, relies on the connection between thyroid iodine content and thyroid disorders.

Table 3.3 Interesting XRF applications

<i>In vivo</i>
Iodine content in benign, euthyroid thyroid glands
Monitoring of iodine program
Monitoring of iodine program in pregnancy
Iodine content in iodine overload
Monitoring amiodarone treatment
Diagnosing and monitoring iodine induced hyperthyroidism
Monitoring subacute thyroiditis
<i>In vitro</i>
Studies on thyroid tissue obtained at surgery of
Benign thyroid disease
Malignant thyroid disease

Note: Applications where XRF analysis may contribute with valuable information of the thyroid iodine content.

A summary of some of the situations where XRF has been useful is presented in **Table 3.3**.

Several studies have been performed on iodine content in normal thyroid glands and in thyroid disease (**Table 3.4**). In a study by Tadros *et al.* (1981) iodine concentration was measured in tissue from normal thyroids, toxic goiter, multi-nodular goiter, colloid nodule/adenoma, Hashimoto's thyroiditis, and thyroid cancer. They found a large variation between normal thyroids, and the mean iodine concentrations for each of the pathological groups were lower than the mean normal value. The lowest concentration was found in thyroid cancer. From the studies presented in **Table 3.4** it can be concluded that the thyroid is able to store varying amounts of iodine under euthyroid conditions. Jockheer (1983) speculated that there would be a lower limit of around 3 mg for sustaining normal thyroid function. In special circumstances with overload, some glands are able to concentrate iodine in large quantities, and in other cases, the overload will lead to thyroiditis or hyperthyroidism with impairment of the iodination control. In iodine deficiency the gland compensates the low access to iodine by goiter growth. In a situation where the individual is exposed to an iodine-deficient environment or where there is a greater need for iodine, that is, during pregnancy, a small iodine pool might lead to serious hypothyroidism sooner than in individuals with a larger iodine pool. It has also been hypothesized that individuals with small iodine pools are more at risk of developing hyperthyroidism when they are exposed to larger amounts of iodine.

Summary Points

- X-ray fluorescence (XRF) analysis can be used for determination of thyroid iodine content and distribution.
- XRF provides important information about the iodine stored in the iodine pool – a parameter that cannot be assessed by other methods, such as urinary iodine or uptake measurements with ^{131}I .

Table 3.4 Iodine concentration and total iodine measured with XRF

Thyroid types	Concentration ($\mu\text{g/g}$)	Total iodine (mg)	n	In vivo/in vitro	References
Normal thyroids	347 \pm 21	5.1 \pm 0.39	58	<i>In vitro</i>	Zaichick (1997)
Normal thyroids	325 \pm 134	4.82 \pm 2.45	149	<i>In vivo</i>	Reiners <i>et al.</i> , (1996)
Normal thyroids	665 \pm 304		50	<i>In vivo</i>	Reiners <i>et al.</i> , (1996)
Normal thyroids	325 \pm 47	10.01	20	<i>In vitro</i>	Reinwein <i>et al.</i> , (1981)
Normal thyroids		9.0 \pm 3.7	48	<i>In vivo</i>	Jonckheer and Deconinck (1982)
Normal thyroids	530 (190–1340)		34	<i>In vitro</i>	Sekita (1983)
Normal thyroids	1030 \pm 670		48	<i>In vitro</i>	Tadros <i>et al.</i> , (1981)
Normal thyroids		15.6 \pm 4.8	18	<i>In vivo</i>	Rougier <i>et al.</i> , (1981)
Normal thyroids	390 \pm 390	5.2 \pm 4.4	37	<i>In vivo</i>	Milakovic <i>et al.</i> , (2006)
Thyroiditis chronic	60 \pm 130		7	<i>In vivo</i>	Sekita (1983)
Thyroiditis chronic	<30		10	<i>In vitro</i>	Tadros <i>et al.</i> , (1981)
Subacute thyroiditis	280 \pm 120		7	<i>In vivo</i>	Sekita (1983)
Graves'	470 \pm 290		33	<i>In vivo</i>	Sekita (1983)
Toxic nodular goiter	20–900		22	<i>In vitro</i>	Tadros <i>et al.</i> , (1981)
Amiodarone euthyroid		10–45	4	<i>In vivo</i>	Fragu <i>et al.</i> , (1988)
Amiodarone hyperthyroid		40	2	<i>In vivo</i>	Fragu <i>et al.</i> , (1988)
Amiodarone hypothyroid		4	6	<i>In vivo</i>	Fragu <i>et al.</i> , (1988)
Malignant	76% <20		21	<i>In vitro</i>	Tadros <i>et al.</i> , (1981)

Notes: Measured values of iodine concentration and total iodine using XRF. The number of patients (*n*) in each study and whether the study was performed *in vitro* or *in vivo* is also stated. Between euthyroid persons, the iodine concentration may vary considerably and differences exist between geographical areas. In thyroid disease, the lowest concentrations were found in thyroid cancer where 76% of the investigated subjects had concentrations less than 20 $\mu\text{g/g}$ (Fragu *et al.*, 1988; Jonckheer and Deconinck, 1982; Milakovic *et al.*, 2006; Reiners *et al.*, 1996, 1998; Reinwein *et al.*, 1981; Rougier *et al.*, 1981; Sekita, 1983; Tadros *et al.*, 1981; Zaichick and Zaichick, 1997).

- The method is noninvasive and can be used *in vitro* as well as *in vivo*.
- The radiation dose involved in a measurement is very low.
- The equipment consists of a radiation source, a detector, electronics, and a spectroscopy program.
- The choice of analyzed volume, positioning, and calibration are important parameters.
- The areas of special interest are monitoring of iodination programs, identification of individuals with a small iodine pool who are at special risk of developing hypothyroidism in iodine deficiency, and also investigations of mechanisms of thyroid disease in iodine overload.

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Plasma Analytes for Determination of Thyroid Status

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Abstract

Plasma tests are essential for determination of thyroid status. The majority of such tests are based on immunochemical techniques, which have advanced rapidly in the last 50 years, enabling the development of sensitive and specific assays for use in hospital laboratories. Thyroid hormone status can be assessed by measurement of total and free hormone concentrations, the latter being technically more challenging. A normal thyroid-stimulating hormone concentration essentially excludes primary hypo- or hyperthyroidism and many laboratories now offer this as a single first-line test of thyroid function. Other tests that are commonly available include thyroid autoantibodies, which can help delineate the cause of thyroid dysfunction and predict future risk of thyroid disease, and measurement of calcitonin and thyroglobulin as tumor markers in patients with thyroid cancers. Measurement of intermediates and degradation products of thyroid metabolism is possible but is not currently performed as part of routine care.

List of Abbreviations

Ab	Antibody
Ag	Antigen
AMP	Adenosine Monophosphate
AST	Aspartate Transaminase
CK	Creatine Kinase
DIT	Diiiodotyrosine (T2)
ELISA	Enzyme Linked Immunosorbent Assay
FT3	Free Triiodothyronine
FT4	Free Thyroxine
IgM	Immunoglobulin, type M
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
MIT	Monoiodotyrosine (T1)
RIA	Radioimmunoassay

rT3	Reverse Triiodothyronine
TBG	Thyroid Binding Globulin
TBII	Thyroid Binding Inhibiting Immunoglobulin
TBPA	Thyroid Binding Prealbumin
TgAB	Thyroglobulin Antibodies
TPO	Thyroid Peroxidase
TRAB	TSH Receptor Antibodies
TRH	Thyroid Releasing Hormone
TSH	Thyroid Stimulating Hormone
T1	Monoiodotyrosine
T2	Diiiodotyrosine
T3	Triiodothyronine
T4	Thyroxine
UDP	Uridine Diphosphate
UK	United Kingdom

Introduction

Plasma tests of thyroid function are an essential tool for patient management. They can be used to detect hypothyroidism and hyperthyroidism and to determine whether any defect detected is primary or secondary. Measurement of plasma analytes can also be undertaken to monitor treatment of thyroid disorders, to investigate their cause, and to predict future risk of thyroid dysfunction. In addition, there are tests on plasma which reflect the effect of thyroid hormones on body tissues ([Table 4.1](#)).

In this chapter tests are referred to as plasma tests, because plasma refers to intravascular water. Once a blood sample has been taken, plasma is obtained by separating the liquid fraction from the blood cells by centrifugation without allowing it to clot. Serum is that part of the blood which remains after clotting has occurred and blood cells have been removed. The majority of tests of thyroid function to be discussed can be performed on plasma or serum, although particular laboratories may state a preferred sample type.

Table 4.1 Plasma tests of thyroid function

Purpose	Example of test
Detection of hypothyroidism and hyperthyroidism	Thyroid hormones
Determine whether any thyroid dysfunction is primary or secondary	TSH
Monitor treatment	TSH, calcitonin, thyroglobulin
Investigate cause	Thyroid autoantibodies
Predict risk of future thyroid disease	Thyroid autoantibodies
Reflect effect on body tissues	Creatine kinase, cholesterol
Others	e.g., reverse T3, erythrocyte zinc

Note: TSH, thyroid-stimulating hormone; T3, triiodothyronine.

Some thyroid assays that are also important in patient care are performed on whole blood rather than plasma and so are not the remit of this chapter, e.g., genetic analyses that are being carried out more frequently as the role of molecular biology in thyroidology develops (Winter and Signorino, 2001) and thyroid function testing is performed on blood spots as part of national neonatal screening campaigns (American Academy of Pediatrics, 2006).

Thyroid Hormone Secretion

It is necessary to understand the physiology of the thyroid gland and the biochemistry of thyroid hormone secretion

before considering tests of thyroid function and interpreting them.

The thyroid gland consists of two lobes joined by a narrow isthmus situated anteriorly in the lower part of the neck. Connective tissue divides each lobe into lobules, the functional units of which are the follicles. Each follicle consists of a layer of epithelium surrounding a cavity containing thyroglobulin, an iodinated glycoprotein precursor of thyroid hormones. Between the follicular cells are C or parafollicular cells, which secrete the hormone calcitonin.

Ingested iodine is absorbed in the small intestine and transported to the thyroid where it is concentrated. It is then oxidized and incorporated into thyroglobulin bound to tyrosyl residues. Monoiodotyrosine (T1) and diiodotyrosine (T2) are coupled to form the thyroid hormones triiodothyronine (T3) and thyroxine (T4), which are stored in the follicles (Figure 4.1).

Thyroid hormones are released into the circulation by a process of proteolysis and reach their target tissues bound to specific carrier proteins. Thyroxine synthesis and release are stimulated by thyroid-stimulating hormone (TSH), which is released from the pituitary. TSH secretion is controlled by negative feedback from thyroid hormones, principally T4, which acts on the pituitary to regulate its response to the hypothalamic tripeptide thyrotropin-releasing hormone.

Significantly more T4 is secreted than T3 and its plasma concentration is about 50 times higher, the plasma concentration of T4 being about 60–150 nmol/l and that of T3 about 1–3 nmol/l. Conversion of T4 to T3 takes place in peripheral tissues, principally the liver,

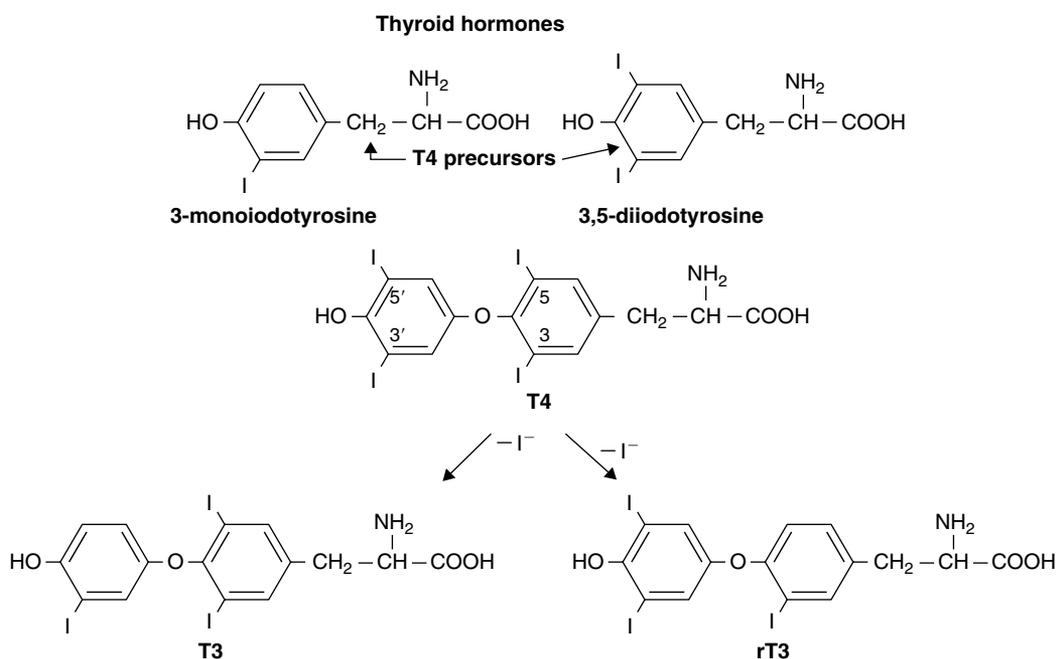
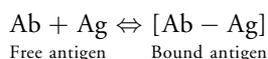


Figure 4.1 The structure of thyroid hormones. T4, thyroxine; T3, triiodothyronine; rT3, reverse triiodothyronine.

kidneys, and muscle and it is T3 that is functionally active. T4 (99.98%) and 99.66% of T3 are protein-bound, mainly to thyroid-binding globulin and to a lesser extent to prealbumin and albumin, hence, the concentration of free T4 (10–25 pmol/l) is only 2–3 times greater than that of free T3 (3–6.5 pmol/l).

Measurement of Thyroid Hormones

The majority of biochemical tests of thyroid function are based on immunochemical techniques that use antibodies as reagents to detect substances of interest and make use of the highly specific binding that occurs between antibodies and their antigens in the following equation:



Once this equilibrium has been established, the amount of antigen (plasma analyte) present in the sample can be determined by separating the bound and the free forms of the antigen.

The immune response and production of antibodies

Antibodies are part of the response of the body to stimulation of the immune system, e.g., any infection by an allergic agent. The “immunogenic” stimulus, or antigen, triggers lymphocytes to form plasma cells, which produce antibodies. Antibodies show specificity for the stimulus that triggered their production. An immunogen, e.g., purified human T4, can be injected into an animal resulting in the production of animal anti-T4 antibodies for use in an immunoassay. However, the result is a heterogeneous antiserum containing a pool of antibodies, because different parts of the T4 molecule have acted as stimuli to different plasma cells. Individual animals would be likely to show different immunological responses to the same stimulus, resulting in potential variation in assay performance. Most modern assays use monoclonal assays. These are produced from hybridomas formed from B lymphocytes taken from an immunized animal and fused with a myeloma cell line. The resulting antibodies are homogeneous and highly specific, and the cells of origin can be preserved ensuring ongoing supply (Burtis and Ashwood, 1999).

Immunoassay techniques

There are two main methods of immunoassay: radioimmunoassay or equivalent in which labeled antigen is used, and immunoassay (IRMA) in which labeled antibody is used.

There are two standard procedures for RIA – competitive and sequential. In competitive assays RIA-labeled antigen is added at the beginning of the reaction to compete with the analyte for the antibody. In sequential or two-step assays, labeled antigen is added after plasma analyte–antibody binding has reached equilibrium. Sequential RIA allows binding of a larger proportion of unlabeled antigen and hence increases sensitivity, but takes longer to perform.

In IRMA, the signal detected from the labeled bound antibody is directly proportional to the concentration of the analyte present.

When immunoassays were first developed radiolabels were used, other labels particularly used are enzymes, e.g., β -galactosidase. After addition of an enzyme substrate, quantification of a colored or fluorescent product can occur (Rongen *et al.*, 1994).

To enable quantification of the analyte in an immunoassay, unbound labeled antigen or antibody must be separated from bound. Various methods exist for this including adsorption or steric exclusion (e.g., ion-exchange resin), precipitation of the bound antigen (e.g., using polyethylene glycol), and the use of solid-phase antibodies (e.g., bound to a tube or bead). In commonly used techniques the analyte is “sandwiched” between two antibodies recognizing different sites (Gosling, 1990), one of which can be linked to a solid phase.

Assay of a calibration solution with known concentration of the analyte enables the preparation of a standard curve from which the patient’s value can be determined.

Measurement of total hormone concentrations

The first tests used to quantify thyroid hormones measured protein-bound iodine. The total amount of thyroxine present in the plasma was proportional to the amount of iodine precipitated with plasma proteins (Chaney, 1958). These assays were replaced by competitive protein-binding assays based on the competition between labeled and unlabeled thyroid hormone for protein-binding sites (Murphy, 1965). RIA methods for measurement of total thyroid hormones became available in the 1970s. Current methods for measurement of total thyroid hormones are mostly IRMA techniques (Demers, 1999).

Measurement of total T4 is generally a good reflection of thyroid status. However, particularly in situations where there is an alteration in plasma-binding proteins, discrepancy can occur between the measured total thyroid hormone concentration and the clinical condition (Table 4.2).

The amount of free hormone (FT4, FT3) is independent of binding protein variations, because in the equilibrium between the bound and the free hormone, the majority of the hormone (>99% for thyroxine) is in the bound form. Familial dysalbuminemic hyperthyroxinemia is an autosomal dominant disorder in which plasma contains an albumin variant that binds T4, but not T3, with

Table 4.2 Circumstances in which discrepancy can occur between total thyroid hormone measurement and thyroid status

<i>Apparent increase in total thyroid hormone concentration</i>	<i>Apparent decrease in total thyroid hormone concentration</i>
Increased TBG Estrogen excess Pregnancy Oral contraceptive, estrogen treatment	Decreased TBG Androgen and anabolic steroid excess
Congenital increase in TBG Familial dysalbuminemic hyperthyroxinemia	Congenital decrease in TBG
Increased concentration of TBPA	Decreased binding capacity of TBG Renal failure Drugs

Note: TBG, thyroid-binding-globulin; TBPA, thyroid-binding prealbumin.

increased avidity. Those with the condition may erroneously be reported to have hyperthyroidism if a T₄ result is interpreted in isolation (Petersen *et al.*, 1994).

Free hormone measurement

Only a small fraction of thyroid hormone circulates as the free form (0.02% as FT₄ and 0.2% as FT₃) not bound to plasma proteins (Schussler, 2000). It is probably the free form that best relates to thyroid status, making its measurement preferable to that of total thyroid hormone. However, measurement of free hormone is more difficult due to reversible equilibrium between the free and the bound forms and relatively low concentration of free hormone in the presence of the more abundant bound form.

Indirect Methods These are intended to be used in combination with a measurement of total hormone to give an estimate of free hormone. With the advent of automated free hormone methods they are no longer widely used. Examples include the T₃ uptake test and T₄:TBG index. They correlate well with free hormone measurement assuming normal binding protein concentrations.

Free hormones can be measured by indirect equilibrium tracer techniques, but such methods are complex (Hay *et al.*, 1991).

Direct Methods The most accurate methods for measurement of free hormone physically separate the free hormone from binding proteins, and measure the free fraction with a highly sensitive T₄ or T₃ assay. Such separation can be achieved using equilibrium dialysis or ultrafiltration (Tikanoja and Liewendahl, 1990), but neither is easily automated for routine use. Analog and two-step (sequential) direct assays for free hormone measurement use

labeled analogs of thyroid hormones that are chemically restricted from binding to proteins. IRMA assays involve direct incubation of the sample with an antibody that only binds to free hormone. A new equilibrium is reached and there is a slight decrease in free hormone concentration, but this is insignificant due to the small amount of the total hormone present in the sample that is sequestered by the antibody (Midgley, 2001).

Interferences in immunoassays

There are a number of potential interferences in immunoassays as a result of cross-reactivity, antibodies, and drug interactivities. Cross-reactivity has been virtually eliminated from modern thyroid hormone assays, but problems with antibodies persist. Antibodies causing interference in thyroid hormone immunoassays may be autoantibodies, e.g., to TSH in patients with autoimmune thyroid disorders, IgM rheumatoid factors, and heterophilic antibodies (Despres and Grant, 1998). Drug interference may be *in vitro*, when the drug results in methodological interference, or *in vivo* (Wenzel, 1996).

Thyroid-Stimulating Hormone

The development of sensitive assays for TSH has enabled biochemical testing of thyroid function to provide not only a measurement of thyroid hormone concentrations, but also an assessment of the hypothalamo-pituitary axis. This allows differentiation between primary thyroid disease and pituitary (central or secondary) pathology, and enables diagnosis of subclinical hypothyroidism and hyperthyroidism where thyroid hormones are within the reference range but TSH is marginally abnormal.

Initially TSH was measured by RIA. There were problems with the sensitivity of the assay due to cross-reactivity with gonadotrophins that share a subunit with TSH. As accurate measurement of low concentrations of TSH was not possible the assay only enabled diagnosis of primary hypothyroidism in which TSH is elevated. Use of the TRH test became a useful way to overcome the limits of assay sensitivity. In this test 200 µg of synthetic TRH is injected. In euthyroid patients it stimulates TSH into the detectable range within 30 min, but not in those with hyperthyroidism. In hypothyroidism, the response is exaggerated, and in hypothalamic disease, it is delayed (Barth *et al.*, 2001) (Figure 4.2).

The introduction of IRMA resulted in increased sensitivity, with assays being able to detect TSH down to 0.1–0.2 mU/l, and the more modern “third generation” assays are further improved as the functional sensitivity is able to detect TSH as low as 0.01–0.02 mU/l.

All but a very small minority of these patients have an intact hypothalamo-pituitary-thyroid axis regulating TSH secretion according to the feedback of thyroid hormones.

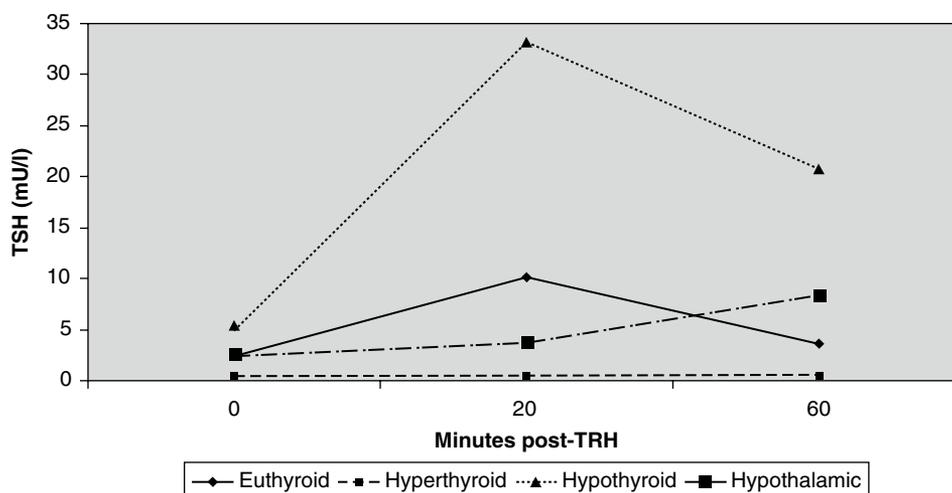


Figure 4.2 TRH test. 200 µg of thyroid-releasing hormone (TRH) is injected; thyroid-stimulating hormone (TSH) concentration is measured prior to injection and 20 and 60 min afterwards. Characteristic responses are shown. TRH, thyroid-releasing hormone; TSH, thyroid-stimulating hormone.

A decrease in thyroid hormone production stimulates TSH secretion while overproduction suppresses it, and at equilibrium, the relationship between the concentrations of TSH and FT₄ is a log-linear one (Spencer *et al.*, 1990). Measurement of TSH is therefore a more sensitive test for early thyroid dysfunction than measurement of thyroid hormone concentrations. After treatment is instituted for hypothyroidism or hyperthyroidism it may take 2 months for the TSH to return to normal. During this time the TSH concentration may not reflect thyroid status and measurement of thyroid hormone concentration is more appropriate for clinical management.

The symptoms and signs of hypothyroidism are non-specific and may be mistakenly attributed to other illnesses, therefore, thyroid function tests form part of the investigation of many presentations. In addition, the prevalence of spontaneous hypothyroidism is between 1% and 2% (Vanderpump *et al.*, 1995), making a case-finding approach appropriate. Our laboratory performs in excess of 100,000 thyroid function tests per annum, of which over half are from Primary Care. A strategy of measuring TSH as the single first-line test of thyroid function is used by some laboratories for screening and case finding. Measurement of FT₄ is performed if the TSH concentration is outside the reference range and in particular circumstances (Table 4.3).

UK Guidelines for the Use of Thyroid Function Tests (2006) indicate that the measurement of TSH alone is appropriate after the first investigation in the sequential follow up of patients who have not been treated for thyroid disorders and who may be at risk of developing thyroid dysfunction. If a laboratory is not able to identify patients who may require measurement of both TSH and FT₄, it is probably not prudent to embark on such a strategy.

Table 4.3 Exceptions to the use of thyroid-stimulating hormone (TSH) as a single first-line test of thyroid function

Testing new patients having symptoms suggestive of thyroid dysfunction
Optimizing thyroxine replacement in the early months of treatment for primary hypothyroidism
Monitoring patients with hyperthyroidism in the early months after treatment
Diagnosing and monitoring thyroid disorders in pregnancy
Patients with known or suspected hypothalamo-pituitary disease
Identifying patients with end-organ thyroid hormone resistance or TSH-secreting pituitary adenoma

Patients with unsuspected central hypothyroidism, due to hypothalamo-pituitary disease, tend to have a TSH within the reference range and will be missed by a frontline TSH strategy. While the number of such patients is low, an estimated 1/20,000 per population, the opportunity to diagnose associated life-threatening hypoadrenalism may be delayed (Squire *et al.*, 2001).

Measurement of FT₃ is required in the presence of a low TSH, in order to assist evaluation of subclinical or overt hyperthyroidism.

Investigation of Cause and Monitoring of Treatment

Thyroid Autoantibodies

The detection of antibodies directed against thyroid-specific antigens can be useful in the diagnosis of autoimmune thyroid disease. Antibodies measured in

clinical practice are those against thyroid peroxidase (TPO), thyroglobulin (TgAB), and the TSH receptor (TRAB).

Thyroid Peroxidase and Thyroglobulin Autoantibodies

Early tests for TPO used semiquantitative complement fixation detecting antibodies against thyroid microsomes. Subsequent manual agglutination tests are now being replaced by immunoassay or immunometric assay.

TgAB was the first thyroid autoantibody observed in the plasma of patients with autoimmune thyroid disease. It was first measured semiquantitatively, but ELISA and chemiluminescent techniques have now superseded this. Measurement of thyroid autoantibodies has a role in the diagnosis of autoimmune thyroid disorders and is particularly useful in predicting those at risk of thyroid dysfunction (Table 4.4).

TPO and TgAB are usually present together in autoimmune disease, but TgAB is more likely to be detected in those without thyroid disease. In view of this it is not necessary to perform both tests as part of the routine assessment, and TPO is thought to be preferable.

Thyroid-Stimulating Hormone Receptor Autoantibodies

Assays are available to measure inhibition of TSH binding to TSH receptors. The antibodies involved include TSH receptor antibodies (TRABs) and thyroid-binding inhibiting immunoglobulins (TBII), but the assays used tend not to distinguish between their stimulating and blocking properties. Specialist laboratories offer assays that measure TSH receptor antibody stimulation by quantifying the cyclic AMP produced in cultured cells (Morgenthaler *et al.*, 1999). Measurement of TRABs is not required in the majority of patients with thyroid disease, but it is useful in particular clinical circumstances (Table 4.5).

Thyroglobulin

Thyroglobulin is detectable in the plasma of the majority of adults. Its concentration is an indicator of secretory activity due to stimulation of the thyroid by TSH, thus, low or absent plasma concentrations indicate a decreased amount of functioning thyroid tissue or suppression of thyroid activity. Measurement of thyroglobulin is not performed routinely, but it is sometimes appropriate. For example, in the investigation of thyrotoxicosis factitia (i.e., thyrotoxicosis due to ingestion of thyroid hormones) activity of the thyroid gland itself is suppressed by the ingested thyroid hormones and the plasma thyroglobulin concentration is low. In neonates diagnosed with congenital hypothyroidism, absence of plasma thyroglobulin is suggestive of thyroid agenesis (Pacini and Pinchera, 1999).

Measurement of thyroglobulin concentration serves as a tumor marker in patients with nonmedullary thyroid cancer. Such patients are treated by total thyroidectomy and prescription of a dose of thyroxine large enough to

Table 4.4 Situations in which the measurement of thyroid autoantibodies may be informative

Diagnosis of autoimmune thyroid disorders
As a risk factor for autoimmune thyroid disease
As a risk factor of thyroid dysfunction during drug treatment, e.g., lithium, amiodarone interferon

Table 4.5 Clinical situations in which measurement of TSH receptor antibodies (TRABs) may be useful

Investigation of thyroid disease of unknown etiology
Investigation of euthyroid Graves' ophthalmopathy
Differentiation of Graves' disease in pregnant patients from pregnancy-associated thyroiditis, and also for better defining the risk of neonatal hyperthyroidism
Identification of transient states in neonatal hypothyroidism due to TSH blocking antibodies

suppress TSH, the presence of which stimulates tumor growth. Serial measurement of thyroglobulin is useful in follow up, a genuine elevation in concentration suggesting tumor recurrence. For routine follow up of low-risk patients, measurement of thyroglobulin is performed while on suppressive therapy (Guidelines for the Management of Thyroid Cancer in Adults, 2002). However, if this is found to be elevated and for patients at high-risk, measurement of thyroglobulin after TSH stimulation, either by temporary withdrawal of thyroid hormone treatment or by injection of recombinant TSH, enables timely detection of recurrence. Thyroglobulin measurement may be useful in patients with metastases, in whom the primary site is unknown, to investigate for thyroid cancer. However, it is not a useful investigation in the differential diagnosis of a primary thyroid cancer as plasma concentrations may be within the reference range at diagnosis. Interference due to thyroglobulin antibodies can complicate serial measurement (Spencer *et al.*, 1996).

Calcitonin

Measurement of calcitonin is used as a tumor marker in the management of medullary thyroid cancer. A fasting morning sample is preferable and it should be placed on ice and the serum or plasma separated and frozen within 30 min of collection (European Group on Tumour Markers, 1999).

α -Subunits

TSH is composed of two subunits, α and β . Most TSH-secreting pituitary tumors (TSHomas) secrete α -subunits in addition to TSH. Patients with TSHomas and TSH

Table 4.6 Commonly performed laboratory measurements which may be abnormal in thyroid disease

Test	Abnormality	Notes
AST, CK, LDH	↑ in hypothyroidism	Multifactorial including release from muscles and decreased clearance (Prakash <i>et al.</i> , 2007)
Bilirubin	↑ in hypothyroidism	Reduced activity of UDP-glucuronyltransferase and decreased bilirubin excretion (Malik and Hodgson, 2002; Van Steenberg <i>et al.</i> , 1989)
Calcium	↑ in hyperthyroidism	Increased osteoclastic activity (Iqbal <i>et al.</i> , 2003)
Cholesterol	↑ in hypothyroidism	Disturbance in synthesis and degradation of lipoprotein with net increase in LDL and triglyceride (Pearce, 2004)
Creatinine	↑ in hypothyroidism	Hypodynamic state and reduced glomerular filtration rate (Kreisman and Hennessey, 1999)

Note: AST, aspartate transaminase; CK, creatine kinase; LDH, lactate dehydrogenase.

resistance, due to mutations in the thyroid hormone receptor gene, may have a normal TSH but raised FT₄ concentration. In thyroid hormone resistance, the molar ratio of α -subunits to TSH is normal, while in TSHoma, it tends to be increased (Brucker-Davis *et al.*, 1999). The assay is only available in specialized laboratories.

Tests Reflecting the Actions of Thyroid Hormones on Body Tissues

Thyroid hormones have multiple effects on peripheral tissues, and abnormalities of thyroid function are therefore reflected in a number of clinical biochemistry tests seemingly unrelated to endocrinology. While these parameters cannot be relied on in order to make a diagnosis of thyroid dysfunction, they may alert previously unsuspecting clinicians to a diagnosis of thyroid dysfunction, and knowledge of tests that may be affected in thyroid disease is important for correct interpretation of the laboratory data (Table 4.6).

Tests not Used as Part of Routine Clinical Practice

3,3',5'-Triiodothyronine (Reverse T₃, rT₃)

rT₃ is secreted in very small amounts by the thyroid gland but its plasma concentration essentially reflects T₄

degradation of which it is a product. rT₃ concentrations tend to be elevated in circumstances where T₃ is low in the absence of clinical hypothyroidism. Measurement is not routinely available in the UK but can be useful to delineate whether low thyroid hormone concentrations are secondary to the effect of nonthyroidal illness or true hypothyroidism.

Other Thyroid Hormones

Other thyroid hormones, such as various forms of T₂ and T₁, DIT and MIT, can all be measured, and reference ranges in the plasma of euthyroid subjects have been established (Nelson and Tomei, 1988). At present their measurement does not have a role in routine clinical practice.

Erythrocyte Zinc

Erythrocyte zinc concentrations reflect integrated thyroid hormone concentrations over the previous few months, similar to the way in which glycated hemoglobin reflects glucose concentrations. This has been used to distinguish Graves' disease from transient hyperthyroidism such as that associated with hyperemesis gravidarum (Batchat *et al.*, 2005).

Summary

The measurement of plasma analytes for the determination of thyroid status is:

1. is an essential part of clinical management.
2. predominantly involves immunoassay techniques.
3. can be used to:
 - detect thyroid disease;
 - investigate cause; and
 - monitor treatment.

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Interpretation of Thyroid Function Tests and Their Relationship to Iodine Nutrition: Changes in TSH, Free T₄, and Free T₃ Resulting from Iodine Deficiency and Iodine Excess

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Abstract

Normal thyroid hormone levels in body tissues are vital for many processes, starting from initial development and differentiation of cells to maintenance of normal homeostasis in adult life. Thyroid disease is common, and the basis of diagnosis of many thyroid conditions is the interpretation of accurate thyroid function tests. It is always important, however, to consider the clinical setting in which the test was performed to correctly interpret these tests. This is particularly the case in various iodine nutrition states, as these can affect test results. For initial discovery and identification of thyroid disease, serum thyroid stimulating hormone (TSH), free T₄, and/or free T₃ are measured. Often serum TSH alone is used to screen for thyroid disease; while this is usually adequate, it may miss some rare diagnoses. The general categories of test results are: normal TSH, free T₄ and T₃ indicating euthyroidism, raised TSH and low free T₄ or T₃ indicating primary hypothyroidism, low TSH and high free T₄ or T₃ indicating primary hyperthyroidism, raised TSH and normal free T₄ and T₃ indicating subclinical hypothyroidism, and low TSH and normal free T₄ and T₃ indicating subclinical hyperthyroidism. The rarer combinations of normal or low TSH and low free T₄ or T₃ suggestive of nonthyroidal illness or secondary hypothyroidism and normal or raised TSH and raised free T₄ or T₃ suggestive of resistance to thyroid hormone or secondary hyperthyroidism are also occasionally seen. Other tests such as for thyroid antibodies can be used to assist diagnosis of specific conditions. Different iodine nutritional states can affect thyroid function tests. In iodine deficiency, generally there is an increase in serum TSH, low free T₄, and raised free T₃ as the body adapts to maintain tissue euthyroidism. Serum thyroglobulin (Tg) is a good marker of iodine intake and is raised according to the level of iodine insufficiency. Acute dietary iodine excess leads to raised serum TSH and low free T₄ and T₃.

Over time this effect usually lessens and results return to normal. The antiarrhythmic drug amiodarone contains a large amount of iodine and inhibits peripheral conversion of T₄ to T₃, resulting in a low free T₃ with raised serum TSH, free T₄, and reverse T₃ (rT₃). Iodine containing radiocontrast agents can affect thyroid function if the dose given is sufficient (depending on the procedure performed); most commonly, they raise serum TSH. The effects are generally transient as they are given as a one-off dose. These situations pertain to people with normal thyroid glands; in the presence of a nodular thyroid gland any of the above causes of iodine excess may induce thyrotoxicosis, while in the presence of autoimmune thyroid disease hypothyroidism may result. In summary, both iodine deficiency and excess may influence thyroid function tests, and should be considered when interpreting these tests at both an individual and a population level.

Abbreviations

CT	Computed tomography
D1	Type 1 deiodinase
D2	Type 2 deiodinase
ERCP	Endoscopic retrograde cholangiopancreatography
IV	Intravenous
rT ₃	Reverse triiodothyronine
SRIH	Somatotrophin releasing-inhibiting hormone
T ₃	Triiodothyronine
T ₄	Thyroxine
TBG	Thyroid binding globulin
Tg	Thyroglobulin
TPOAb	Antithyroid peroxidase antibody
TRAb	Anti-TSH receptor antibody

TRH	Thyrotropin (or TSH) releasing hormone
TSH	Thyroid-stimulating hormone
UI	Urinary iodide

Introduction

Thyroid hormones play an important role throughout our life; they are vital for normal development and for regulation of many body systems in adult life. Thyroid disease is common, and an important part of the diagnosis and management of these conditions involves thyroid function tests, i.e., measurement of levels of the hormones produced by and stimulating the thyroid gland in the serum. These tests have become increasingly sensitive and are now vital in the diagnosis of thyroid abnormalities. However, it is not the test results themselves, but proper interpretation in the clinical setting which is particularly important. The methodology and accuracy of these tests are covered elsewhere in this text.

Iodine plays an important physiological role in the functioning of the thyroid gland. The production of thyroid hormone has been discussed elsewhere in this text. It has also been discussed that many populations remain iodine deficient, have only recently been supplemented, or have excess iodine intake in the form of diet, medication, or treatment. As these can all affect thyroid function testing, they should be taken into consideration when interpreting these tests to avoid missing pathology or making the incorrect diagnosis.

In this chapter, we first cover a general interpretation of thyroid function tests and then move on to how these tests should be interpreted in situations of abnormal iodine nutrition.

General Interpretation of Thyroid Function Tests

Routinely available thyroid function tests include measurement of serum thyroid-stimulating hormone (TSH) also known as thyrotropin, free thyroxine (free T₄), and free triiodothyronine (free T₃) concentrations. In some centers, estimation of thyroglobulin (Tg) and assessment of the presence of thyroid antibodies including antithyroid peroxidase (TPOAb), antithyroglobulin, and antiTSH-receptor (TRAb) antibodies may be available. Some laboratories still measure total T₄ and total T₃. Interpretation of all these tests requires an understanding of basic thyroid physiology.

The normal thyroid gland produces and releases T₄ and T₃ into the circulation in response to stimulation by TSH released by the anterior pituitary gland. TSH, in turn, is released in response to thyrotropin releasing hormone (TRH) released by the hypothalamus. Both TRH

and TSH are subject to negative feedback of T₄ and T₃ on the hypothalamus and anterior pituitary gland, respectively. This is displayed in **Figure 5.1**. The thyroid secretes predominantly T₄, approximately 14 times as much as T₃; however, due to the action of the deiodinase enzymes type 1 and 2 (D1 and D2) which peripherally convert T₄ to T₃, the normal serum T₄:T₃ ratio is 4–5:1 (Pilo *et al.*, 1990; Schimmel and Utiger, 1977). T₃ is the active thyroid hormone, which causes effects in cells by binding to thyroid hormone receptors in cell nuclei and altering transcription. Many cells contain deiodinase enzymes themselves, and can thus activate T₄ by deiodinating it to T₃ within the cell.

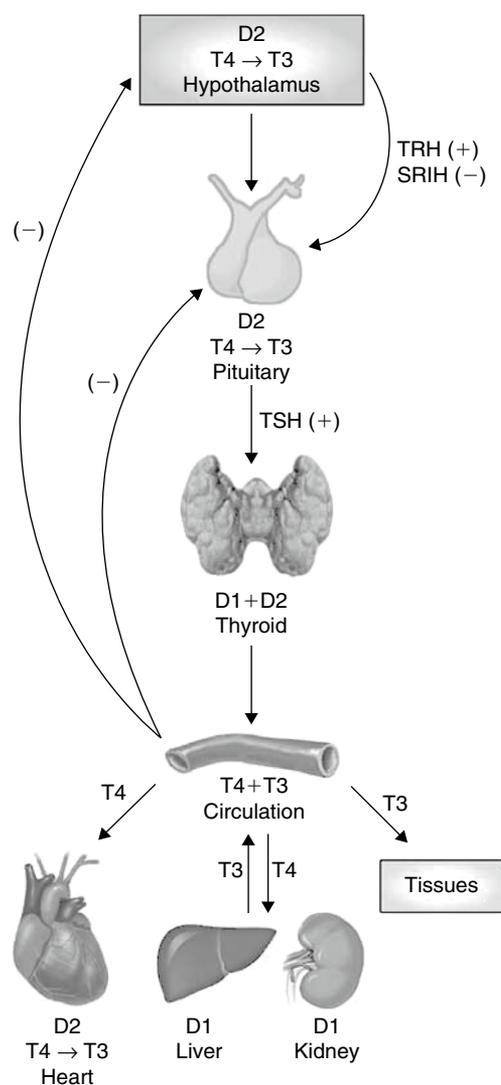


Figure 5.1 Thyroid hormone feedback regulation. D1, type 1 deiodinase; D2, type 2 deiodinase; SRIH, somatostatin (somatostatin) releasing-inhibiting hormone; T₃, triiodothyronine; T₄, thyroxine; TRH, thyrotropin releasing hormone; TSH, thyroid-stimulating hormone. Reprinted from Biondi *et al.*, (2005) with permission from Macmillan Publishers Ltd.

Only approximately 0.02% of T4 and 0.3% of T3 in serum is free; the rest is bound to protein, mostly thyroid binding globulin (TBG), transthyretin, or albumin. The bound hormone is not available to cells; it must be in unbound (or free) form to enter cells and produce actions. In normal physiological states, free hormone concentrations are stable, a reflection of hormone production by the thyroid, available binding sites, and uptake (and metabolism) by tissues. Total hormone concentrations, however, are dependent on binding protein levels, which are variable and influenced by some physiological states (e.g., pregnancy) and many drugs (e.g., estrogen, phenytoin, carbamazepine, salicylates). Therefore, free hormone levels are preferable when using thyroid function tests to diagnose thyroid disease, although they also have limitations. For the rest of this chapter, reference will be made to free thyroid hormone levels only.

The first-line test for thyroid dysfunction is serum TSH, which can now be measured very accurately using highly sensitive assays. Due to cost issues, many centers use this as an initial screening test for thyroid disorders. There are rare conditions however, predominantly in the presence of pituitary disease, which may be missed with this approach (Dayan, 2001); these are displayed in Table 5.1. Therefore, using this approach one must be aware of the clinical situations in which it may lead to error. For confirmation of thyroid disease and to identify rare abnormalities, this is then combined with a free T4 and, in some cases, free T3 measurement. Free T3 assays are generally not as robust as free T4 (and levels are lower); however, they are useful in some clinical situations, particularly hyperthyroidism. Measurement of serum TSH, and free T4 and T3

concentrations allow diagnosis of thyroid status in almost all cases. Table 5.2 provides a matrix for interpretation of these results. In summary, normal TSH, and free T4 and T3 indicate euthyroidism; raised TSH and low free T4 or T3 suggest hypothyroidism; and low TSH and raised free T4 or T3 suggest hyperthyroidism. Abnormally raised TSH with normal free T4 and T3 is suggestive of mild (often referred to as subclinical) hypothyroidism, and low TSH with normal free T4 and T3 suggests mild (subclinical) hyperthyroidism. The other combinations of thyroid function tests are rare. The main causes of these test results are shown in Table 5.3.

It is always important that the clinical situation be taken into consideration when thyroid function tests are interpreted. This is particularly the case in the syndrome of nonthyroidal illness, where severe illness elsewhere in the body may cause unusual thyroid function tests due predominantly to impaired conversion of T4 to T3. This is one example when the relationship between free T4 and T3 can be disproportionate, while they generally maintain their relationship with one another. Other instances include T3 thyrotoxicosis, use of certain drugs, and some iodine states covered later in this chapter. Rarer conditions include familial dysalbuminemic hyperthyroxinemia and the presence of anti-T4 (or T3) immunoglobulins, which bind the radiolabeled T4 (or T3), resulting in a falsely elevated level when measured by radioimmunoassay. These are listed in Table 5.4. In these situations, testing both free T4 and T3 may be useful and prevent misdiagnosis. In addition, several drugs can affect thyroid function tests. High-dose glucocorticoids and dopamine infusions can suppress serum TSH acutely without causing hypothyroidism. High doses of some β -adrenergic antagonists (such as propranolol) and glucocorticoids can slightly decrease T3 levels by decreasing peripheral conversion of T4 to T3. Phenytoin, carbamazepine, and phenobarbitone increase the hepatic clearance of T4 and T3 and may therefore slightly decrease the levels. There are many drugs

Table 5.1 Conditions in which assessment of serum TSH alone (without free T4 or T3 measurement) may miss the diagnosis

Condition	TSH	Free T4/T3	Result
Recent treatment of thyrotoxicosis, becoming hypothyroid	Normal	Low	Delay in commencing replacement therapy
Pituitary hypothyroidism	Normal (often)	Low	Missed diagnosis
Nonthyroidal illness	Normal	Low	
TSH-secreting pituitary tumor (pituitary hyperthyroidism)	Normal (often)	High	Missed diagnosis
Resistance to thyroid hormone	Normal	High	Missed diagnosis

Notes: TSH, thyroid-stimulating hormone; T4, thyroxine; T3, triiodothyronine.

Source: Adapted from Dayan (2001) with permission from Elsevier.

Table 5.2 General interpretation of thyroid function tests

	Raised free T4 or T3	Normal free T4 and T3	Low free T4 or T3
Raised TSH	Pituitary hypothyroidism	Mild (subclinical) hypothyroidism	Primary hypothyroidism
Normal TSH	Resistance to thyroid hormone Recent ingestion of T4	Euthyroid	Pituitary hypothyroidism Nonthyroidal illness
Low TSH	Primary hyperthyroidism	Mild (subclinical) hyperthyroidism	

Notes: TSH, thyroid-stimulating hormone; T4, thyroxine; T3, triiodothyronine.

Table 5.3 Causes of thyroid function test abnormalities

	<i>raised free T4 or T3</i>	<i>normal free T4 and T3</i>	<i>low free T4 or T3</i>
Raised TSH		Subclinical hypothyroidism Heterophile antibodies Poor compliance with T4 therapy Amiodarone Recovery phase of nonthyroidal illness Inherited TSH receptor defects	Chronic autoimmune thyroiditis (Hashimoto's) Post radioiodine/thyroidectomy Hypothyroid phase of transient thyroiditis Drugs (amiodarone, lithium, interferon) Iodine deficiency
Normal TSH	Interfering antibodies to thyroid hormone Resistance to thyroid hormone TSH secreting pituitary tumor Early acute psychiatric illness Amiodarone	Inherited resistance to TSH Euthyroid	Iodine excess Congenital thyroid dysgenesis Congenital functional thyroid defects Nonthyroidal illness Recent treatment for hyperthyroidism with suppressed TSH Secondary (pituitary) hypothyroidism Congenital TSH or TRH deficiency
Low TSH	Graves' disease Multinodular goiter Toxic nodule (adenoma) Transient thyroiditis (postpartum, lymphocytic, postviral) Thyroxine ingestion Amiodarone or iodine induced Ectopic thyroid tissue Struma ovarii Gestational thyrotoxicosis	Subclinical hyperthyroidism Thyroxine ingestion High-dose glucocorticoids Dopamine infusion Nonthyroidal illness	

Notes: TSH, thyroid-stimulating hormone; T4, thyroxine; T3, triiodothyronine; TRH, thyrotropin releasing hormone.

Source: Adapted from [Dayan \(2001\)](#) with permission from Elsevier.

that can affect the levels of TBG or the binding of thyroid hormones to binding proteins, but these rarely affect free hormone levels. The effect of drugs containing iodine on thyroid function is considered later in this chapter.

Additional thyroid tests that may be performed include tests for thyroid antibodies such as TPOAb and TRAb. These can be useful to confirm certain diagnoses and, in some situations, to predict clinical outcomes. The effects of iodine deficiency on thyroid antibodies are considered elsewhere in this text.

Thyroid Function Tests in Iodine Deficiency

In subjects with iodine deficiency, adaptive mechanisms attempt to keep tissue thyroid hormone activity sufficient. These include increased serum TSH, increased iodine trapping by the thyroid, preferential synthesis of T3 by the thyroid ([Stevenson et al., 1974](#)), increased

peripheral conversion to T3, and increased thyroid volume ([Vanderpas, 2006](#)). In isolated mild iodine deficiency, a picture of low serum T4, normal or slightly raised serum T3, and normal or raised serum TSH is seen, with adaptive mechanisms generally maintaining tissue levels of T3, except in those tissues particularly reliant on T4 to convert to T3 ([Vagenakis et al., 1973](#); [Obregon et al., 2005](#)). In this situation, there is an inverse relationship between T4 and TSH, however not between T3 and TSH, suggesting that the negative feedback on the pituitary is controlled in this situation by T4 ([Figure 5.2](#)). Eventually however, with prolonged severe iodine deficiency, the adaptive mechanisms may be overcome and biochemical and clinical hypothyroidism will occur. A study by [Patel et al. \(1973\)](#) has suggested that in severe iodine deficiency, subjects with significant goiter were better able to maintain serum T3 levels than their nongoitrous counterparts. This physiological response to iodine deficiency is similar to that seen in autoimmune hypothyroidism in iodine-sufficient subjects.

Table 5.4 Conditions in which there may be an altered T4:T3 ratio

Cause	Result	Explanation
Nonthyroidal illness	↑ T4:T3 ratio	Impaired peripheral conversion of T4 to T3
T3 thyrotoxicosis	↓ T4:T3 ratio	Increased thyroidal secretion of T3 ± increased peripheral conversion of T4 to T3 by D1 in Graves' thyrotoxicosis
Amiodarone	↑ T4:T3 ratio	Impaired peripheral conversion of T4 to T3
Iodine deficiency	↓ T4:T3 ratio	Increased thyroidal secretion of T3 and conversion of T4 to T3 as an adaptive measure to maintain T3 levels
Familial dysalbuminemic hyperthyroxinemia	↑ T4:T3 ratio	Inherited altered structure of albumin, which results in increased binding of T4 but not T3 (clinically euthyroid)
Anti-T4 immunoglobulins	↑ T4:T3 ratio	Bind radiolabeled T4 and give falsely elevated T4 but not T3 results on radioimmunoassay
Anti-T3 immunoglobulins	↓ T4:T3 ratio	Bind radiolabeled T3 and give falsely elevated T3 but not T4 results on radioimmunoassay

Notes: T4, thyroxine; T3, triiodothyronine; D1, type 1 deiodinase.

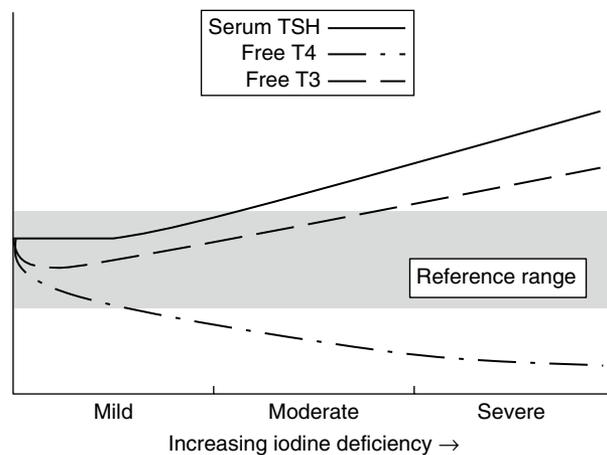


Figure 5.2 Thyroid function tests in iodine deficiency. TSH, thyroid-stimulating hormone; T4, thyroxine; T3, triiodothyronine.

Some studies in areas of iodine deficiency have paradoxically shown serum TSH to be significantly lower than those in iodine-sufficient areas, even when comparing moderate iodine-deficiency areas to mild (Knudsen

et al., 2000; Gutekunst *et al.*, 1986). This is mainly due to decreasing serum TSH levels with age in iodine-deficient areas resulting from increased nodularity and autonomy of thyroid glands in this region (Knudsen *et al.*, 2000). In fact, when people with nodular thyroids are excluded from this evaluation, the differences disappear. There was no significant difference shown in free T3 and free T4 concentrations. Hence, this is not an effect of iodine deficiency on thyroid function tests, but rather an indication of the increased prevalence of nodular thyroid disease in these populations. There is, therefore, a need for thyroid ultrasound to exclude nodular disease when setting laboratory reference ranges for these populations.

This suggests that we should interpret thyroid function tests in populations from iodine-deficient, and even recently supplemented areas, with caution. A study by Volzke *et al.* (2005) has shown that the reference ranges applicable to iodine-sufficient populations and those supplied by manufacturers of tests may not be applicable to such populations as displayed in Figure 5.3. In this recently iodine-supplemented region of Germany, the reference ranges calculated from the population were different from those supplied by the manufacturer of the tests and the reference range from the NHANES population in the United States (an iodine-sufficient population). In particular, the reference range for serum TSH is much smaller and suggests generally lower TSH values, which would be consistent with an increased prevalence of nodular thyroid disease as compared to the iodine-sufficient populations.

It should be noted, however, that while abnormalities in thyroid function tests do often occur in iodine deficiency, free T4 and TSH were not shown to be associated with iodine intake, as measured by spot urinary iodide (UI) in a generally iodine-sufficient population (Soldin *et al.*, 2005). This most likely represents the differing ability of individuals to adapt to iodine deficiency. We know from studies that individuals respond differently to similar levels of iodine deficiency in terms of goiter development, maintenance of serum T3 levels, and progression to hypothyroidism (Patel *et al.*, 1973). Adaptive mechanisms rely on the ability to increase iodine trapping within the thyroid gland and up-regulate deiodinase activity, and it is likely that these are, at least in part, genetically determined. Hence, at the population level there is no direct correlation between TSH and T4 changes and mild-to-moderate iodine deficiency, suggesting that thyroid function tests themselves are not appropriate measures of iodine intake.

Serum Tg is generally used as a marker for recurrence of thyroid cancer. However, it has been shown to be a sensitive measure of iodine sufficiency; the levels increase with the severity of iodine deficiency (Knudsen *et al.*, 2001; Gutekunst *et al.*, 1986). The test is not routinely used for this purpose, but has been used in epidemiological studies.

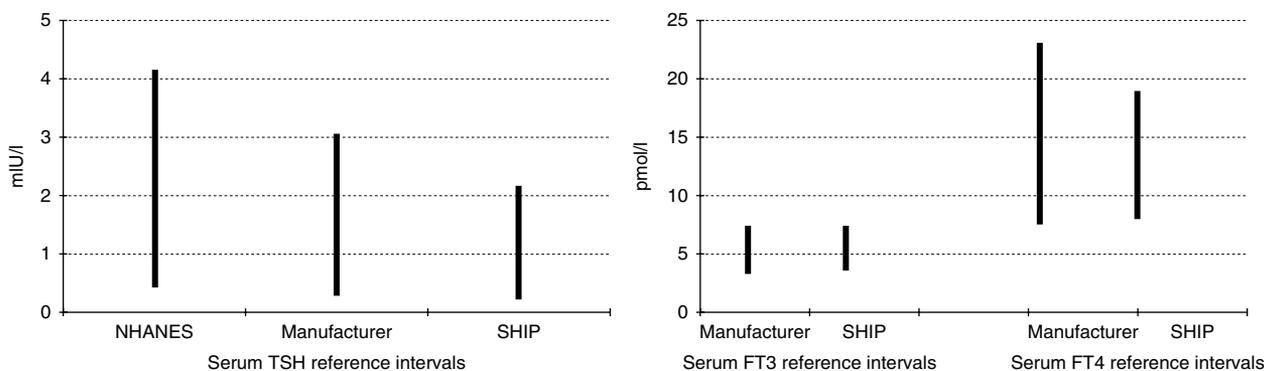


Figure 5.3 Comparisons of serum TSH, FT3, and FT4 reference intervals for the U.S. population (obtained from the NHANES database), those provided by the manufacturers of the assays, and a formerly iodine-deficient population from Germany (Study of Health in Pomerania, SHIP). TSH, thyroid-stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; NHANES, National Health and Nutrition Examination Survey. Reprinted from *Volzke et al. (2005)* with permission from Mary Ann Liebert Inc. publishers.

Thyroid Function Tests in Iodine Excess

Acute iodine excess results in decreased iodine transport, intrathyroidal organification, and release of thyroid hormones from the thyroid gland, a process known as the Wolff–Chaikoff effect. This is at least in part due to inhibition of the peroxidase enzyme inhibiting iodide to iodine conversion (*Burman and Wartofsky, 2000*). The resultant effect of acute administration of iodine (15 days of 80 mg oral iodine) on thyroid function is an increase in serum TSH, while T4 and T3 may be slightly decreased, although all usually remain within the reference range (*Theodoropoulou et al., 2007*; see *Figure 5.4*). Reports vary as to the dose required to produce these significant differences, but most suggest the requirement of greater than 500 $\mu\text{g}/\text{day}$ (*Gardner et al., 1988*; *Paul et al., 1988*). This is a relatively small amount and includes dosages often used for low-dose iodine supplementation programs (*Chow et al., 1991*).

Chronic iodine excess is generally associated with euthyroidism due to “escape” from the Wolff–Chaikoff effect and resumption of normal organification of iodide. There may be increased incidences of goiter, hyperthyroidism (in people with preexisting nodular thyroid disease or Graves’ disease) (*Roti and Uberti, 2001*), and hypothyroidism (in people who do not display the escape phenomenon) (*Markou et al., 2001*); however, these are covered elsewhere.

Amiodarone

The antiarrhythmic agent amiodarone contains a significant amount of iodine (75 mg per 200 mg tablet), which represents about 500 times the recommended daily intake of iodine in each tablet. The high iodine load can affect thyroid function, but amiodarone itself also inhibits T4 to T3 conversion. In the acute phase, in euthyroid individuals, amiodarone produces a reduction in serum T3,

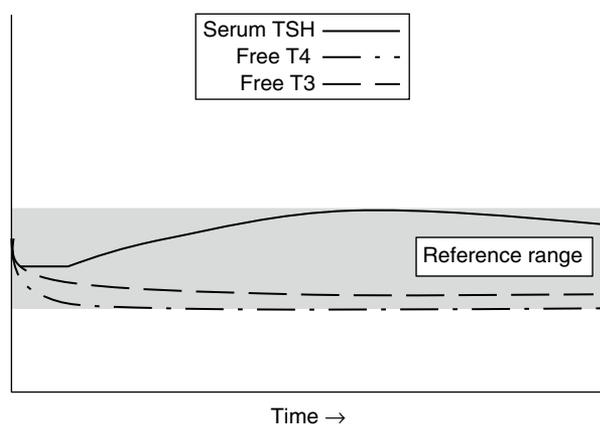


Figure 5.4 Thyroid function tests in iodine excess. TSH, thyroid stimulating hormone; T4, thyroxine; T3, triiodothyronine.

while TSH, serum T4, and reverse T3 (rT3) are increased (*Melmed et al., 1981*; *Iervasi et al., 1997*). After 3 months, when a steady state is reached, serum TSH levels return to normal; however, serum T4 and rT3 levels can remain in the upper range of normal or slightly elevated while serum T3 remains in the low normal range (*Basaria and Cooper, 2005*). Due to the long half life of amiodarone and accumulation in body tissues, these effects may persist for some months after the drug is discontinued. Amiodarone can also cause overt hyperthyroidism (in people with multinodular goiter, Graves’ disease, or via a destructive thyroiditis in people with no previous thyroid pathology) and hypothyroidism (especially in people with pre-existing autoimmune thyroiditis).

Radiocontrast and topical iodine

Radiocontrast agents also contain significant amounts of iodine, and as a result can affect thyroid function tests,

although the effects are relatively minor in patients who previously had a normal thyroid. Trials have shown that a reasonable dose of contrast, e.g., that given during coronary angiography and thorax/abdominal computed tomography (CT) scans (300–1221 mg of iodine per kilogram body weight), can raise serum TSH levels significantly to either high normal or slightly abnormal values. These doses are many times greater than those studied in the above-mentioned trials of acute iodine excess. The rise in TSH generally occurs within 3–5 days and returns to normal levels within a similar time period. In patients with a normal thyroid gland initially, no change in T4 or T3 levels was reported (Gartner and Weissel, 2004). Contrast given during endoscopic retrograde cholangiopancreatography (ERCP) (Mann *et al.*, 1994), intravenous (IV) urography, and IV cholangiography (Burgi *et al.*, 1976) did not show any changes in thyroid function after the procedures. The doses of iodine given in these procedures were lower, ranging from 5.6 to 29.6g for each procedure. It is felt that in euthyroid patients, there is no reason to assess thyroid function when undergoing these studies, given that at worst a mild transient rise in TSH is not a worrying complication.

The situation is different in patients with abnormal thyroids, notably Graves’ disease or multinodular goiter, who are undergoing radiocontrast investigations. Since nodular disease is common in the elderly and thyrotoxicosis carries a significant risk due to coexistent cardiac disease in this age group, elderly patients are also considered to be at “high-risk” of developing abnormal thyroid function after radiocontrast. It has been suggested that such high-risk patients should have a baseline assessment of thyroid function and be followed up after a procedure requiring significant contrast use (van der Molen *et al.*, 2004). Oral cholecystography with sodium-iopanoate alters thyroid function in a different manner. Although it has a high iodine content, its main effect is inhibition of deiodinase 1 and 2 enzymes, which activate T4 peripherally by deiodination into T3. Indeed, the drug has been used to treat some forms of hyperthyroidism. The result is a characteristic rise in T4, rT3, and TSH, while T3 declines. This effect may last for some weeks after administration (Burgi *et al.*, 1976). The use of this agent is, however, becoming rarer with new imaging techniques.

Topical iodine has been used as a treatment for sulfur-mustard-induced skin lesions and as an antiseptic for wounds. Typically, the amount of iodine contained, and hence absorbed, is small and unlikely to cause thyroid function test abnormalities. The only cases where there have been reports of this resulting in transient significant rises in TSH have been in neonatal populations; however, not all studies have demonstrated this.

In summary, doses of iodine used in radiological procedures are generally safe; however, due to the potential for the development of thyrotoxicosis and its complications in elderly patients and those with pre-existing nodular

Table 5.5 Effect of various iodine nutritional states on thyroid function tests

<i>Iodine nutritional states</i>	<i>TSH</i>	<i>Free T4</i>	<i>Free T3</i>	<i>Other</i>
Iodine deficiency	↑	↓	↑	↓ rT3, ↑ Tg
Acute iodine excess	↑	↓	↓	
Acute iodine excess in nodular thyroid gland	↓	↑	↑	Possible development of thyrotoxicosis
Chronic iodine excess	–	–	–	
Amiodarone	↑	↑	↓	↑ rT3
IV radiocontrast agents	↑	–	–	Depending on dose

Notes: TSH, thyroid-stimulating hormone; T4, thyroxine; T3, triiodothyronine; rT3, reverse triiodothyronine; Tg, thyroglobulin; IV, intravenous.

thyroid disease or Graves’ disease, these patients should be carefully monitored.

Table 5.5 summarizes the effect of various iodine states on thyroid function tests.

Effect of Iron Deficiency on Iodine and Thyroid Function

Iron deficiency is a common accompaniment to iodine deficiency in some populations, and furthermore has been shown to impair response to iodine supplementation in these people. Iron deficiency itself can affect thyroid function even in the absence of iodine deficiency. Studies in humans have shown that moderate-to-severe iron deficiency significantly lowers both T3 and T4 (although T3 to a greater extent) and reduces TSH responsiveness (Zimmermann, 2006). This is thought to be due to impaired thyroid peroxidase activity (Hess *et al.*, 2002) and deiodinase activity, and responds to iron replacement; however, this is covered elsewhere.

Summary Points

- Thyroid function tests can vary in various iodine states, and should be interpreted carefully, taking the clinical situation into account.
- Iodine-deficient populations may have raised serum TSH, low free T4, and raised free T3; however, these are not reliable indicators of the severity of iodine deficiency.
- Raised Tg is a good marker of iodine deficiency.
- Acute iodine excess in people with normal thyroid glands results in raised serum TSH and low free T4 and T3; this usually resolves when iodine excess is chronic.

- In people with pre-existing nodular thyroid or Graves' disease, iodine excess may precipitate thyrotoxicosis, while it may precipitate hypothyroidism in those with autoimmune thyroiditis or treated Graves'.
- The antiarrhythmic agent amiodarone causes raised serum TSH, which resolves, and low free T₃ and raised free T₄ and rT₃, which may persist.
- Moderate-to-severe iron deficiency can lower free T₃ and T₄ due to impaired thyroid peroxidase activity even in the presence of iodine sufficiency.

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6

Thyroglobulin as an Indicator of Iodine Intake

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Abstract

Nearly 2 billion individuals have inadequate iodine nutrition, and occurrence of iodine deficiency has been observed in 36.4% of school-aged children. A major indicator corresponding to iodine nutrition and reflecting recent changes in iodine intake is the concentration of iodine in urine. According to population studies, thyroglobulin (Tg) as a product of thyroid epithelial cells also seems to be a valuable indicator of thyroid status, due to its sensitivity to recent changes in iodine nutrition. Serum Tg was found to be elevated in iodine-deficient areas. Tg belongs to the most difficult serum assays in current routine diagnostics, because of inhomogeneity in the large Tg molecule. In addition, the prevalence of autoantibodies against Tg in more than 10% of healthy individuals is an important limitation concerning both the precision and the accuracy of immunoanalytically measured serum Tg, mainly if immunometric techniques are used. Variability in polyclonal antibodies and inhomogeneity of ^{125}I -tracers are factors that impair competitive determination of Tg. Standardization of specific antibody and Tg reference material are necessary to obtain accurate, precise, and reproducible kits providing comparable results. According to our results, Tg is a valuable indicator of iodine nutrition in a population where thyroid disorders are infrequent.

Abbreviations

CBR	The Community Bureau of Reference
CRM-457	Tg standard of the Community Bureau of Reference of the Commission of the European Communities
ExPASy	Expert Protein Analysis System
^{125}I	Radioactive iodine
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDD	Iodine deficiency disorders

IRMA	Immunoradiometric assay
NADPH	Nicotinamide adenine dinucleotide phosphate
NIS	Sodium-iodine symporter
Pax-8	Paired box gene 8
RER	Rough endoplasmic reticulum
RIA	Radioimmunoassay
Tg	Thyroglobulin
TSH	Thyrotropin
UI	Urinary iodine
UNICEF	United Nations Children's Fund
TTF-1, 2	Thyroid transcription factor 1, 2
WHO	World Health Organization

Introduction

The WHO estimated the worldwide prevalence of iodine deficiency using urinary iodine (UI) data collected for 92% of the world's population in the period from 1993 to 2003. Nearly 2 billion individuals have inadequate ($<100\mu\text{g}/\text{l}$ urine) iodine nutrition, and occurrence of iodine deficiency was observed in 36.4% of school-aged children (Zimmermann, 2004a). Several indicators are used to assess the iodine status of a population: thyroid size, UI, and the blood constituents, thyrotropin (TSH), and thyroglobulin (Tg) (WHO Global Database on Iodine Deficiency, 2004). A major indicator corresponding to iodine nutrition and reflecting recent changes in iodine intake is the concentration of iodine in urine. UI below $20\mu\text{g}/\text{l}$ denotes severe iodine deficiency, between 20 and 49 moderate, between 50 and 99 mild iodine deficiency, between 100 and 199 adequate iodine intake, UI between 200 and 299 more than adequate, and UI more than $300\mu\text{g}/\text{l}$ excessive iodine intake (WHO, UNICEF, ICCIDD, 2001). Thyroid volumes reflect a population's history of iodine nutrition, but not present iodine status (Zimmermann, 2004a). Thyroid volume may not return to normal size for months or years after correction of iodine deficiency (Delange *et al.*, 2001);

thus, it is not a good indicator of iodine deficiency disorders (IDD) after introduction of iodized table salt. Iodine deficiency lowers circulating T4 and raises serum TSH, so that iodine-deficient populations generally have higher serum TSH concentrations than iodine-sufficient groups, but the difference is not great and much overlap occurs between individual TSH values. Therefore, the blood TSH concentration in school-aged children and adults is not recommended for routine use as an indicator of iodine intake (WHO, UNICEF, ICCIDD, 2001). Tg originates only in the thyroid gland, and its disposition to incorporate and store available iodine in the form of iodotyrosyl residues or inactive thyroid hormone is unique. The value of Tg as an indicator of global IDD status has yet to be fully explored (WHO Global Database on Iodine Deficiency, 2004), but as the results from population studies indicate, Tg seems to be a valuable indicator of thyroid status in respect to its sensitivity to recent changes in iodine nutrition (Zimmermann, 2004a).

Thyroglobulin: The Major Iodoglycoprotein of the Thyroid Gland

Structure of Tg homodimer

Tg is one of the largest proteins in the body (molecular weight of the soluble dimer is about 660 kDa, sedimentation coefficient 19S, isoelectric point 4.4–4.7). It is the major iodoglycoprotein (0.1–2.0% iodine; 8–10% total carbohydrate with galactose, mannose, fucose, *N*-acetyl glucosamine, and sialic acid residues (Venkatesh and Deshpande, 1999)) of the thyroid gland, which consists of two identical subunits (homodimer), and belongs to the type-B carboxylesterase/lipase family (Park and Arvan, 2004).

The primary structure of human Tg (Figure 6.1) was deduced from the sequence of its 8448-base complementary DNA (Malthiéry and Lissitzky, 1987) and from human Tg gene cloned using cosmid and phage libraries (Baas *et al.*, 1986). The Tg gene containing 48 exons is located on the long arm of chromosome 8 between positions 8q24.2 and q24.3 (from base pair 133 948 386 to base pair 134 216 324); the length of one subunit of Tg homodimer is 2749 amino acids. A finished sequence and gene catalog for chromosome 8 was recently described (Nusbaum *et al.*, 2006). According to the Expert Protein Analysis System (ExPASy) proteomics server (Gasteiger *et al.*, 2003) of the Swiss Institute of Bioinformatics (access number P01266) the N-terminal segment of the Tg subunit (Figure 6.1, part I), which consists of the first 1190 residues, contains 10 highly conserved homologous regions referred to as the Tg type 1 domains. The 11th Tg type 1 domain is in the central part of the Tg subunit. These domains have also been described in relation to proteins unrelated to Tg, for example, in insulin-like growth factor binding proteins (Kiefer *et al.*, 1991), in HLA class II associated invariant chain (Koch *et al.*, 1987), and so on.

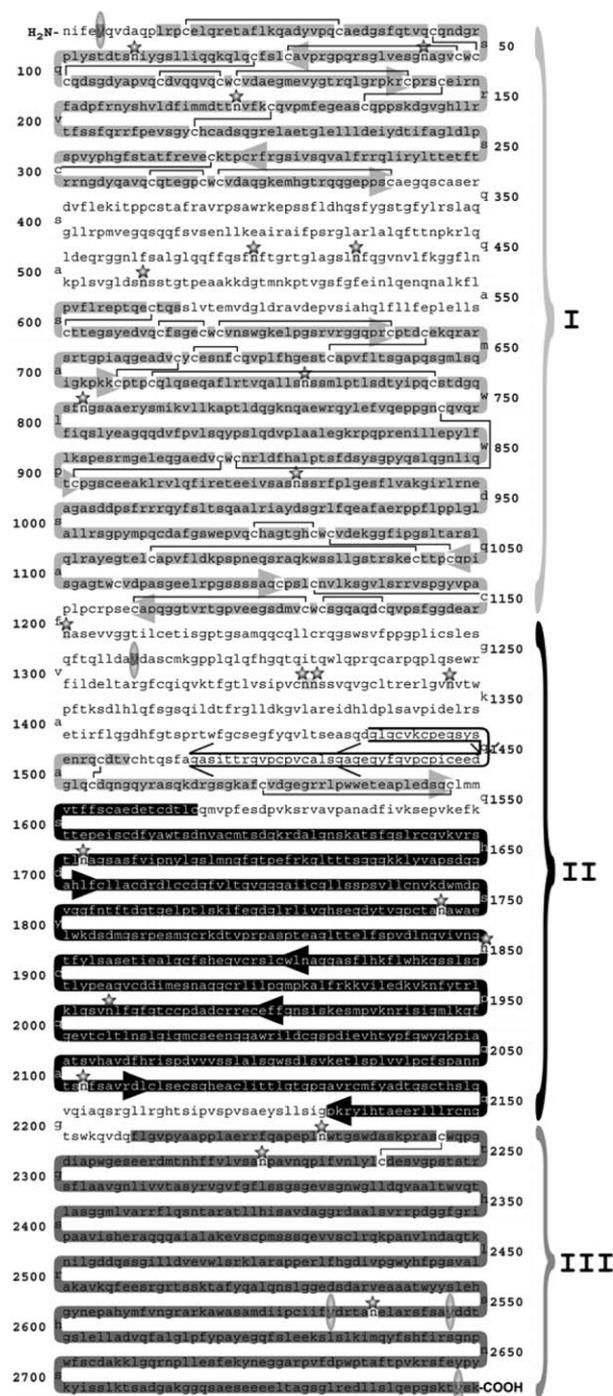


Figure 6.1 The primary structure of the thyroglobulin (Tg) homodimer subunit. The primary structure of the Tg subunit (2749 amino acids) was prepared according to the ExPASy proteomics server (Gasteiger *et al.*, 2003) of the Swiss Institute of Bioinformatics (access number P01266). Disulfide bridges are shown as black lines connecting cysteines (c), potential glycosylation sites are marked with stars, and hormonal glycosylation sites are shown by narrow ellipses. Part I of the Tg subunit contains 10 Tg type 1 domains (part II has 1) marked as light shadow arrows, while part II contains 3 Tg type 2 domains (bold lined arrows) and 5 Tg type 3 domains (solid black arrows) with internal homology. Part III of the Tg subunit, shown as a shaded arrow, shares approximately 30% homology with cholinesterases.



Figure 6.2 Experimentally-determined three-dimensional structure of thyroglobulin (Tg) domain type 1 contained in residues 160 to 234 of insulin-like growth factor-binding protein 6 precursor. Three-dimensional structure of insulin-like growth factor-binding protein 6 precursor (amino acids 160–234) corresponding to Tg domain type 1 was experimentally determined using nuclear magnetic resonance (Headey *et al.*, 2004; ExPASy access number P24592). Model of the molecule was constructed using CAChe software (Fujitsu Ltd, Japan) according to the XYZ coordinates from Protein Data Bank file (code 1RMJ.pdb).

Tg type 1 domains appear to be associated with specific structural features that enabled them to inhibit proteases, mainly cysteine proteases, which are prominent in Tg proteolysis (Galesa *et al.*, 2003). The experimentally-determined three-dimensional structure of the Tg type 1 domain is shown in [Figure 6.2](#).

The central segment of Tg between residues 1191 and 2208 ([Figure 6.1](#), part II) does not have any known similarity to other proteins, but does have internal homology described as Tg type 2 domain occurring three times or type 3 encountered five times in the central part of the Tg subunit. The C-terminal segment of the Tg monomer, consisting of about 541 residues ([Figure 6.1](#), part III), shares approximately 30% homology with acetylcholinesterases. According to the biological role of acetylcholinesterases and their known experimentally-determined three-dimensional structures, it has been suggested that this region is important for binding to the cell membrane, and might contain the Tg dimerization domain (Park and Arvan, 2004). The experimentally-determined crystal structure of homodimer of mouse acetylcholinesterase is shown in [Figure 6.3](#).

Thyroglobulin gene expression

Tg gene expression is regulated by the same thyroid-specific transcription factors that control synthesis of thyroid

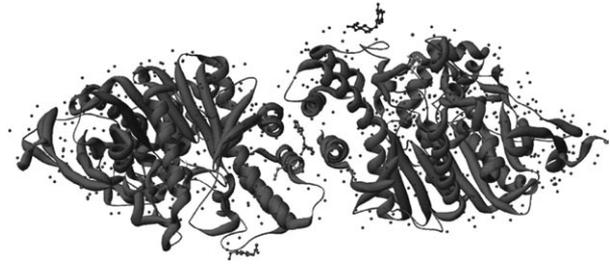


Figure 6.3 Crystal structure of the mouse acetylcholinesterase-2 gallamine homodimer complex with 30% homology of 532 residues from the C-terminal cholinesterase part of human thyroglobulin (Tg). The three-dimensional structure of mouse acetylcholinesterase homodimer complex homological to part III of human Tg in [Figure 6.1](#) was experimentally determined at a resolution of 2.20 Å using X-ray diffraction (Bourne *et al.*, 2003; ExPASy access number P21836). A model of the molecule was constructed using CAChe software (Fujitsu Ltd, Japan) according to the XYZ coordinates from Protein Data Bank file (code 1N5M.pdb).

peroxidase and TSH receptor. The three most important factors are thyroid transcription factor 1 (TTF-1) (Saiardi *et al.*, 1995), thyroid transcription factor 2 (TTF-2) (Macchia *et al.*, 1999), and paired box gene 8 (Pax-8) (Fabbro *et al.*, 1998). The regulation of these factors is mainly under the control of TSH, which stimulates expression of transcription factors, and Tg, which suppresses expression of these factors. It is known that iodine deficiency is the most important etiological factor for euthyroid endemic goiter. However, family and twin pair studies indicate a genetic predisposition. Molecular defects have been identified in the Tg gene of subjects suffering from euthyroid goiter (Neumann *et al.*, 2003), which results in alteration of the Tg three-dimensional shape, reducing the amount of properly structured protein that is available for thyroid hormone production. As demonstrated by Ban (2003a) the 8q24 locus containing the Tg gene is strongly linked with autoimmune thyroid diseases.

Thyroglobulin and biosynthesis of thyroid hormones

Tg is produced by the thyroid epithelial cells (thyrocytes), which form spherical follicles. Tg is secreted and stored in the follicular lumen. The rough endoplasmic reticulum (RER) of the thyrocyte synthesizes the Tg polypeptide chain, carbohydrates are added in the Golgi, iodination and hormone formation occur at the apical membrane, and the mature hormone-containing molecule is stored in the follicular lumen, where it makes up the bulk of the thyroid follicle's colloid. Thyroid hormone biosynthesis is dependent on iodide uptake and its incorporation into the Tg. From this point of view the expression of Tg, TSH receptor, sodium-iodine symporter (NIS), pendrin, thyroid peroxidase, NADPH-oxidase family generating hydrogen peroxide, and iodothyronine deiodinases are major factors determining thyroid hormone biosynthesis. Tg takes part

in these actions because it is a potent autocrine regulator of thyroid-specific gene expression (Suzuki and Kohn, 2006). Thyroid iodine uptake is mediated by NIS at the basolateral side of the follicular cells. Expression and activity of NIS are up-regulated by TSH, but it was found that follicular Tg is a potent suppressor of thyroid-specific gene expression, and can overcome TSH-increased gene expression. It seems to be a negative feedback, an autoregulatory mechanism that counterbalances TSH stimulation of follicular function (Suzuki *et al.*, 1999). It was shown that the expression of NIS and pendrin genes is differentially regulated by Tg concentration and exposure time (Suzuki and Kohn, 2006).

Release of thyroglobulin into circulation

Thyroid hormone secretion depends on apical endocytosis of Tg (van den Hove *et al.*, 2006). Endocytosis can take place by nonselective fluid phase uptake and receptor-mediated processes (de Vijlder, 2003). Two receptors that play a role in the endocytosis of Tg have been identified: megalin (Zheng *et al.*, 1998) and the thyroid asialoglycoprotein receptor (Ulianich *et al.*, 1999). Tg binds to megalin with high affinity, and megalin mediates its uptake in part by thyrocytes (Marino *et al.*, 2001). Tg internalized by megalin avoids the lysosomal pathway and is delivered by transepithelial transport (transcytosis) to the basolateral membrane of thyrocytes, from where it is released into the bloodstream. This process competes with the pathways leading to thyroid hormone release from Tg molecules, which occurs following the internalization of Tg molecules from the colloid by other means of uptake (fluid-phase endocytosis or endocytosis mediated by low-affinity receptors) that result in proteolytic cleavage in the lysosomes. During transcytosis of Tg, a portion of megalin (secretory component) remains complexed with Tg and enters the circulation. Tg endocytosis via megalin is facilitated by the interaction of Tg with cell surface heparan sulfate proteoglycans, which occurs via a carboxyl terminal heparin-binding site of Tg functionally related with a major megalin-binding site. Megalin expression in thyroid cells is TSH-dependent (Marino *et al.*, 2001). The process involves especially hormone-poor Tg, which may favor hormone secretion by preventing competition with hormone-rich Tg for proteolytic degradation (Lisi *et al.*, 2005).

Molecular Forms of Circulating Thyroglobulin Tg is stored within the follicular lumen of active follicles mainly in a soluble form, but globules made of insoluble multimers are also present, mainly in hypofunctioning follicles and are considered to be a mechanism to store prohormone at high concentration (Gerard *et al.*, 2004). Analyses of the molecular forms of serum Tg have shown that circulated Tg of patients with differentiated thyroid cancer is remarkably homogeneous, and in the form of dimers dissociable into

uncleaved monomers. In contrast, serum Tg from patients with Graves' disease or subacute thyroiditis is heterogeneous with respect to its sedimentation properties and/or the structural integrity of its polypeptide chains (Druetta *et al.*, 1998). Tg is present in all thyroid carcinomas, but all types of carcinomas lose the capacity to synthesize thyroxine-rich, iodinated Tg. In follicular carcinomas, this might be due to a defect in iodide transport at the basolateral pole of the cell. In papillary carcinomas, this defect seems to be coupled to an altered apical transport of iodide, and probably thyroid peroxidase activity (Gerard *et al.*, 2003).

Determination of Circulating Thyroglobulin

Tg belongs to the most difficult of serum assays in current routine diagnostic tests, because of the inhomogeneity of the large Tg molecule, where various isoforms of Tg exist with differences in both primary structure and iodine or carbohydrate content. These factors determine the three-dimensional conformation of the molecule, and thus can reduce the epitopes important for immunoanalytical interactions. In addition, the presence of circulating autoantibodies against Tg may substantially interfere with the determination of serum Tg. In the 1960s, the first hemagglutination techniques were developed for the measurement of Tg in serum (Torrighiani *et al.*, 1969). More convenient radioimmunoassay (RIA) techniques were introduced in the 1970s (Van Herle *et al.*, 1973). However, problems were detected in RIA determination of Tg, mainly due to variability in polyclonal Ab, and in regard to the inhomogeneity of ^{125}I -tracers, where the contamination of ^{125}I -9S Tg with a smaller, partially immunoactive, degradation product (530 kDa) was observed and nonparallelism was shown between diluted high Tg sera and the Tg RIA standards, irrespective of the iodination method used (Spencer *et al.*, 1985a, b). Immunoradiometric assays introduced since the mid-1980s (Mariotti *et al.*, 1982) have improved the functional sensitivity of Tg from 3 to 5 ng/ml (Schlumberger and Baudin, 1998) to less than 1 ng/ml (Smallridge *et al.*, 2007), and correlation with previous RIAs was excellent, but only in sera in which there was no interference in the assay (Schlumberger and Baudin, 1998). Dissonance was found between serum Tg measurements made by competitive techniques (RIA) vs. immunometric techniques (immunoradiometric assay, IRMA) in samples containing interferential agents.

Interference of autoantibodies against Tg in the determination of Tg

Circulating autoantibodies against Tg interfere in the determination of serum Tg, and they are an important limitation concerning both precision and accuracy of

immunoanalytically measured serum Tg, mainly if immunometric techniques are used.

Occurrence of Tg Autoantibodies Genetic susceptibility in combination with external factors (e.g., dietary iodine) is believed to initiate the autoimmune response to thyroid antigens including Tg (Ban and Tomer, 2003b). The iodination of Tg has been known to increase its immunopathogenicity, presumably through the formation of iodine-containing neoantigenic determinants that can elicit an autoimmune response (Barin *et al.*, 2005; Li and Carayanniotis, 2006; Li *et al.*, 2007). Tg with no detectable iodine did not induce proliferation of lymphocytes (Rasooly *et al.*, 1998). Chronic autoimmune thyroiditis, the most frequent cause of acquired hypothyroidism with goiter in iodine-sufficient areas, and Graves' disease, are marked by the production of autoantibodies against Tg. High titers of autoantibodies against Tg are found in over 90% of patients with Hashimoto's thyroiditis, and low-to-moderate titers are found in half of the patients with Graves' disease (Gentile *et al.*, 2004). Autoantibodies against Tg have also been detected in 10% of 13 344 disease-free subjects in the USA; their prevalence was higher in females and increased with age (Hollowell *et al.*, 2002). Similar, slightly higher, results were found in Denmark in the area of mild-to-moderate iodine deficiency (13% of 4649 randomly selected adult subjects) (Pedersen *et al.*, 2003) and in Turkey (17.6% of 993 adolescents from an iodine-sufficient area and 6.4% of 740 adolescents from an iodine-deficient area) (Bastemir *et al.*, 2006). In contrast, elevated Tg antibodies were found in only 0.9% of an international group of iodine-sufficient school-aged children ($n = 700$) (Zimmermann *et al.*, 2006).

Interference of Tg Autoantibodies The serum Tg concentrations of autoantibodies against Tg-positive subjects measured by RIA methods were similar to those of autoantibodies against Tg-negative euthyroid subjects. On the other hand, all of the IRMA methods reported generally lower values, some of which were paradoxically undetectable, using 11 different IRMA methods and 4 different RIA methods (Spencer, 2004).

Standardization of Tg immunoassays

A collaborative effort, sponsored by the Community Bureau of Reference of the Commission of the European Communities, produced a Tg standard CRM-457 (Feldt-Rasmussen *et al.*, 1996a, b). The applications of this serum Tg reference material can improve the interassay variability (Zimmermann *et al.*, 2006), but in respect to the complicated three-dimensional structure of Tg, and also in respect to the various Tg isoforms, the standardization of antibodies used for the determination of circulating Tg can be a more important step for obtaining comparable

results across many commercially available Tg assays. The Community Bureau of Reference (CBR) standardization reduced the interassay coefficient of variations from 43% to 29%, which means that while significant differences among various kits remain, the sensitivity of different assays cannot be compared on a numeric bias, and reproducibility of the measurements erodes over time, because of changes in reagent lots, calibrations, instrument factors, and a myriad of other less well-defined variables (Spencer and Wang, 1995). A recently developed Tg assay for use on dried whole blood spots with CRM-457 Tg standards had a reference range (the 3rd and 97th percentiles, 5–14-year-old children ($n = 700$), euthyroid, anti-Tg antibody negative, residing in areas of long-term iodine sufficiency) of 4–40 ng/ml (Zimmermann *et al.*, 2006). This reference range is nearly the same as the adult reference range of 3–40 ng/ml using the immunoassay kits with CRM-457 Tg standards (Demers and Spencer, 2003).

Thyroglobulin and Iodine Intake

As was mentioned previously, transcytosis of Tg-containing endosomes across the thyrocyte results in small amounts of Tg being released into the blood after stimulation of the thyroid gland. The serum Tg concentration primarily reflects three factors: (a) the mass of differentiated thyroid tissue present; (b) any physical damage to, or inflammation of, the thyroid gland; and (c) the magnitude of TSH receptor stimulation (Spencer *et al.*, 1996). The WHO proposed, in 1994, that a median Tg concentration of less than 10 ng/ml in a population indicated iodine sufficiency (WHO, UNICEF, ICCIDD, 1994). This recommendation was not included in the revised 2001 WHO guidelines (WHO, UNICEF, ICCIDD, 2001), but it is mentioned in materials from the Scientific Committee on Food of the European Commission (European Commission, 2002). The main problem is the specification of Tg cutoff value corresponding to various levels of iodine intake. The use of Tg for monitoring iodine status is limited by large interassay variability and lack of reference data for Tg in healthy, iodine-sufficient individuals (Zimmermann *et al.*, 2006). However, serum Tg reflects abnormalities in thyroid function, and Tg is a sensitive marker of iodine deficiency in a population (Benmiloud *et al.*, 1994; Knudsen *et al.*, 2001; European Commission, 2002). Its concentration is thought to respond quickly to stimulation of the thyroid, increasing when iodine supply to the thyroid is depleted, and returning to normal levels when the supply is sufficient. Serum Tg and UI concentration are the most appropriate indicators of iodine status and thyroid function under conditions of increasing iodine supply (van den Briel *et al.*, 2001) because thyroid volume, thyroid nodularity, or iodine excretion have close associations to serum Tg (Knudsen *et al.*, 2001).

Iodine and concentration of serum Tg

In iodine-deficient areas the serum Tg concentration is elevated due to TSH hyperstimulation or thyroid hyperplasia (Zimmermann *et al.*, 2006). The proteome analysis of thyroid tissues has shown that benign cold thyroid nodules, which represent a frequent endocrine disorder accounting for up to 85% of thyroid nodules in a population living in an iodine-deficient area, exhibit significant up-regulation of proteins involved in thyroid hormone synthesis, yet are deficient in thyroxine-containing Tg (Krause *et al.*, 2007). Significant inverse correlations were found for relationships between measures of urinary iodide excretion and serum Tg (Thomson *et al.*, 2001; Rasmussen *et al.*, 2002; Simsek *et al.*, 2003). For example, Buchinger *et al.* (1997) have shown that the mean serum Tg of 2311 untreated euthyroid patients decreased progressively as the UI concentration rose, but the change did not reach statistical significance.

On the other hand, excess iodine intake may also inhibit thyroid function, by either inhibition of iodide organification (Wolff–Charkoff effect) or inhibition of Tg proteolysis with reduction in hormone secretion, and may manifest itself either as a goiter, as hypothyroidism with/without goiter, or as hyperthyroidism (0.01–0.6% in populations on iodine prophylaxis), the outcome depending on the initial and current iodine status and current thyroid dysfunction (European Commission, 2002). The comparison of iodine intake and concentrations of serum Tg at various physiological or pathophysiological conditions is shown in Table 6.1.

Table 6.1 Influence of thyroid status and iodine intake on concentration of circulating thyroglobulin (Tg)

	<i>Iodine intake</i>	<i>Serum Tg concentration</i>
Eufunction of thyroid gland	Deficiency	Increased
	Adequate	Normal
	Excess	Normal or increased
Hypofunction of thyroid gland	Deficiency	Decreased
	Adequate	Decreased
	Excess	Decreased
Hyperfunction of thyroid gland	Deficiency	Increased
	Adequate	Increased
	Excess	Increased

Notes: Overactivity, inflammation or proliferation of thyroid gland reflects in elevated serum Tg. Suppressed activity of thyroid gland results in decrease of serum Tg. However, there is also dependence on initial and current iodine status and thyroid dysfunction.

Population Studies Concerning Iodine Intake and Serum Tg

Iodine Deficiency The increase of serum Tg was determined in newborns from iodine-deficient areas in comparison with newborns from iodine-sufficient area (Sava *et al.*, 1986). In subjects living in areas with severe iodine deficiency, high median Tg concentrations were found in school-aged children (Zimmermann *et al.*, 2004b). However, the administration of iodized oil (Benmiloud *et al.*, 1994; Mirmiran *et al.*, 2002; Markou *et al.*, 2003) or potassium iodide (Todd and Dunn, 1998) decreased serum Tg values in children or in adult goitrous patients (Knobel and Medeiros-Neto, 1986; Lima *et al.*, 1986). In population studies concerning subjects living in areas of moderate or mild iodine deficiency, mean serum Tg levels were increased (Gutekunst *et al.*, 1986; Knudsen *et al.*, 2001; Aydin *et al.*, 2002). The mean Tg concentration of 9.1 ng/ml was determined in the normal adult populations living in mild iodine-deficient areas ($n = 609$), but Tg concentration in the subgroup of subjects with diffuse and nodular goiter was significantly higher (12.0 or 50.1 ng/ml) than that of the normal population (Hu *et al.*, 2003). Subjects ($n = 488$) living in iodine-deficient areas were reported to have elevated serum Tg concentrations and 80% prevalence of goiter (Fenzi *et al.*, 1985).

Adequate or Excessive Iodine Intake In normal children of more than 1 year of age and in adults, 5–35 ng/ml of Tg was found in plasma (Gons *et al.*, 1983). The median of serum Tg was 14.5 ng/ml in an international population study of euthyroid autoantibody against Tg-negative school-aged children ($n = 700$) with adequate iodine intake (Zimmermann *et al.*, 2006). Mean Tg concentration of 7.7 ng/ml was found in the normal adult population ($n = 1136$) with adequate iodine intake (Hu *et al.*, 2003). In pregnant women aged 20–40 years with adequate iodine intake the mean concentration of Tg was 16 ng/ml ($n = 124$). The mean Tg value of 14 ng/ml in 62 healthy nonpregnant controls was nearly the same (Mitchell *et al.*, 2003). Excessive iodine intake in school children (median of UI: 631 $\mu\text{g/l}$, $n = 313$) resulted in the increase of serum Tg (Chong *et al.*, 2004).

Pathophysiological States Changing Serum Tg Concentrations

A transient increase of serum Tg is found in neonates (Gons *et al.*, 1983). Increased serum Tg was also detected in children suffering from primary congenital hypothyroidism, and in patients with defects of iodine organification (European Commission, 2002). Elevated serum Tg concentrations have been determined in patients with differentiated thyroid cancer, and their measurements are useful for detecting metastases of this disease (European Commission, 2002). Low to normal levels are found in cases with hypoplasia of the gland, and plasma Tg levels vary from undetectable to normal in patients with a

disturbed synthesis of Tg. In the absence of thyroid gland plasma Tg is undetectable (Gons *et al.*, 1983).

Our Population Studies Concerning Iodine Intake and Serum Tg Concentrations

Our results are in agreement with the fact that serum Tg is elevated in subjects with deficient iodine intake. UI and serum Tg were determined in a general healthy population of 3902 randomly selected individuals aged 5–98 years (1675 males, 2215 females), or in a hospital population ($n = 4642$), which consisted of individuals aged 0–95 years (595 males, 4047 females) attending the Institute of Endocrinology, Prague. This population study was conducted between the years 1995–2002 in the Czech Republic, which was historically in the area of iodine deficiency; however, since the year 2000 it has been in the area of adequate iodine intake (Zamrazil *et al.*, 2004). A histogram of age for both groups is shown in Figure 6.4.

The level of UI was determined using the Sandell–Kolthoff reaction, which was preceded by the alkaline

ashing of urinary samples. Details about the method were published formerly (Bilek *et al.*, 2005). Serum Tg was determined in 1995–1996 using our laboratory RIA kit (reference range: 0–30 ng/ml), in 1997–2000 Tg was measured using the enzymeimmuno-metric system of Boehringer-Mannheim, Germany (reference range: 0–53 ng/ml), and from 2000 to 2002 the electrochemiluminometric system of Roche Diagnostics, Switzerland was used (reference range: 0–53 ng/ml). The individuals were divided into groups according to their level of iodine deficiency, that is, to the group with UI concentration < 50 (moderate), 50–100 (mild), 100–200 (adequate), and > 200 (more than adequate, excessive) $\mu\text{g}/\text{l}$ of urine. In these groups the mean and median of Tg were calculated. Figure 6.5 shows that, in particular groups, both the mean and the median of Tg changed in relation to the increase of UI. As can be seen in Figure 6.5a, in normal populations living in areas of mild iodine deficiency or adequate iodine intake, the increase of UI is accompanied by a decrease of serum Tg concentrations. However, the situation is more complicated in the hospital population, as demonstrated in Figure 6.5b. Due to thyroid abnormalities of various etiology and the treatment of these disorders, the concentration of serum Tg is not suitable for the evaluation of iodine intake.

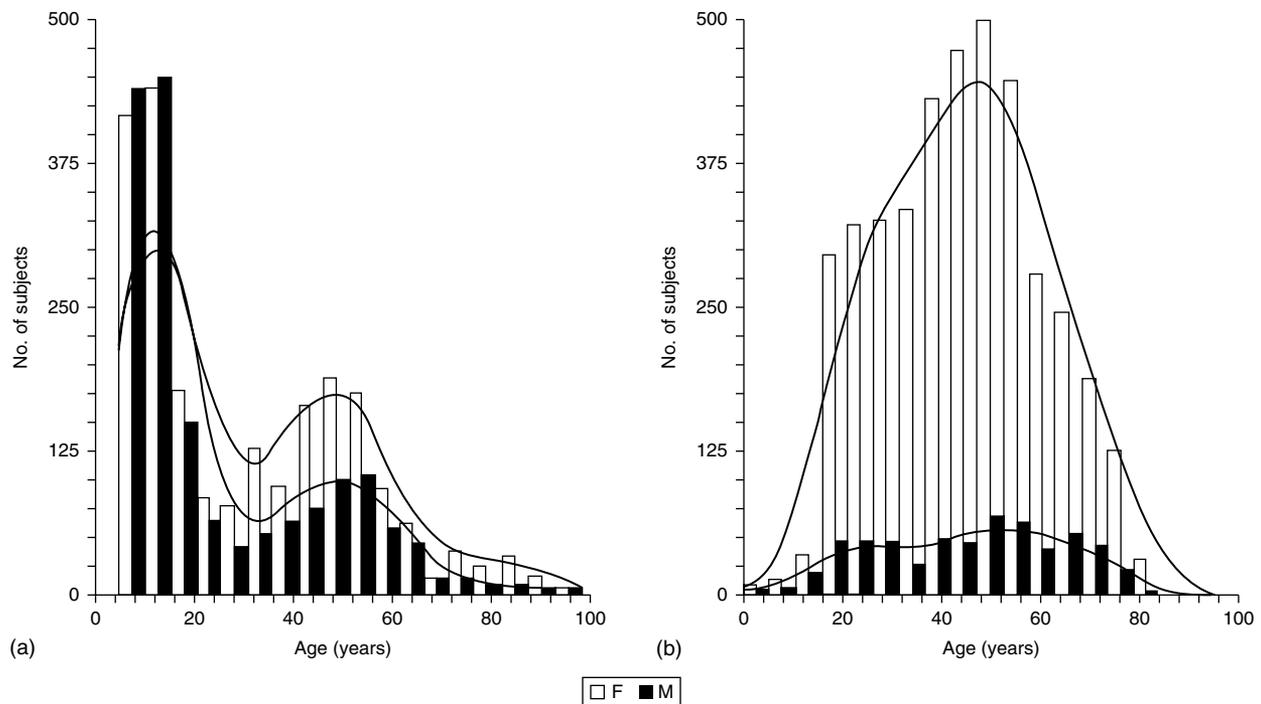


Figure 6.4 Histogram of age by gender (F, female; M, male) in general (a) and hospital (b) populations. Results of a population study conducted in the Czech Republic during the period 1995–2002 concerning iodine intake in the country. Three thousand nine hundred and two individuals participated in this study from a general, randomly selected population (a) and 4642 individuals from a hospital population, which consisted of patients from the Institute of Endocrinology in Prague, The Czech Republic.

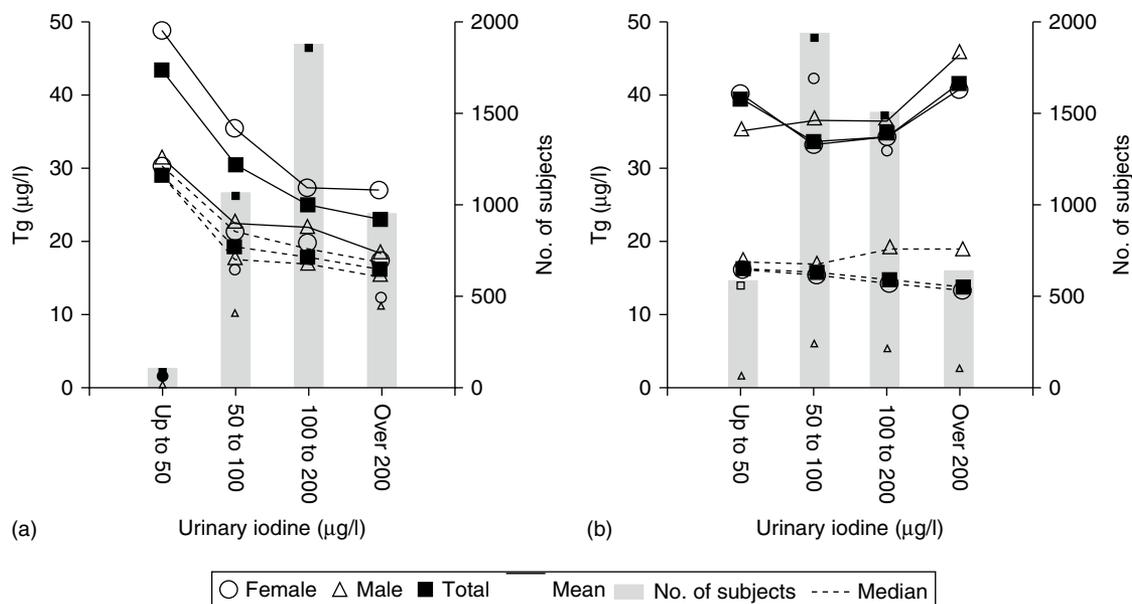


Figure 6.5 The relationship of mean and median concentrations of serum thyroglobulin (Tg) to urinary iodine in general (a) and hospital (b) populations. Subjects were divided into groups based on their iodine intake, and the mean or median of serum Tg were calculated in total groups, and also in corresponding subgroups consisting of men or women.

Summary Points

- Nearly 2 billion individuals have inadequate ($< 100 \mu\text{g I/l}$ urine) iodine nutrition in the world.
- Several indicators are used to assess the iodine status of a population: thyroid size, UI and the blood constituents, TSH, or Tg.
- A major indicator corresponding to iodine nutrition is the concentration of iodine in urine.
- Thyroid volume reflects a population's history of iodine nutrition.
- Thyroid volume, thyroid nodularity, or iodine excretion have close associations to serum Tg, one of the largest iodoglycoproteins in the body with a complex three-dimensional structure, which originates only in the thyroid gland.
- Tg reflects primarily three factors: (a) the mass of differentiated thyroid tissue present; (b) any physical damage to or inflammation of the thyroid gland; and (c) the magnitude of TSH receptor stimulation.
- Serum Tg was found to be elevated in iodine-deficient areas.
- Tg belongs to the most difficult of serum assays in current routine diagnostics because, of inhomogeneity in the large Tg molecule, where various isoforms of Tg exist with differences in both primary structure and iodine and carbohydrate content. In addition, the presence of circulating autoantibodies against Tg may substantially interfere with the determination of serum Tg.

- We can conclude that Tg is a valuable indicator of iodine nutrition in a population, if thyroid disorders are infrequent. However, precision, reproducibility, and accuracy of immunoanalytical kits must be improved to obtain comparable results among various laboratories.

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Computer Systems for Monitoring Effects of Iodine/Thyroid Status in Populations

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Abstract

Low and high iodine intake are both associated with thyroid disease. The World Health Organization (WHO) recommends that iodine supplementation in a population should be followed by a monitoring program, which comprises linkage of different records or databases, including diagnoses of discharge from hospitals, records of treatment for thyroid disorders, laboratory databases, and results of thyrotrophin (TSH) screening in the newborn. However, the availability of records and the practical possibilities for follow-up differs widely. A specific computer-based system linked to diagnostic laboratories for prospective registration of new cases of hyper- and hypothyroidism is described.

Abbreviations

Bs	Blood samples
CHN	Community health number
CPR	Civil personal registry
GP	General practitioner
ICCIDD	International Council for Control of Iodine Deficiency Disorders
ID	Iodine deficiency
MEMO	Medicines Monitoring Unit
RAI	Radioactive iodine
SMR1	Scottish morbidity record
T3	Triiodothyronine
T4	Thyroxine
TSH	Thyrotrophin
WHO	World Health Organization

Introduction

The World Health Organization (WHO) and the International Council for Control of Iodine Deficiency

Disorders (ICCIDD) recommend that initiation of iodine supplementation in a population should be followed by a monitoring program to evaluate its effectiveness, and to detect and counteract any unintended effects of the iodine enrichment (World Health Organization, 2001). Such monitoring should also detect unplanned alterations in iodine intake due to changes in farming practice or use of iodine-containing chemicals in the food industry (Phillips, 1997). Iodine deficiency is associated with many abnormalities (Delange, 1994), but an uncontrolled increase in iodine intake may also cause disease (Konno *et al.*, 1994; Stanbury *et al.*, 1998). One way of monitoring the effect of iodine supplementation could be to register the incidence or prevalence rates of hyper- and hypothyroidism in the population. In a classic incidence study, a cohort is actively screened and followed for a given time period. The advantage is that all new cases, with or without symptoms, are registered, except for those that might be lost from follow-up. As the incidence of overt thyroid dysfunction is relatively low, the size of the cohort studied and the follow-up time need to be considerable to maintain a reasonable robustness of the study, or mostly cases with subclinical disease will be detected.

Other possibilities for identification (ID) of patients with thyroid disorders are searching in different records or databases, such as records of diagnoses of discharge from hospitals, prescriptions of thyroid medicaments (antithyroid drugs and levothyroxine), and records of treatments for thyroid disorders including thyroid surgery and radio-iodine treatments. Finally, diagnosis of overt thyroid dysfunction is based on a biochemical thyroid function test, and laboratory databases with results of analyses of thyrotrophin (TSH) and thyroid hormones in a population cohort, and records of serum TSH in newborns may be used to identify new patients (Kempers *et al.*, 2006).

There are, however, various epidemiological problems associated with the use of nearly all types of records and databases. Furthermore, the availability of records and

the practical possibilities for follow-up differ widely from country to country.

TSH Screening in Neonates

Neonatal TSH screening is well-established in many developed countries and is being introduced in relatively prosperous developing countries (World Health Organization, 2001). As part of monitoring the iodine status in a population, evaluation of the results of TSH screening of newborns has been recommended by the WHO (World Health Organization, 2001). As a general rule, the frequency of neonatal TSH above 5 mU/l blood is below 3% where iodine supply is normal (World Health Organization, 2001). In mild ID the frequency may be 3–19.9%, and frequencies of 20–39.9% and above 40% may be found in moderate and severe iodine deficiency, respectively. It has been suggested that neonatal thyroid screening is a particularly sensitive index in the monitoring of iodine supply at population level. Sufficient iodine intake in the fetus and small infants is very important, because iodine deficiency with impaired thyroid function may lead to brain damage (Delange, 1994). There are, however, some uncertainties and other factors than the maternal iodine status that may influence the TSH value measured in the newborn. Technical details, such as the TSH assay and the collection paper used, may influence TSH distribution. Moreover, the time interval between birth and the blood test is crucial for results (World Health Organization, 2001). The use of iodine-containing antiseptics in the mother or the newborn may lead to transient high serum TSH in the newborn (Mahillon *et al.*, 1989). Because of these limitations more research on the use of TSH screening of newborns for evaluation of iodine intake has been recommended by the WHO.

Hospital Records

The tradition for mandatory registration of disorders, such as infectious diseases and different types of cancers, differs widely between countries. In many countries including Denmark, all diagnoses at discharge from hospitals have been registered in a National Patient Register for years (Nickelsen, 2001). The USA was the first country to implement casemix systems and many other countries have followed (www.sundhedsstyrelsen.dk). The system was primarily developed as a basis for financial reimbursement, and therefore contains both diagnosis-specific codes and codes referring to specific procedures performed regardless of the patient's status as either in- or outpatient. Several studies have been based on hospital registers; however, the usefulness of the registers is highly dependent on the disease studied, as many disorders may be diagnosed and treated other than in hospitals.

Hospital records and records from specialist clinics have been used in a number of epidemiological studies on thyroid diseases (Laurberg *et al.*, 1991; Manji *et al.*, 2006; Mostbeck *et al.*, 1998). Thyroid disorders are, however, diagnosed and treated at all levels of the health care system. Patients with a milder degree of thyroid disease especially are not usually referred to hospitals and are therefore not included in the hospital records. To compensate for this, some authors have combined the results from the hospital register with information obtained from general practitioners (GPs) and specialists in the areas studied, to ensure that all patients not referred to hospital were registered (Laurberg *et al.*, 1991).

In Austria, the incidence of different subtypes of hyperthyroidism was registered before (retrospective) and after (both retrospective and prospective) the iodization of table salt was increased in the mid-1990s (Mostbeck *et al.*, 1998). In Austria, thyroid examinations were mostly carried out in nuclear medicine centers, and the registration was based on all patients with newly diagnosed hyperthyroidism referred to one of 19 nuclear medicine departments in the study area. No linkage to other hospital records or contact with GPs was made, to ensure that all patients were referred to hospital and thereby registered in the study.

In a number of studies, the registration of new cases of hyperthyroidism has been based on a retrospective review of results from thyroid function tests carried out by biochemistry laboratories. In some studies, the biochemical results have been confirmed by review of the patient records (hospital or GPs). Unfortunately, the methods have only been briefly described with few methodological details (Barker and Phillips, 1984; Mogensen, 1980).

Example of Record Linkage

Record linkage is the systematic bringing together of records of individuals in a large population (Flynn *et al.*, 2004).

Flynn *et al.* used record linkage technology to identify incident and prevalent cases of treated thyroid dysfunction in Tayside, Scotland, with a population of about 370 000 subjects. The following six principal databases were used to identify patients: (1) The community health number (CHN) master patient index, which contained information on all GPs in the area and data registered with them – data were used to define the study population. (2) The Medicines Monitoring Unit (MEMO) – dispensed prescription database – MEMO is a university-based organization that uses record linkage techniques to carry out pharmaco-epidemiological research in Tayside. The database contains validated subject-specific data on all prescriptions dispensed from all community pharmacies in Tayside. (3) The radioactive iodine (RAI) database – all RAI treatments with patient ID and doses and

dates of administration are contained in the database. (4) The Scottish morbidity record 1 (SMR1) – the database includes diagnostic and procedural codes related to all hospital inpatient episodes using the International Classification of Disease. (5) Biochemistry database – electronic data on TSH were not available in all areas and the TSH results were only used in some cases to confirm the diagnosis. (6) The Tayside Thyroid Register – the register contains automatic follow-up information on patients on thyroid replacement therapy.

Patients were registered as “hyperthyroid” if they had been treated for hyperthyroidism by surgery, RAI or medication, or if they had a history of hyperthyroidism on the thyroid register. Patients on long-term thyroid replacement therapy according to the MEMO database were registered as “hypothyroid.” A validation of the database system was performed in 450 patients from general practice. A positive predictive value of 0.98 and 0.96 was found for treated hyperthyroidism and hypothyroidism, respectively.

Example of a Specific Register Database

We developed a specific register database to prospectively register all new cases of hyper- and hypothyroidism in two areas of Denmark before and during the first years after the implementation of iodine fortification of salt.

Background and study cohorts

Two well-defined open subcohorts were chosen for monitoring. One subcohort with mild iodine deficiency consisted of 225 734 subjects living in the vicinity of Bispebjerg Hospital, Copenhagen. The other subcohort had moderate iodine deficiency and comprised 310 125 subjects from the city of Aalborg and the surrounding municipalities in Northern Jutland. Information including updates on the composition of the cohort was obtained from the Danish Bureau of Statistics.

In Denmark a 10-digit civil personal registry (CPR) number is assigned to all citizens shortly after birth. The number is used in all interactions with public services, including hospitals and laboratories. About 98% of the population is registered with and consulting one GP only. All GP and hospital departments have a specific referral-identity number to be used with all laboratory contacts. It is, therefore, possible to identify patients from the geographical cohort by means of referral identity numbers from the GPs and hospitals in the area.

All blood tests from the two areas were analyzed in one of four laboratories (one in Aalborg, three in Copenhagen), who all participated in the study (Figure 7.1).

For many years all diagnostic laboratories in Denmark have kept electronic records of blood test results in in-house databases. Each result is linked to the patient’s CPR number

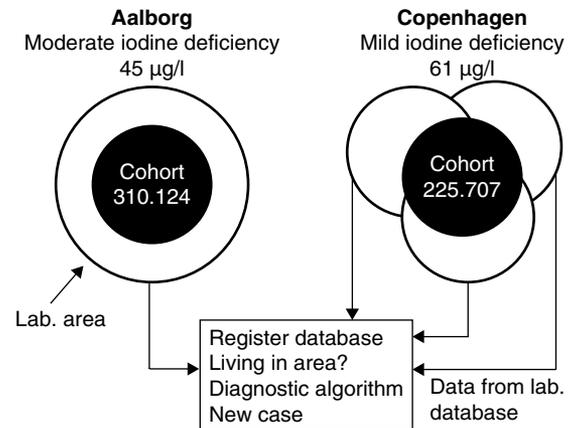


Figure 7.1 Principle of cohort coverage by diagnostic laboratories. The two open subcohorts were defined by geographical boundaries. The diagnostic laboratories covered all diagnostic activity in the cohort (one laboratory in Aalborg, three in Copenhagen). Data from laboratory databases were imported into the register database. Data taken from Pedersen *et al.*, (2002) with permission from Elsevier.

and to the physician or hospital department specific referral identity number. At all the laboratory databases, subroutines enabling automated daily withdrawal of the TSH and thyroid hormone results into ASCII files were established. All resulting data were subsequently filtered to ensure that only results arising from GPs and hospital departments in the study areas were included in the files.

The register database

A register database was developed by a programmer experienced in the development of biomedical databases. It was primarily made on the basis of Delphi Developer 2.0 software, but after about 3 years converted to run on the basis of Progress.

The database was built on the following diagnostic algorithms: hyperthyroidism, low serum TSH combined with a high serum triiodothyronine (serum T3) and/or a high serum thyroxine (serum T4) value; hypothyroidism, high serum TSH combined with a low serum T4. The first parameter evaluated in the register database was TSH. T3 and T4 were only evaluated (using reference ranges of the laboratory performing the analyses) in case of an abnormal TSH.

The database comprised three cross-tables: blood samples (“Bs”), “Man,” and “Man2” (Figure 7.2). The results from the laboratory databases were imported on a continuous basis into cross-table “Bs,” and evaluated according to the diagnostic algorithm.

Data on subjects classified as hyper- or hypothyroid were automatically copied to cross-table “Man,” which contained data on all cases of biochemical hyper- and hypothyroidism. The table consisted of two subsections: “known cases” including cases previously evaluated and

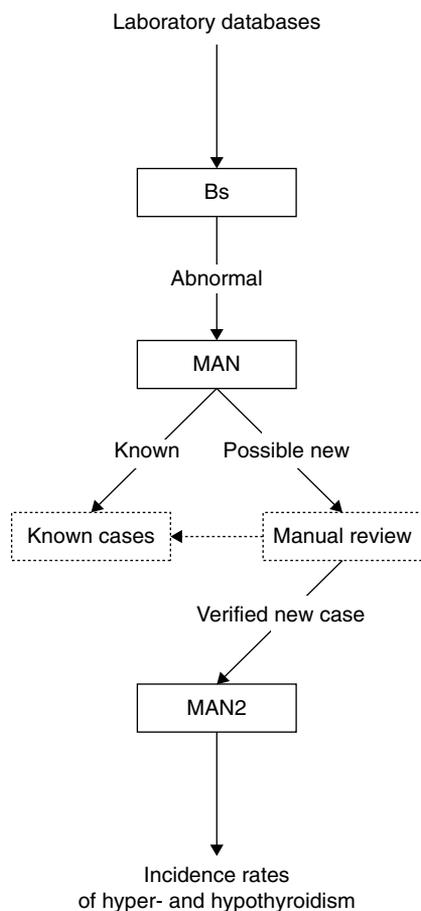


Figure 7.2 Principle of the project database. The register database comprises three cross-tables: “Bs,” “Man,” and “Man 2.” All serum TSH, T3, and T4 results are imported into “Bs,” where primarily TSH and, in case of abnormal value, T3 and T4 are evaluated. Cases of biochemical overt hyper- or hypothyroidism are copied to cross-table “Man,” which consists of two subsections: “known cases” (already registered in the database) and “possible new case” (without any prior database registration). All “possible new cases” are manually cross-checked (see text). All new cases are copied to cross-table “Man 2” and after that registered as known cases. Data taken from Pedersen *et al.*, (2002) with permission from Elsevier.

registered by the database to have hyper- or hypothyroidism, and “possible new cases” including cases not previously evaluated by the database. Entries in this subsection were manually cross-checked and evaluated by a physician (Pedersen *et al.*, 2002). If the diagnosis was verified, the case was copied to “Man2.” Subjects registered in “Man2” were seen by a physician for a diagnosis of the subtype of hyper- or hypothyroidism to be obtained.

The system generates figures at several levels (Table 7.1). Continuous information on new cases of biochemical hyper- or hypothyroidism can easily be obtained to observe trends in incidences of overt thyroid dysfunction. More detailed information (corresponding to levels 5 and

Table 7.1 Levels of information obtained by the computer-based system

Data source	Parameter
Statistical bureau	Cohort size and composition ^a
Database generated	Diagnostic activity in the area ^b (number of TSH, T4, and T3 analyses) Number of subjects with abnormal blood test results ^b Number of subjects with new biochemical hyper- or hypothyroidism ^b
Participating physician	Number of new cases of hyper- and hypothyroidism verified by physician ^c Number of new cases of hyper- and hypothyroidism with subtypes ^c

^aInformation regularly obtained from The Danish Bureau of Statistics.

^bRegistered directly by the database.

^cDetailed information necessitating involvement of a physician.

6 in Table 7.1) needs further evaluation by the physician and can be obtained periodically (Carlé *et al.*, 2006).

Results of evaluation

The methods involved were evaluated before and during the registration (Pedersen *et al.*, 2002). This included evaluation of the diagnostic performance of the participating laboratories. A reference panel of normal sera ($n = 100$) was analyzed in the four laboratories before registration was initiated (Figure 7.3), after 1 year, and when new procedures had been implemented at a laboratory (Figure 7.4). No systematic differences in the distribution of values within reference ranges were found. The database identity of new cases was tested by a 60-day run in Aalborg with parallel manual and computer-based evaluation of all abnormal TSH results ($n = 2159$). The results were identical. Further, we evaluated the risk of non-detection because of a GP outside the study area. This was found to be negligible. Finally the use of TSH as a primary test in the diagnostic algorithm was evaluated. No cases of thyroid dysfunction had been diagnosed based only on T3 and T4.

Backup and database security

The security of the database is an important issue. The register database program is run in two copies, one in Aalborg and another in Copenhagen, where data from the two localities are imported directly, processed, and stored. On a regular basis data are withdrawn from the Copenhagen database and imported into the complete database in Aalborg, which is connected to a server. Access to the data in the database is restricted to a few persons,

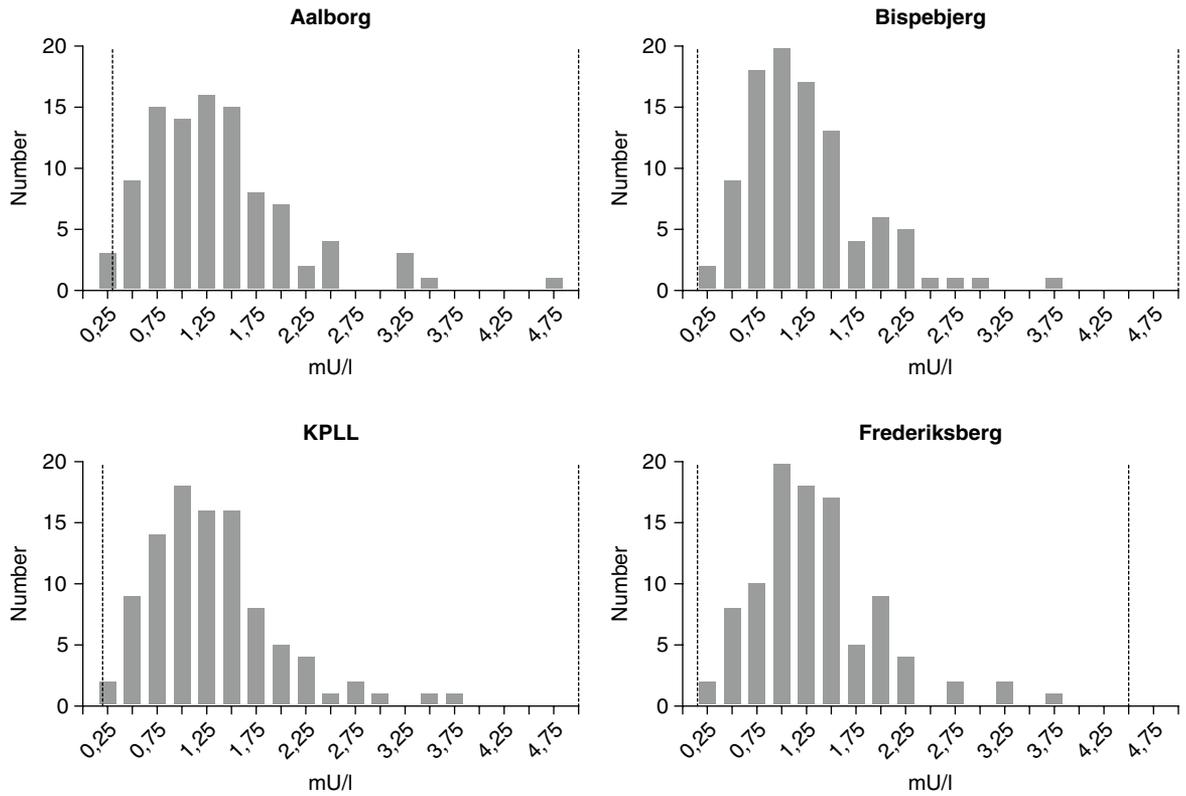


Figure 7.3 The distribution of reference panel ($n = 100$) serum TSH analyzed by the four participating laboratories at the start of the study. Almost all values lie within the respective reference range of the laboratories (dotted vertical lines). Data taken from Pedersen *et al.*, (2002) with permission from Elsevier.

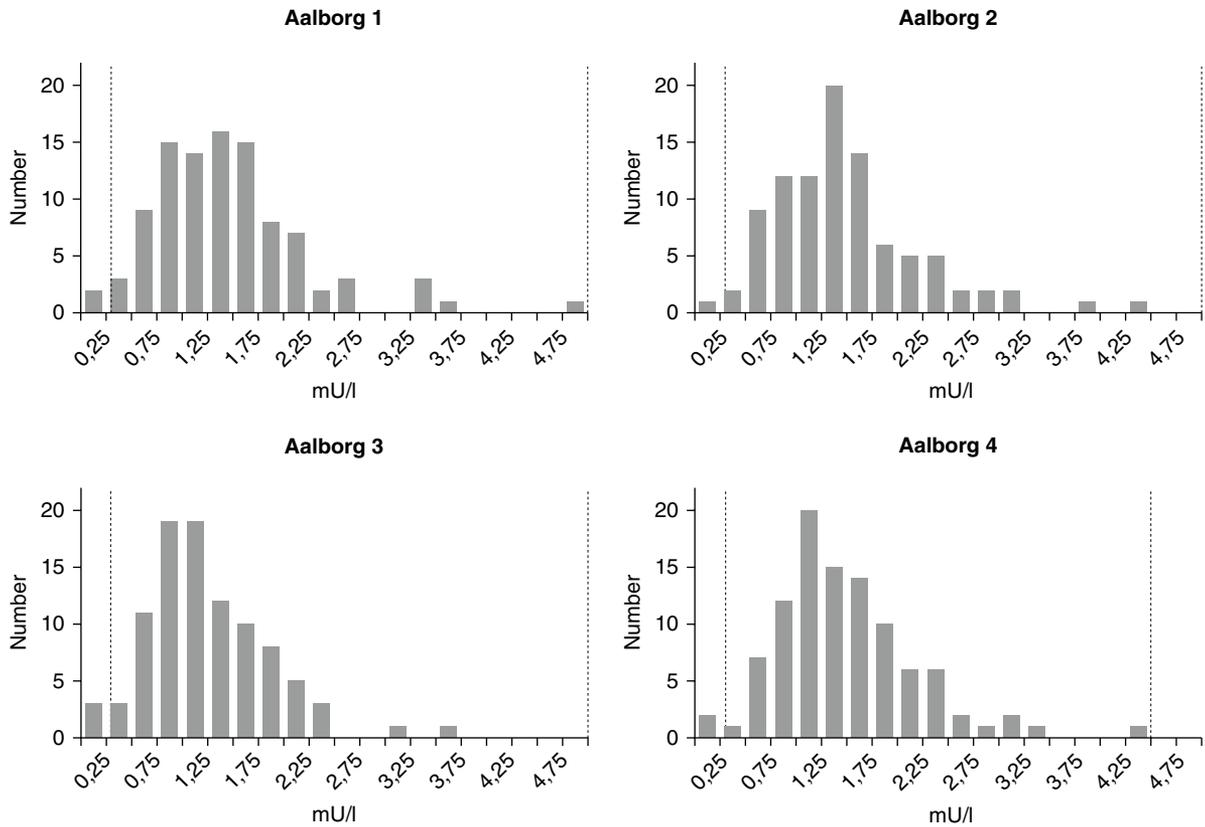


Figure 7.4 The distribution of reference panel ($n = 100$) serum TSH analyzed in Aalborg at (1) the start of the study, (2) after 1 year, (3) after 2 years, and (4) after change of method of analysis. No systematic differences were seen.

and it is not possible to change imported data. Alterations in the software are only made by the programmer or, if necessary, another software specialist with knowledge of the construction of the database.

Export of data from the database

Data can be directly imported from the database into Excel or SPSS for further statistical analyses. Data are transferred as the three cross-tables: “Bp,” “Men,” and “Men 2.” When data are exported, the patients’ specific identity numbers are encrypted to comply with Danish laws of data protection.

Information on the diagnostic activity in the areas is important, because this may influence the incidence of diagnosed thyroid dysfunction. Due to the data encryption it is not possible to evaluate whether an increase in the number of blood tests performed is due to more tests in an unaltered number of persons or to more persons being investigated.

Summary Points

- Different types of records and databases can be useful in monitoring the effect of iodine supplementation.
- The tradition for registering populations and diseases within the population, and thereby the records and databases available, differs between countries.
- For thyroid disorders, which are diagnosed and treated at all levels of the healthcare system, some type of record linkage is necessary to be sure that all cases are included.
- In Denmark, a computer-based system for prospective registration of new cases of hyper- and hypothyroidism has been developed.
- The system is linked to diagnostic laboratory databases in the cohort area and based on the tradition that nearly all inhabitants are registered with and consulting one GP, and that all inhabitants have a specific CPR number that is used in all contacts to the hospital.

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- www.sundhedsstyrelsen.dk

Section 2

**General Aspects of Iodine Sources
and Intakes in the Diet, Main Routes
of Iodine Metabolism, and Metabolic
Roles**

Section 2.1

Iodine Cycle and Chemistry

Iodine in the Air: Origin, Transformation, and Exchange to Mammals

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Abstract

As part of the biogeochemical cycle, the injection of iodine-containing gases into the atmosphere, and their subsequent chemical transformation therein, play a crucial role in environmental and health aspects associated with iodine – most importantly, in determining the quantity of the element available to the mammalian diet. This chapter focuses on these processes and the variety of gas- and aerosol-phase species that constitute the terrestrial iodine cycle, through discussion of the origin and measurement of atmospheric iodine in its various forms (“Sources and Measurements of Atmospheric Iodine”), the principal photo-chemical pathways in the gas phase (“Photolysis and Gas-Phase Iodine Chemistry”), and the role of aerosol uptake and chemistry and new particle production (“Aerosol Chemistry and Particle Formation”). Potential health and environmental issues related to atmospheric iodine are also reviewed (“Health and Environment Impacts”), along with discussion of the consequences of the release of radioactive iodine (I-131) into the air from nuclear reactor accidents and weapons tests that have occurred over the past half-century or so (“Radioactive Iodine: Atmospheric Sources and Consequences”).

Abbreviations

BDE	Bond dissociation energy
CCN	Cloud condensation nucleus/nuclei
DMS	Dimethyl sulfide
MBL	Marine boundary layer
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
RGM	Reactive gaseous mercury

Introduction

Iodine is an essential trace element in the endocrine system, necessary for the production of the hormones triiodothyronine (T3) and thyroxine (T4) in the thyroid gland. Mammals thus provide the termination step for the cycling of iodine in the biosphere. This biogeochemical cycle (Figure 8.1) involves processes of oceanic release, sea-air transfer, photochemical transformation, aerosol uptake, and deposition on the land where iodine is adsorbed onto the soil and vegetation (Fuge and Johnson, 1986).

The primary source of iodine is marine flora, with microalgae (phytoplankton and cyanobacteria) in open oceans and macroalgae (seaweed) in coastal areas releasing a range of gas-phase iodine-containing organic species, including methyl iodide (CH₃I), diiodomethane (CH₂I₂), and molecular iodine (I₂), to the atmosphere (Vogt, 1999; Carpenter, 2003). The predominant loss of these molecules occurs through photolysis, with subsequent oxidation by ozone (O₃) and reaction with other atmospheric species (e.g., OH and NO₂) in the marine boundary layer

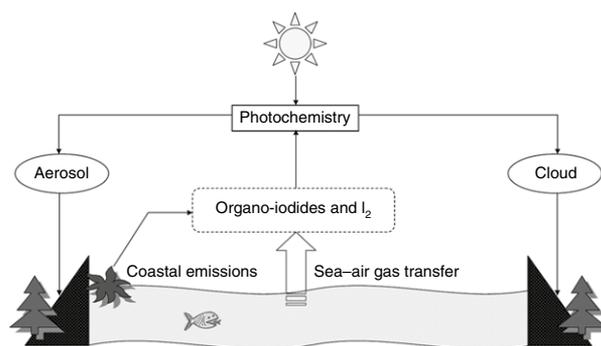


Figure 8.1 Schematic of the production, transfer, and loss processes for atmospheric iodine.

(MBL) leading to the formation of iodine sinks in the form of more stable gas-phase species and aerosol particles (Vogt *et al.*, 1999; McFiggans *et al.*, 2000; O'Dowd and Hoffmann, 2005). There is, therefore, an intricate interplay between physical and chemical atmospheric processes prior to the eventual deposition of iodine on the soil and ultimate ingestion by animals and humans.

Sources and Measurements of Atmospheric Iodine

Despite the first detailed measurements of air-borne iodine dating back to the 1930s (Cauer, 1936, 1939) and observations of iodine enrichment (with respect to chlorine) in rainfall and aerosol (compared with seawater) in the 1960s (Duce *et al.*, 1963), it was only a decade later, with the direct measurement of CH_3I in Atlantic water and air (Lovelock *et al.*, 1973), that details of the origin and speciation of atmospheric iodine began to be resolved. Since then, and particularly in the last decade, there has been extensive research in this area, and the role and potential climatic effects of iodine in the atmosphere have been placed in a more accurate context (Kolb, 2002; von Glasow, 2005). A current estimate of the total emission rate of organo-iodide gases into the atmosphere lies in the range 2–4.5 Tg (10^{12} g) per year (Saiz-Lopez and Plane, 2004a), with CH_3I and CH_2I_2 accounting for > 90% of this atmospheric input. For comparison, the estimated input from the major anthropogenic source of iodine (fossil fuel burning) is some 2–3 orders of magnitude smaller (Vogt, 1999).

A number of organo-iodide species are of biological origin (Gribble, 2003) and have been identified at different locations around the globe – see Vogt (1999) or Carpenter (2003) for a summary of some of these more recent measurements. Measured atmospheric volume mixing ratios e.g., of CH_3I , show an order of magnitude increase at sea-weed-rich coastal locations compared with typical oceanic air values of 1–3 parts per trillion or ppt ($1 \text{ ppt} = 2.5 \times 10^7 \text{ molecules} \cdot \text{cm}^{-3}$). Only very recently, molecular iodine (I_2) was measured in the MBL for the first time, at Mace Head, Ireland (53°N), with peak mixing ratios of 80–90 ppt at evening low-tide periods (Saiz-Lopez and Plane, 2004b) (Figure 8.2). These last two observations, along with reported correlations between measured gas-phase iodine oxide (IO and OIO) levels and rapid aerosol particle nucleation events or “bursts” (O'Dowd and Hoffmann, 2005), have established that the release of these iodine-containing gases into the marine air is initiated by a stress-induced biochemical pathway. For example, at Mace Head, the coastline is rich in the brown kelp, oarweed (*Laminaria digitata*). These accumulate very high concentrations of iodine within their structure (Leblanc *et al.*, 2006) and recent laboratory studies have shown that light-, chemical-, and oxidative-induced stress in such seaweed species results

in the release of gases including CH_2I_2 and I_2 (Palmer *et al.*, 2005). Saiz-Lopez and Plane (2004b) estimated an annual flux for I_2 of coastal origin of 0.5 Tg, assuming that 20% of the Earth's total coastline ($1.6 \times 10^6 \text{ km}$) is habitable for these iodine-rich seaweed types.

In terms of the open ocean, while algal sources are most likely for the organo-iodides, a number of alternative processes have been suggested for volatilization of I_2 from seawater to the air. These include (i) the reduction of iodide ions (I^-) by UV photo-oxidation in the presence of O_2 , originally proposed by Cauer (1939) and verified experimentally by Miyake and Tsunogai (1963); (ii) oxidation of I^- by O_3 (Garland and Curtis, 1981); and (iii) decomposition of iodine-rich organics at the ocean surface and on the surface of sea salt particles, generated and passed into the air via wind-driven wave breaking (Moyers and Duce, 1972; Seto and Duce, 1972). Although estimates were made in the cited studies for likely global annual production rates of iodine from such mechanisms, (i) 0.4 Tg/year and (ii) 0.1 Tg/year, no concerted effort has been undertaken to more accurately characterize these processes and quantify the resultant gas emissions. Consequently, even though these sources are potentially far greater than the localized coastal emissions, due to the large sea surface area available, the contribution of the open ocean to the atmospheric iodine burden remains an unresolved issue.

Photolysis and Gas-Phase Iodine Chemistry

Sunlight ($\lambda > 290 \text{ nm}$) penetrating down to the troposphere initiates the photo-oxidation of iodine-containing gases. Table 8.1 lists some of the precursor species along with key parameters associated with the photolysis process, which releases a reactive iodine (I) species in each case. The threshold wavelength (λ_T) corresponds to the minimum photon energy required to cause bond dissociation in the respective molecule, while λ_{max} is the wavelength at which the absorption cross-section has its highest value (σ_{max}). Note that, although the organo-iodides have very similar bond dissociation energies (BDE), because the λ_{max} values fall below the actinic cut-off wavelength of 290 nm for CH_2ICl , CH_2IBr , and CH_3I , these molecules have comparatively small photolysis rates/long lifetimes in the lower atmosphere.

Figure 8.3 illustrates this point and shows the variation in the cross-section values for these molecules compared with a representative MBL solar (actinic) flux variation (values corresponding to cloud-free sky, overhead sun conditions – see Saiz-Lopez *et al.*, 2004) with wavelength.

Clearly, the overlap between the flux and the cross-section profiles is greatest for CH_2I_2 and I_2 , which consequently have the shortest lifetimes of the iodine-containing gases emitted from the oceans (~2 min and 10 s, respectively).

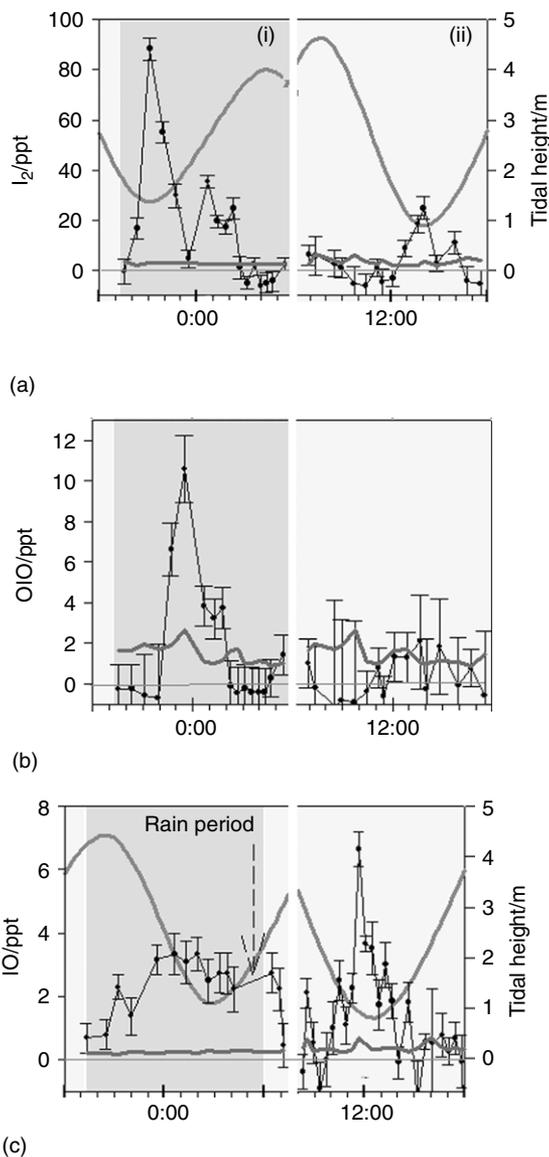
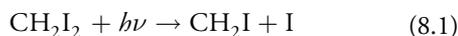


Figure 8.2 Mixing ratio profiles of (a) I_2 , (b) OIO, and (c) IO measured at Mace Head, Ireland, during August 2002. The instrumental detection limit and the tidal variation are represented by solid light and dark gray lines, respectively. Daytime and nighttime measurement periods are plotted as light and dark gray backgrounds, respectively (adapted from Saiz-Lopez and Plane, 2004b).



In the lower atmosphere, iodine destroys ozone via the reactions:

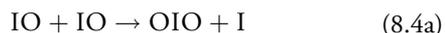
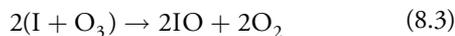


Table 8.1 Molecular photolysis data

	λ_{7a} (nm)	BDE ^b (kJ · mol ⁻¹)	$\lambda_{max}c$ (nm)	$(10^{20}) \sigma_{max}^d$ cm ² · molecule ⁻¹
CH ₃ I	541	239	< 290	112
CH ₂ I ₂	547	219	290	381
CH ₂ ClI	551	217	< 290	122
CH ₂ BrI	559	214	< 290	567
I ₂	792	151	525	285

Notes: Key parameters for consideration of the photolysis of iodine-containing gases in the air (data taken from the International Union of Pure and Applied Chemistry (IUPAC) Subcommittee for Gas Kinetic Data Evaluation online database: <http://www.iupac-kinetic.ch.cam.ac.uk/>).

a Photo-dissociation threshold wavelength.

b Bond dissociation energy (298 K).

c Wavelength of maximum absorption.

d Peak molecular absorption cross-section (298 K).

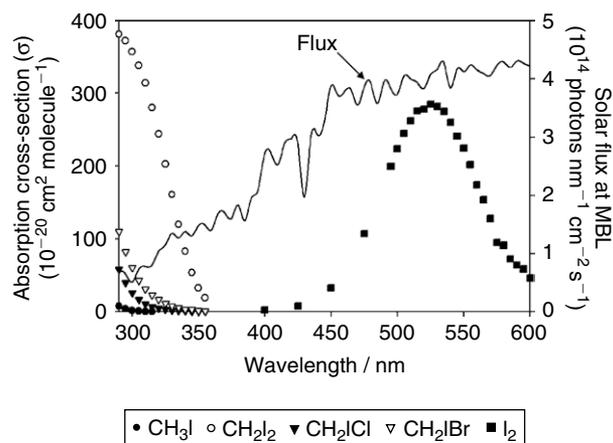
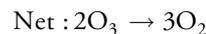
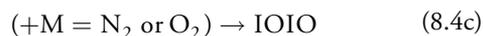


Figure 8.3 Plot of molecular absorption cross-sections for the primary iodine-containing gases detected in the atmosphere, showing the relative overlap with the solar actinic flux in the MBL.

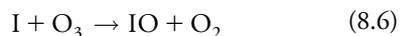
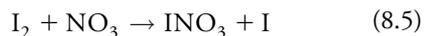


The iodine monoxide (IO) self-reaction is relatively fast and yields mainly iodine dioxide (OIO) or I_2O_2 at the range of pressures in the lower atmosphere. The IO dimer formed via (8.4c) is likely to undergo photolysis, be taken up onto existing aerosol surfaces or take part in nucleation processes leading to the formation of new particles in coastal marine environments (O'Dowd and Hoffmann, 2002).

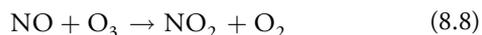
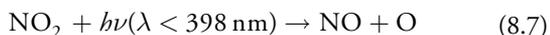
Figure 8.2 shows an example of the diurnal concentration profiles of I_2 , OIO, and IO measured recently at the mid-latitude coastal marine location of Mace Head,

Ireland (Saiz-Lopez and Plane, 2004b). The correlation in the data with the tidal cycle is evident and gives strong support for a biological origin for the gases, as discussed previously.

The reaction of I_2 with the nitrate radical (NO_3) during nighttime provides a nonphotolytic pathway to the formation of IO, and hence OIO (Saiz-Lopez and Plane, 2004b; Saiz-Lopez *et al.*, 2006b).



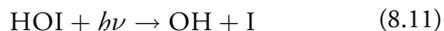
Iodine can have an effect on the oxidizing capacity of the atmosphere via modulation of the ratios of NO_2/NO and HO_2/OH . The ratio of NO_2 to NO in the atmosphere is controlled principally by the following reactions:



In the presence of significant iodine concentrations, the ratio is shifted toward NO_2 :



However, the HO_2/OH balance will be shifted toward OH:



Iodine can also participate in the removal of gaseous elemental mercury (Hg^0) from the atmosphere. Hg^0 , a toxic pollutant of the environment, is emitted primarily from coal combustion (Lindberg and Stratton, 1998) and has an atmospheric lifetime of about 1 year, except in polar regions where reaction with halogen species converts it to reactive gaseous mercury or RGM (Hg^{2+}) on a timescale of only a few hours (Calvert and Lindberg, 2004a). RGM will then deposit and accumulate in the snowpack as a bioavailable species, hence providing a pathway for the input of mercury to the Arctic biota (Brooks *et al.*, 2006).

Over oceanic regions, iodine (in the form of IO) may oxidize dimethyl sulfide or DMS (Chatfield and Crutzen, 1990), a product of marine algae and the primary biogenic source of atmospheric SO_2 (and subsequently H_2SO_4), and hence be a possible route to the formation of cloud condensation nuclei or CCN (see "Aerosol Chemistry and Particle Formation" for further discussion). Although recent laboratory work indicates that this is unlikely to be significant over the open ocean (Gravestock *et al.*, 2005), the higher measured iodine levels in the air at coastal sites may be conducive to this reaction pathway.

Aerosol Chemistry and Particle Formation

The production of the gas-phase iodine oxides IO and OIO, as described in the previous section, initiates a number of possible processes that determine the ultimate speciation of iodine prior to deposition back on the land. These include (i) further gas-phase reactions, followed by (ii) uptake of the products onto existing background aerosol, primarily sea salt particles (Vogt *et al.*, 1999), and (iii) resultant chemical cycling in the aerosol phase and activation of other halogens, followed by the release of the interhalogen species IBr and ICl, or (iv) further oxidation with O_3 to form higher oxides such as I_2O_4 and I_2O_5 (Saunders and Plane, 2005), leading to (v) the homogeneous nucleation of new particles in the atmosphere (O'Dowd and Hoffmann, 2005), and growth through condensation of other species or via Brownian collision-coagulation. Pathways (i–iii) are illustrated in Figure 8.4, which shows the overlap of iodine chemistry with that of chlorine and bromine in both gas and aerosol phases, and gives an idea of the complexity of the chemical transformation pathways for iodine while in the atmosphere.

In marine aerosol, iodine exists mainly in the inorganic forms, iodate (IO_3^-) and iodide (I^-) (Baker *et al.*, 2001), although recently the presence of soluble organic iodine, formed through the reaction of hypoiodous acid (HOI) and organic matter, has also been identified in aerosol samples collected from air above the Atlantic Ocean (Baker, 2005).

A more recently studied phenomenon is that of new particle formation, reported at coastal sites possessing abundant iodine-rich seaweeds (O'Dowd *et al.*, 2002). The episodic production of very high concentrations ($>10^5 \text{ cm}^{-3}$) of ultrafine aerosol over comparatively short periods of time (a few hours) has been shown to be correlated with daytime, low-tide conditions at these locations. This is consistent with the scenario of the photooxidation of iodine-containing gases, emitted by exposed marine flora. Subsequent laboratory and modeling studies have indicated that I_2 and CH_2I_2 are the dominant precursor gases in this process, due to their relatively rapid photolysis in the MBL (O'Dowd and Hoffmann, 2002; McFiggans *et al.*, 2004), and that the resulting particles are composed of polymeric iodine oxide structures consistent with either I_2O_4 (Jimenez *et al.*, 2003) or I_2O_5 (Saunders and Plane, 2005) composition and possess nonspherical, noncompact structures with densities significantly lower than the respective bulk material value. Theoretical calculations (Saunders and Plane, 2005) indicate that the formation of these two iodine oxides in the gas phase is thermodynamically favored by a sequential oxidation route featuring more transient species such as I_2O_2 and I_2O_3 (Figure 8.5).

The precise mechanism responsible for the transition from gas to stable solid phase remains an area of some

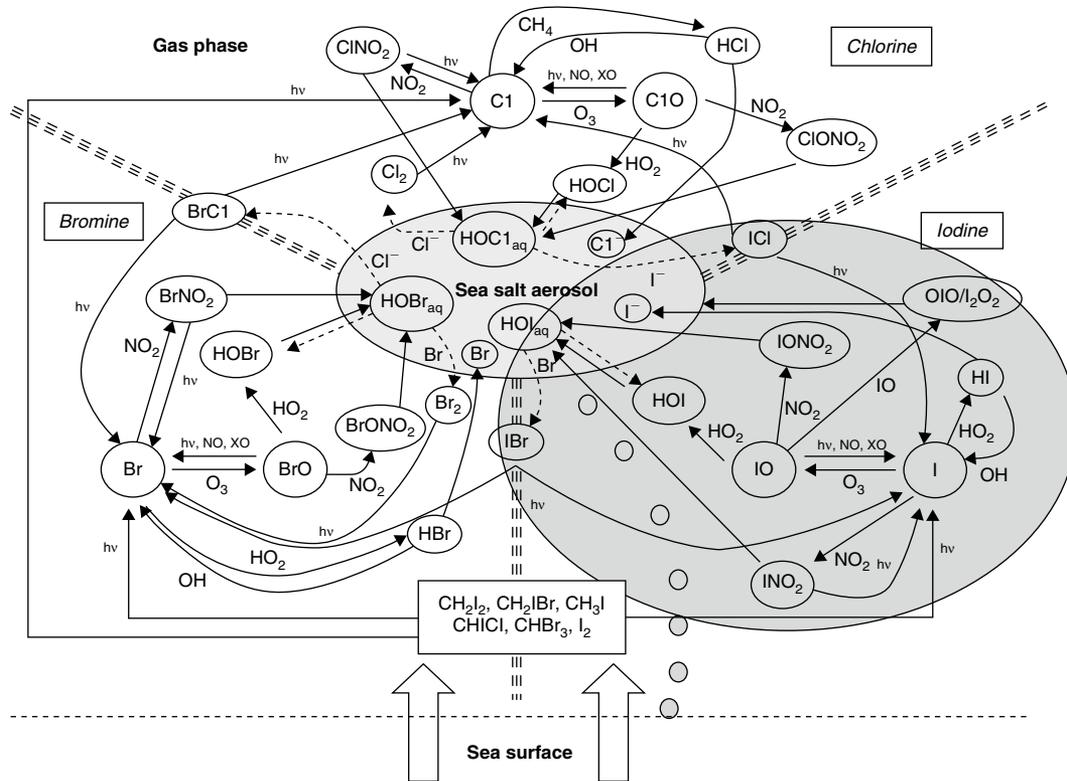


Figure 8.4 Schematic showing the chemical cycling of the gas-phase halogens (iodine highlighted in dark gray) in the atmosphere, and the links to aerosol formation and chemistry (adapted from Saiz-Lopez and Plane, 2004a).

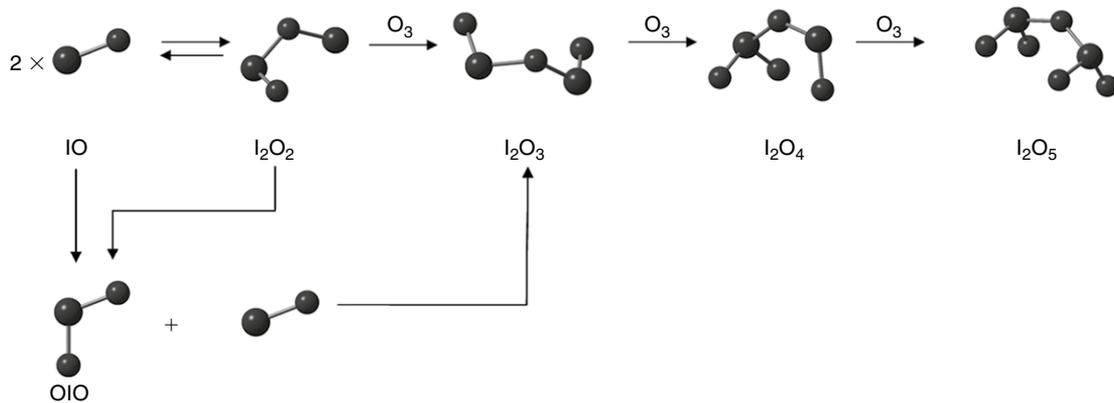


Figure 8.5 “Stick and ball” representations of the lowest energy, most stable molecular structures for gas-phase iodine oxides (iodine atoms are represented by the larger balls).

uncertainty, although laboratory studies of I_2 photo-oxidation indicate that the homogeneous nucleation of either single I_2O_5 molecules or molecular clusters of the same species is a viable pathway (Saunders and Plane, 2006).

The subsequent fate of these particles is uncertain, although continued growth to larger sizes is likely to lead to either deposition or chemical modification through reactive uptake of species, such as water or sulfuric acid vapors. Another possibility is that the particles may grow

to large enough sizes for them to bring about the activation of cloud formation, due to the ability of the particles to act as cloud condensation nuclei or CCN – “seeding” species that can initiate cloud droplet growth. The CCN potential of these particles is likely to be composition-dependent as I_2O_4 is nonhygroscopic while I_2O_5 is hygroscopic, and therefore aerosol composed of the latter species would be more likely to promote water uptake and condensation.

Finally, as with gas emissions to the atmosphere, the potential levels of and impacts from particle formation over open waters are far greater than for coastal locations. However, to date, this phenomenon has been exclusively observed at the latter; therefore, the global significance remains to be assessed.

Health and Environment Impacts

In the 1960s, a series of studies reported the beneficial role of iodine in the atmosphere with regard to issues such as the inhibition of urban photochemical smog production and artificial weather modification. *Stephens et al.* (1962) showed that addition of elemental iodine dramatically suppressed the formation of organic products, such as aldehydes and peroxyacetyl nitrate (PAN), produced from photochemical reactions between alkenes and NO₂, but concluded that the high concentrations of iodine required (parts per million, ppm) precluded any large-scale practical application for its use in smog reduction. Soon after, *Hamilton et al.* (1963) reported the effective inhibition of O₃ formation by trace amounts of iodine in a controlled smog-filled environment containing a mixture of car exhaust fumes and NO₂ and the resulting health benefits observed in test subjects exposed to the chemical pollutants. This method was suggested as a practical solution to the removal of ozone in supersonic aircraft, and indeed the authors subsequently took out a patent (3,084,024 – US Patent Office) on behalf of the Lockheed Aircraft Corporation.

Schaefer (1966) reported the activation of large numbers of ice nuclei on the addition of trace levels of iodine vapor to car exhaust (containing lead oxide nanoparticles) at temperatures from –3 to –20°C in the laboratory. The formation of lead iodide was concluded to have a “seeding” effect similar to that of silver iodide particles (*Vonnegut*, 1947), which had been used in an attempt to artificially modify cloud properties and enhance precipitation. Consequently this method was proposed as a means to remove harmful aerosol formed in polluted urban areas, and also in artificial weather modification. However, the development of unleaded fuels, for which no similar ice nucleating ability was shown to occur in the presence of iodine (*Hogan*, 1967), provided a better long-term solution to this problem.

Vikis and MacFarlane (1985) reported on reaction rates between I₂ and O₃ and the resultant formation of solid-phase iodine oxide aerosol. Coming from the opposite direction to the earlier work of *Hamilton et al.* (1963), this led the authors to suggest that the addition of O₃ to nuclear reactor environments should be considered as a practical route for the removal of air-borne radioactive iodine species produced as fission by-products (see “Radioactive Iodine: Atmospheric Sources and Consequences”).

Almost certainly however, the most important impact of the presence of iodine in the atmosphere is its potential for depletion of O₃ in the troposphere and lower stratosphere. Results of laboratory studies of the photo-oxidation of I₂ (*Jenkin et al.*, 1985) supported the notion that oxidation of iodine released by photolysis of precursor gases would lead to a catalytic destruction cycle with the net result that two molecules of O₃ are converted to three molecules of O₂. *Davis et al.* (1986) predicted that, for equatorial to mid-latitude regions (0–42°), a total gas-phase iodine mixing ratio (ΣI) of just 1.5 ppt in the upper troposphere would result in a 6% depletion of ozone, increasing to 30% for $\Sigma I = 7$ ppt. It should be noted that their calculations did not include an I₂ source from the oceans in addition to the organo-iodides. A decade later, with improved knowledge of reaction pathways and rate constants, a study by *Saiz-Lopez et al.* (2006a) of coastal, daytime I₂ emissions (average value of 6.5 ppt) at Mace Head, Ireland predicted an O₃ loss rate in the MBL, along a 4.2 km path length from the coast, of 1 part per billion or ppb (10^{–9}) per hour, corresponding to an hourly depletion of ~4%. Modeling of iodine chemistry under a polar scenario (Barrow, Alaska 71°N), which included I₂ as a source, also predicted a significant O₃ depletion contribution in the springtime (*Calvert and Lindberg*, 2004b).

Solomon et al. (1994) implicated iodine in lower stratospheric ozone loss as a result of the rapid vertical transport of precursor gases via convection currents, resulting in photolysis and subsequent chemical transformations at altitudes up to 20 km, and concluded that this route would be 3 orders of magnitude greater than O₃ loss resulting from chlorine chemistry.

Radioactive Iodine: Atmospheric Sources and Consequences

The stable ¹²⁷I isotope constitutes 100% of naturally occurring iodine. However, a number of artificial, radioactive isotopes are formed as by-products of nuclear fission pathways (*Figure 8.6*). Of these, ¹³¹I is the most insidious in the biogeochemical cycle (via its transport in the atmosphere) due to its subsequent assimilation in the thyroid gland and relatively short half life of ~8 days.

Once released into the air, wind dispersal followed by deposition on the soil via rainfall and aerosol uptake and adsorption onto the vegetation (*Chamberlain et al.*, 1960) leads to the primary source of ¹³¹I in humans originating from milk produced by grazing animals. Infants are therefore especially susceptible to the accumulation of this isotope to levels at which carcinoma can occur.

Jenkin et al. (1985) estimated that if radioactive iodine is released primarily as I₂, then during the daytime, 95% will be photolyzed within 1 min of release, and hence deposition during daylight hours is likely to occur in the form of species such as IONO₂ or aerosol, whereas at nighttime,

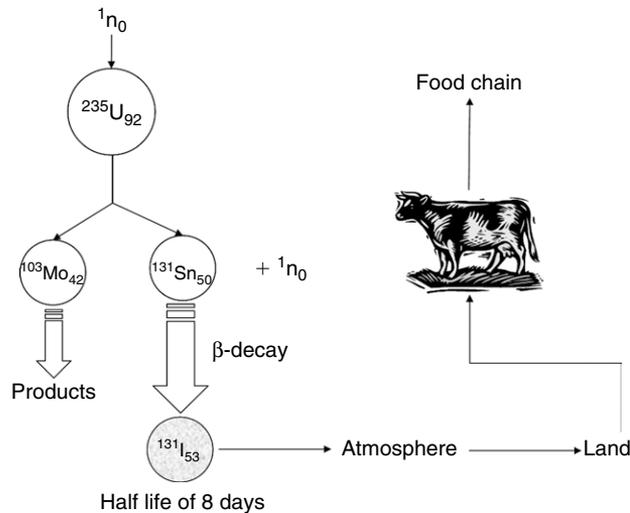


Figure 8.6 Nuclear fission route to the formation of radioactive I-131 and subsequent pathways to its ingestion.

the major deposition channel is more likely to be in the elemental form.

The major atmospheric source of ^{131}I originates from nuclear reactors in which the radioactive by-products are normally contained by the reactor coolant. However, since the first electricity-generating power plant went online in 1954, a handful of accidents resulting in the release of radioisotopes into the air have occurred, and subsequent instances of contaminated milk and cases of increased thyroid cancer have been well-documented. For example, the accident at the Windscale reactor in Cumbria, England, in 1957 is estimated to have released 2×10^4 Curies (Ci) of ^{131}I into the atmosphere (Williams, 2006), equivalent to $\sim 7.5 \times 10^{14}$ disintegrations per second. Approximately 1 year after the accident, measurements of different radioactive isotopes collected in air filters and from grass samples showed that ^{131}I had by far the highest activity (Chamberlain and Dunster, 1958). Wind dispersal carried the fallout along the coastline and further inland, i.e., to Leeds, Yorkshire, where subsequent studies indicated elevated ^{131}I concentrations in milk and in infant thyroids (Burch, 1959).

However, this incident was dwarfed in scale by the meltdown that took place at the Chernobyl reactor in the former Soviet Union or modern-day Ukraine in 1986. Setting Windscale at 1 on an ^{131}I release scale, Chernobyl comes in at ~ 2300 (Williams, 2006), and within a few years reports were made of a marked increase in cases of thyroid cancer in neighboring areas, such as modern-day Belarus (Kazakov *et al.*, 1992). Studies of the aftermath of the incident continue today, although much uncertainty remains as to the long-term environmental and health effects (Baverstock and Williams, 2006).

Even greater than this event, in terms of radioactive iodine release, was the cumulative release (Windscale

$\times 7500$) from the atomic weapons test program conducted in Nevada, USA, from 1951 to 1962. Again, fallout of the isotope was directly linked with radioactivity detected in cattle (Van Middlesworth, 1956), and in later studies, air trajectory calculations of fallout from underground explosions showed how effectively and rapidly air-borne ^{131}I could be transported over large distances prior to deposition (Martell, 1964).

In conclusion, such relatively short-term studies only hint at the likely scale of effects possible following such incidents if fast, remediative action is not taken, and harmful levels of ^{131}I are transported through the atmosphere prior to deposition back on the land and incorporation into the food chain. No further major reactor accidents have taken place since Chernobyl, and nuclear weapon tests are much less common than 40–50 years ago. However, in the light of the current build-up of such weapons in places such as North Korea, Pakistan, and the Middle East, and serious consideration, at least in the UK, of recommencing a program of nuclear reactor construction as a means of combating reliance on fossil fuel burning, lessons from the recent past must be kept in mind.

Summary Points

- The main sources of atmospheric iodine are biogenic, i.e., phytoplankton in the open ocean and certain seaweed species at coastal sites, with some likely contribution from chemical transformation of I_2 in seawater.
- These marine species release organic iodine gases (i.e., CH_3I and CH_2I_2) and molecular iodine (I_2).
- The total flux of these iodine-containing gases from the oceans into the air is $3.0\text{--}5.5 \times 10^{12}$ g/year.
- All of these gases are subject to dissociation in the atmosphere, primarily via photolysis, with I_2 having the shortest lifetime and therefore being the dominant source of reactive iodine, particularly in coastal locations.
- Oxidation of I atoms with ozone (O_3) in the lower atmosphere produces the IO molecule, which is subject to a number of subsequent reaction channels.
- These channels lead to further gas-phase reactions, aerosol uptake, and chemical cycling or particle nucleation, and ultimately deposition on the land via rainfall or aerosol.
- The chemical transformation of iodine overlaps with the chemistry of other halogen species, chlorine and bromine.
- The major impact of atmospheric iodine chemistry is the resultant depletion of O_3 , while other consequences, such as enhanced cloud formation, remain to be established.
- The release of radioactive iodine isotopes, particularly ^{131}I , into the atmosphere, and their subsequent transport and deposition, has conclusively been linked to increases in cases of infant thyroid cancer.

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Iodine and Iodine Species in Seawater: Speciation, Distribution, and Dynamics

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Abstract

It is well known that iodine, which is a micronutrient, is present mainly as iodate (IO_3^-) and iodide (I^-) with minor dissolved (nonvolatile) organic iodine (DOI) in seawater, where the distribution varies with depth and geographical location. On the other hand, the total dissolved iodine concentration is almost constant ($0.4\text{--}0.5\ \mu\text{M}$) in most stations, making the ocean a huge reservoir of iodine. Iodine in seawater is evaporated into the atmosphere as volatile organic iodine, such as methyl iodide (CH_3I) and methylene diiodine (CH_2I_2) and also molecular iodine (I_2), and is transferred and deposited onto soils and into fresh water, and is then incorporated into plants and animals. This is one of the major pathways of the iodine cycle and one of the main routes for iodine to enter into the human food chain. Iodine in seawater is also incorporated into microalgae (phytoplankton) and macroalgae (seaweed). This chapter first describes the distribution of iodine in the hydrosphere, especially seawater. Secondly, the distribution of dissolved iodine species in seawater is discussed from various viewpoints. Thirdly, the factors controlling the speciation of iodine species in seawater are presented with respect to biological and abiological processes.

Abbreviations

DOI Dissolved (nonvolatile) organic iodine
DO Dissolved oxygen

Introduction

Seawater is a saline solution and contains major cations, such as sodium and magnesium ions, and major anions, such as chloride and sulfate ions. **Table 9.1** shows the composition of standard seawater with a salinity of 35‰

(Riley, 1975). The 11 ions, except for iodine, compose more than 99.9% of salinity. Iodine is one of the most abundant micronutrients in seawater, with a total concentration of 5×10^{-5} to $6 \times 10^{-5}\ \text{g}\cdot\text{l}^{-1}$ ($0.4\text{--}0.5\ \mu\text{M}$). Other micronutrients, such as nitrogen and phosphorus and many trace ions, are also contained therein. The three main oceans (i.e., the Atlantic, Indian, and Pacific oceans), including adjacent seas, occupy 70.8% of the earth's surface (total sea area of $360.8 \times 10^6\ \text{km}^2$). Of the sea area, the ratio of the continental shelf (less than 200 m deep), between 200 and 2000 m deep, and over 2000 m deep is 7.6, 8.5, and the remaining 83.9%, respectively. The total volume of seawater is $1.37 \times 10^{18}\ \text{m}^3$ (mean depth 3795 m) (Bowden, 1975). The ocean is thus a huge reservoir of iodine.

Figure 9.1 shows the iodine cycle with the amounts in each sphere and level of transportation (Fuge and Johnson, 1986). Almost iodine in the hydrosphere comes from seawater, because iodine concentration in freshwater

Table 9.1 Composition of seawater with 35‰ salinity

Composition	Concentration ($\text{g} \cdot \text{kg}^{-1}$)	Weight (%)
Cl^-	19.344	55.02
Na^+	10.773	30.64
SO_4^{2-}	2.712	7.71
Mg^{2+}	1.294	3.68
Ca^{2+}	0.412	1.17
K^+	0.399	1.13
HCO_3^-	0.142	0.40
Br^-	0.067	0.19
Sr^{2+}	0.008	0.02
B	0.004	0.01
F^-	0.001	0.003
I	0.00005–6	
Summation	35.156	

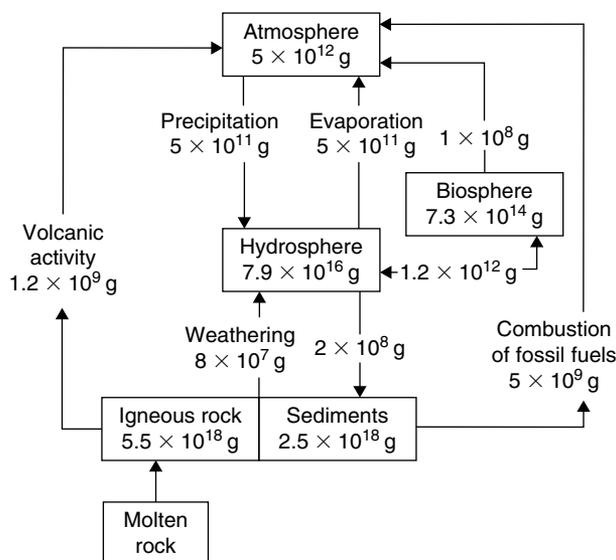


Figure 9.1 The iodine cycle on earth. Reproduced from Fuge and Johnson (1986) and Johnson (1980) with permission.

is very low and the water volume is very small compared to seawater. The amount of iodine in seawater (97.5% of the hydrosphere) is almost the same as that in the hydrosphere.

Although iodine in oceans is only 0.8% of the total iodine on the earth's crust (Muramatsu and Wedepohl, 1998), it enters the human food chain via three important processes: (1) the evaporation of iodine from seawater into the air, its subsequent deposition onto soils and in fresh water, and final incorporation into terrestrial plants and animals; (2) a higher incorporation into microorganisms, seaweeds, and fish, which are used as food and nutritional supplements; and (3) extraction of iodine from pore water that contains no sediments and higher concentrations of iodine and using it for various purposes, e.g., as an additive to edible salt.

Since iodine in these processes is present in various chemical forms, the chemical speciation of iodine is important for understanding its geochemistry in oceans. Further, iodine is redox sensitive and the most abundant biophilic minor element in the oceans. Here, the behaviors of various iodine species in seawater will be described in regard to biological and abiological processes.

Distribution of Iodine Species in Seawater

The distribution of iodine species in seawater will be described with other data, such as temperature and nutrients, to understand the characteristics better. As an example, the data from the Pacific Ocean by Huang *et al.* (2005) will mainly be described.

Vertical profiles of temperature, nutrient, dissolved oxygen (DO), and salinity

Figure 9.2 shows vertical profiles of temperature, nutrient, and DO in the north Pacific Ocean, observed by the Japan Marine Science and Technology Center (JAMSTEC) during the cruise named MR03-K01 from February 20 to March 30 of 2003. The temperature, a key factor in biological activity, decreased with depth regardless of sampling locations, Sta. 11 (41°N, 155°E), Sta. 17 (36°N, 155°E), and Sta. 36 (22°N, 155°E). Generally, the temperature decreased with depth down to about 1.5°C near the bottom, with the most pronounced temperature change between 150 and 600 m. Above 1000 m, a significant temperature decline was observed when going from south (Sta. 36) to north (Sta. 11). On the other hand, below 2400 m, the temperature was between 1.7°C and 1.4°C, exhibiting negligible vertical and latitudinal deviations.

Figure 9.3 shows the vertical distribution of salinity (‰) at three sampling locations. Above 1000 m, salinity varies between 33.8‰ and 35.3‰, whereas no latitudinal change in salinity is seen below 1000 m. Salinity increases with depth; however, below 2400 m the salinity levels (34.7‰) become invariable. Thus, salinities at the three stations are almost similar to the data in Table 9.1. All the concentrations shown were normalized to 35‰.

Distribution of iodate (IO_3^-) and iodide (I^-)

As is clear from Figure 9.4, iodate is the predominant form of dissolved iodine in the north Pacific Ocean and its vertical profiles are fairly similar. The IO_3^- concentrations in water columns were between 0.333 and 0.463 μM (Sta. 11), 0.350 and 0.452 μM (Sta. 17), and 0.218 and 0.442 μM (Sta. 36). Iodate was depleted in surface waters where it was found at the lowest level of 0.218 μM at the lowest latitude (Sta. 36). Below 1000 m, the concentration of iodate increased to 0.430–0.463 μM at Sta. 11, 0.430–0.452 μM at Sta. 17, and 0.430–0.442 μM at Sta. 36, forming a broad maximum between 500 and 2400 m. At greater depths, the iodate content tended to decrease again, reaching ca. 0.410–0.420 μM .

In contrast to iodate, iodide concentrations in surface water were higher (see Figure 9.4), with maximum levels of 0.110, 0.047, and 0.154 μM at Sta. 11, 17, and 36, respectively. Below 200 m, iodide is present at a very low, but still measurable, concentrations (ca. 0.010 μM).

These depth profiles for iodate and iodide are, in general, consistent with early measurements in the same ocean (Nakayama *et al.*, 1989; Ito, 1997). A similar pattern was also reported in the Gulf of Mexico (Schwehr and Santschi, 2003) and the seas around Iceland (Waite *et al.*, 2006), although the iodide concentrations in surface water were relatively lower in the latter case.

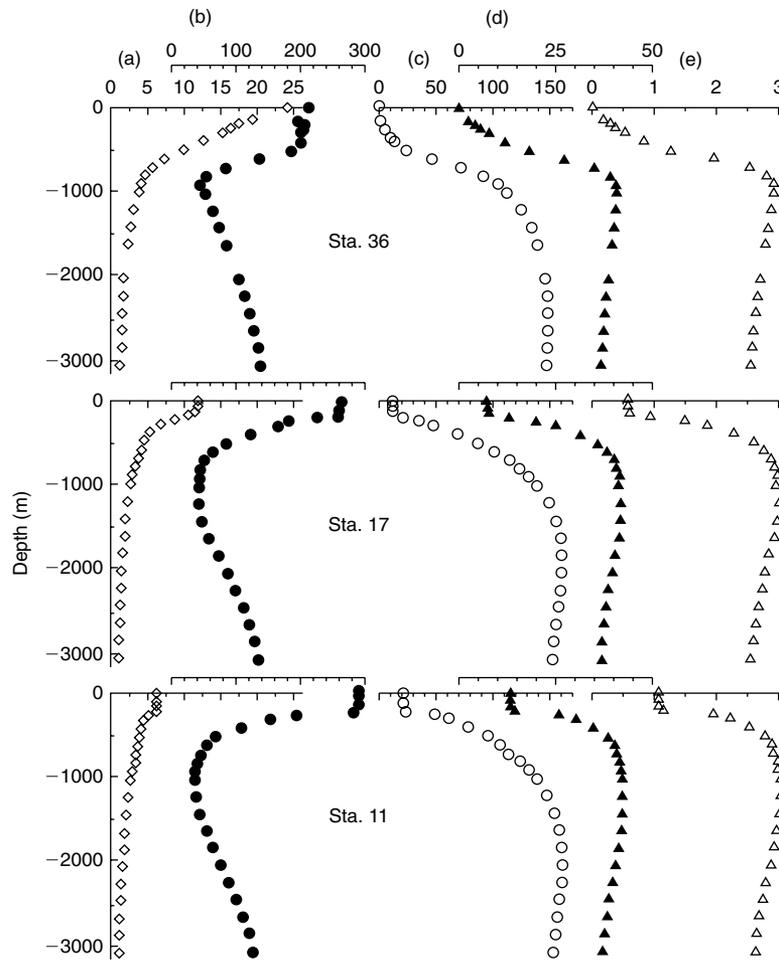


Figure 9.2 Vertical variation of (a) temperature (°C; open diamonds), (b) DO (μM ; solid circles), and (c)–(e) nutrient concentrations (μM) at three stations. Nutrients: (c) silicate (open circles), (d) nitrate (solid triangles), and (e) phosphate (open triangles) at three sampling points, i.e., Sta. 11 (41°N , 155°E), Sta. 17 (36°N , 155°E), and Sta. 36 (22°N , 155°E). Reproduced from Huang *et al.*, (2005).

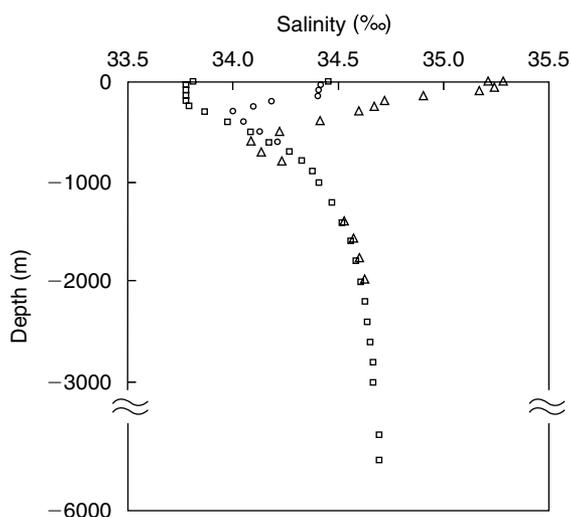


Figure 9.3 Vertical distribution of salinity (‰) at three sampling locations: Sta. 11 (open squares), Sta. 17 (open circles), and Sta. 36 (open triangles). Reproduced from Huang *et al.*, (2005).

A comparison of Figures 9.2 and 9.4 reveals that the depth-dependent distributions of temperature, DO, and nutrient elements at the same latitude resemble those of iodate. Geographically, the iodate vertical profiles are consistent with those of nutrients, whereas the oxygen concentration decreases on increasing the nutrient concentration in the water column. Relationships between iodate and nutrient contents show a significant positive correlation. A similar pattern was also obtained in the South Atlantic water, as is shown in Figure 9.5 (Wong and Brewer, 1974). Iodate concentration varied from $0.39\mu\text{M}$ in the surface seawater to $0.50\mu\text{M}$ in the deep sea, covaried with apparent oxygen utilization, phosphate, and nitrate. This pattern is due to the fact that iodate uptake by phytoplankton leads to a release of dissolved iodine from the cells, presumably in the form of iodide, as described later (Tsunogai and Sase, 1969; Moisan *et al.*, 1994; Wong *et al.*, 2002; Chance *et al.*, 2007). Thus, iodate, like nutrients, can also be removed from the surface waters by phytoplankton or biologically produced particulate matter, the growth

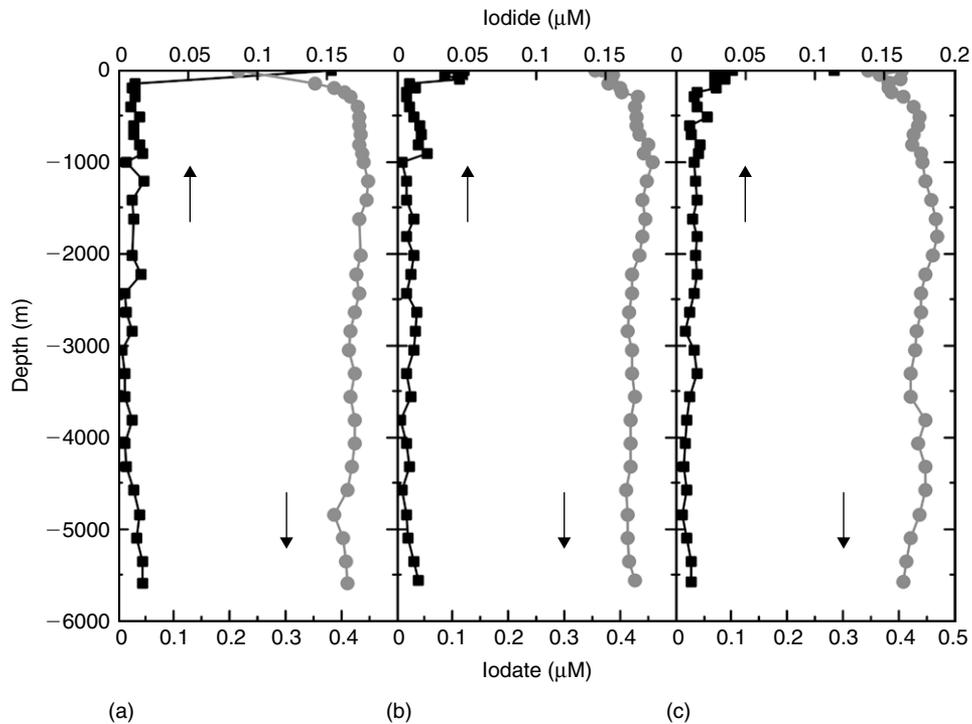


Figure 9.4 Vertical distribution of iodide (μM ; open circles) and iodate (μM ; solid circles) at (a) Sta. 36, (b) Sta. 17, and (c) Sta. 11. Reproduced from Huang *et al.*, (2005).

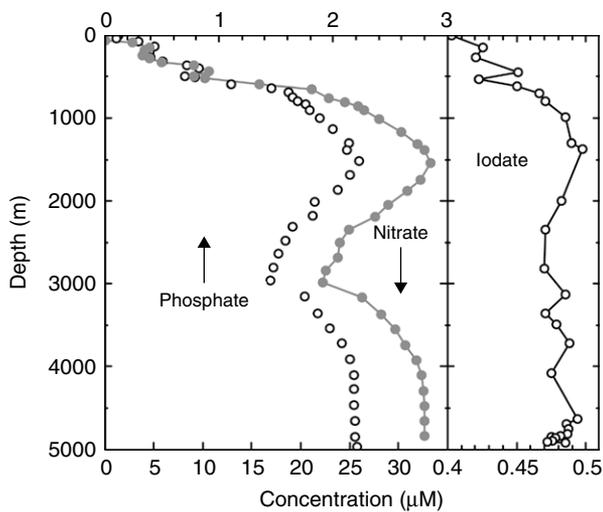


Figure 9.5 Vertical profile of iodate, phosphate, and nitrate at Stn. 61 (36°S , 45°W). Reproduced from Wong and Brewer (1974) with permission.

of which is controlled by the concentrations of essential nutrients in seawater.

Total iodine and dissolved organic iodine

Figure 9.6 shows the vertical variation of dissolved (nonvolatile) organic iodine (DOI) at Sta. 17. DOI was obtained from the difference in TI (total iodine = $\text{I}^- + \text{IO}_3^- + \text{DOI}$) and

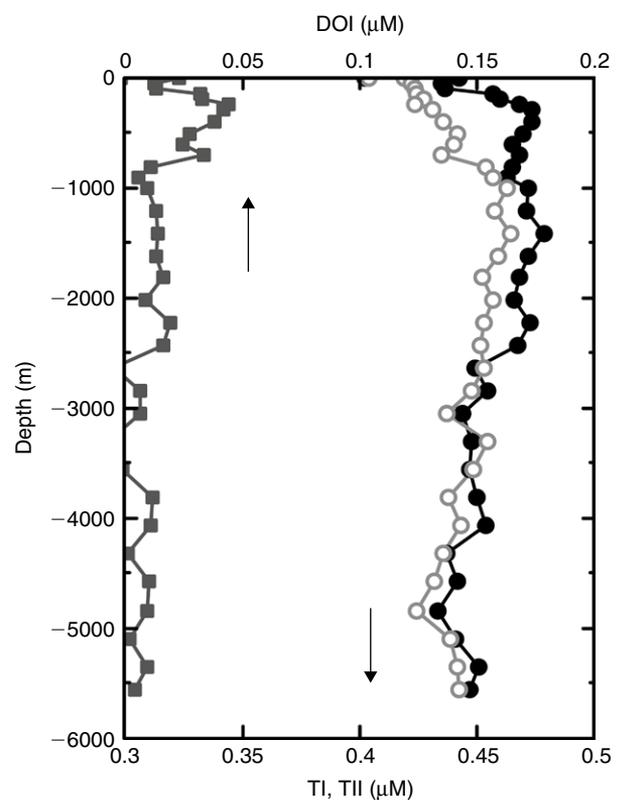


Figure 9.6 Vertical distribution of TI (μM ; solid circles), TII (μM ; open circles), and DOI (μM ; solid squares) at Sta. 17. Reproduced from Huang *et al.*, (2005).

TII (total inorganic iodine = $I^- + IO_3^-$). DOI in the near-surface water reached a maximum of $0.043\ \mu\text{M}$, which is in close proximity to the value of $0.040\ \mu\text{M}$ reported elsewhere (Cook *et al.*, 2000; Truesdale *et al.*, 2001). The DOI concentration was ca. $0.015\ \mu\text{M}$ at mid-depths (800–2400 m). Although there were some differences between TI and TII observed at greater depths, these are about the standard deviation of the method, which is $0.006\ \mu\text{M}$ for DOI. Thus, one cannot conclude with confidence whether DOI is present in deep seawater.

Vertical variations of iodine species

In surface waters, the formation of iodide is directly related to biological activities, but not solar photochemistry and strict chemical redox reaction, because the UV radiation and open oceanic environment are favorable for the oxidation of iodide to iodate. In subsurface waters, the rapid decrease of DO reflects the presence of more oxidizable organic matter, such as humic substances and biological debris. It is conceivable that, being associated with the greater decomposition of biological debris or metabolites from sinking organic matter, DOI attains its maximum concentration of $0.043\ \mu\text{M}$ at 400 m (Figure 9.6).

Below 400 m, the DOI concentration declines quickly and approaches the limit of determination. It is generally recognized that due to lower biological activities at these depths, there is only a slight vertical variation of iodine-like nutrients, and iodate is still predominant as the thermodynamically preferred form in oxic seawater.

Latitudinal variations of inorganic iodine

The concentrations of iodate (Figure 9.4) and nutrients (Figure 9.2) in the euphotic zone increase from south to north. Their levels are lowest in the surface water near the equator (22°N), $0.218\ \mu\text{M}$ for iodate, $0.65\ \mu\text{M}$ for silicate, $0.07\ \mu\text{M}$ for nitrate, and undetectable for phosphate. These values are considerably lower than those found at the north station (41°N), where the same seawater constituents are found at 0.333, 21.60, 13.58, and $1.05\ \mu\text{M}$, respectively. The temperature and iodide concentration (24°C and $0.154\ \mu\text{M}$) in the surface water at the south station are much higher than those at latitude 41°N (6.4°C , and $0.110\ \mu\text{M}$). The latitudinal variations in DO in the euphotic layer result in its increase from 214.3 to $294.4\ \mu\text{M}$ toward the north water, but the surface waters still contain the highest DO concentration (see Figure 9.2). It should be emphasized that such latitudinal variations can contribute to increased biological activity due to a higher temperature and more solar radiation, which are the dominant physical factors controlling the production of phytoplankton by driving the process of photosynthesis in the ocean. The biological activity is significantly

influenced by sunlight, and may thereby enhance the biologically mediated reduction of iodate to iodide. More nutrients and DO may be consumed at lower latitudes, giving rise to greater biological productivity and subsequently to the production of more iodide in surface waters (Waite *et al.*, 2006).

Below 3000 m, no substantial differences in temperature, salinity, DO, iodate, and nutrient concentrations were observed between the three sampling locations. This reflects almost the same biochemical behavior at the uniform temperature at these depths. These findings prove the conservative behavior of nutrients in deep seawater.

Factors Controlling Speciation and Distribution in Seawater

Figure 9.7 shows the iodine cycle in seawater (Isshiki, 2005, partly modified). It is considered that the following iodine species, I^- , IO_3^- , DOI, volatile organic carbons such as CH_3I , I_2 , and particulate iodine are present in seawater, whose species are represented by circles in the figure. Bold solid lines show the change of chemical species, and thin lines the transport of each component. Here, we will describe the factors controlling the speciation and distribution of iodine in seawater.

Chemical control of the dissolved I^- and IO_3^-

Five oxidation states are possible for inorganic iodine in the laboratory: (a) -1 as hydroiodic acid (HI) and iodides (I^-); (b) 0 as molecular iodine (I_2); (c) $+1$ as hypoiodous acid (HIO) and hypoiodites (IO^-); (d) $+5$ as iodic acid (HIO_3) and iodates (IO_3^-); and (e) $+7$ as periodic acid (HIO_4) and periodates (IO_4^-).

In a slightly basic solution (ca. pH 8) such as seawater, both iodate and iodide are present, but iodate is more stable than iodide under oxic conditions (Wong, 1991). However, direct oxidation of iodide to iodate is very slow due to the kinetic barrier, and thus iodide is stable for a long time in seawater once it is formed by biological and/or abiological process. On the other hand, as iodate is thermodynamically stable, the reduction of iodate to iodide does not occur spontaneously.

Biological control of dissolved I^- and IO_3^-

The biophilic nature of iodine is an important factor, but biological processes are not simple.

- i. Most of the dissolved inorganic iodine in seawater is IO_3^- , and its reduction to I^- is readily attained by both bacteria and phytoplankton (Tsunogai and Sase, 1969; Moisan *et al.*, 1994; Wong *et al.*, 2002; Chance *et al.*, 2007) and by the enzyme extracts that are capable of reducing nitrate to nitrite (Tsunogai and Sase,

1975). This compound can be detected by gas chromatography using an electron capture detector (GC-ECD) and mass spectroscopy (GC-MS). Biological methylation is a ubiquitous tendency, and mean CH_3I in the Atlantic Ocean ($135 \times 10^{-12} \text{ ml} - \text{CH}_3\text{I}$ per ml water) is much higher than that ($1.2 \times 10^{-12} \text{ ml}$) in the air (Lovelock *et al.* 1973), suggesting that this compound originated biologically from the oceans. A two-film model of the air-sea interface, together with this observed concentration differential, was used to calculate the total flux of CH_3I from the oceans to the atmosphere (Liss and Slater, 1974). The value ($2.4 \times 10^{11} \text{ g} \cdot \text{I} \cdot \text{yr}^{-1}$) obtained was about half of the value ($5 \times 10^{11} \text{ g} \cdot \text{I} \cdot \text{yr}^{-1}$) achieved by Miyake and Tsunogai (1963) who constructed a global budget for iodine with a balance.

Moore and Groszko (1999) measured CH_3I in air samples and surface and subsurface waters of the Atlantic and Pacific Oceans. It is substantially oversaturated in surface waters, and the estimated CH_3I flux from the ocean to the air was 0.9×10^9 to $2.5 \times 10^9 \text{ mol} \cdot \text{yr}^{-1}$. Figure 9.8 shows the vertical profiles of CH_3I concentration in the Pacific Ocean at three points, together with the latitude and temperature at the depth of each CH_3I maximum. It is considered that the pronounced subsurface maxima in CH_3I was due to production and accumulation of the gas in the poorly ventilated water. The disappearance of maximum at higher temperature was ascribed partly to the temperature-dependent chemical loss and not to the production rate of CH_3I .

Manley and de la Cuesta (1997) reported CH_3I production from 15 species of marine phytoplankton cultures. The CH_3I production largely varied from a low of $10^{-4} \text{ amol CH}_3\text{I cell}^{-1} \cdot \text{day}^{-1}$ to a high of $8 \times 10^{-3} \text{ amol CH}_3\text{I cell}^{-1} \cdot \text{day}^{-1}$. Other possibilities, such as bacterial contribution (production and consumption), were also looked into the experiment. Moore and Zafriou (1994) showed the photochemical production of CH_3I in filtered (through 0.45 or $0.22 \mu\text{m}$ membrane) seawaters by either sunlight or an artificial light source having a spectral output close to sunlight at sea level. The production was enhanced when the water sample was deoxygenated and also when iodide and organic matter were added. Thus, they suggested the interaction between photochemically produced methyl radicals and iodine atoms, and also suggested the importance of their reaction in the global iodine cycle.

In surface seawater, the concentrations of biogenic methylene diiodine (CH_2I_2) are higher than that of CH_3I (Schall and Heumann, 1993). Klick and Abrahamsson (1992) suggested that the annual oceanic global flux of CH_2I_2 would be of the same order of magnitude as CH_3I .

Volatile organic iodines such as CH_3I and CH_2I_2 in seawater undergo a substitution reaction with halides, such as chlorides and bromides, to form I^- (Zafriou, 1975; Jones and Carpenter, 2005).

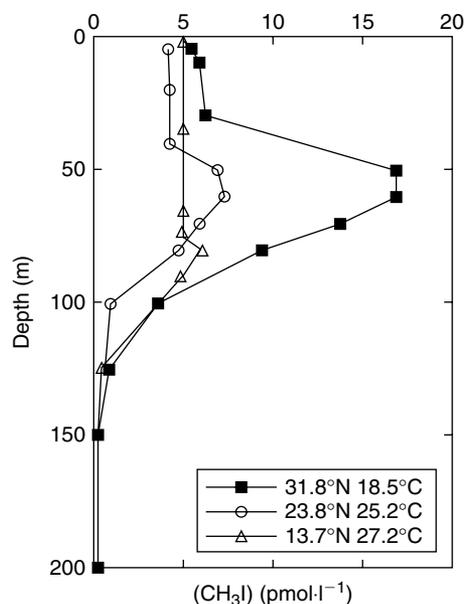
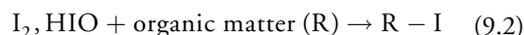
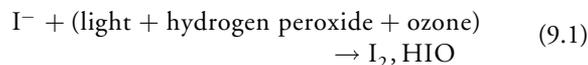


Figure 9.8 Vertical profiles of CH_3I concentration in the Pacific Ocean. Reproduced from Moore and Groszko (1999) with permission.

Dissolved (nonvolatile) organic iodine

Organic iodine in seawater can be divided into (i) volatile compounds and (ii) nonvolatile compounds. Here, DOI will be used to refer to nonvolatile compounds. The presence of DOI in seawater was first described by Truesdale (1975). He observed an increase in the “reactive” iodine when seawater was irradiated with high-intensity UV light. In the 13 samples studied, its concentration ranged from <0.01 to $0.04 \mu\text{M}$. The ratio of DOI in total iodine was from $<1\%$ to 11% .

Detritus of marine organisms and the bacterial decomposition of organic matter may release DOI into the marine environment. Luther *et al.* (1991, 1995) found higher DOI of up to 70% of the total iodine in oxygen-saturated surface waters of higher biological activity. I^- at oxygen saturation may react with naturally occurring reactive transient oxidants, such as hydrogen peroxide (H_2O_2) and ozone (O_3), leading to the formation of I_2 and HIO . These products may then react with organic molecules to form DOI as follows:



Cook *et al.* (2000) suggested from the field data that humic material may be involved in this reaction. Francois (1987) suggested from laboratory experiments that IO_3^- may react with humic material of reducing properties in sediments to form an electrophilic iodine

species, probably I_2 and hypiodous acid (HIO). HIO has a strong electrophilic character and would readily react further with organic matter to form iodinated organic compounds.

On the other hand, Luther *et al.* (1991, 1995) suggested a nucleophilic displacement of iodide by sulfide and the bacterial decomposition of DOI to form I^- in sulfidic and anoxic waters, respectively:



where R^* is an organic compound which has undergone chemical change. Wong and Cheng (2001) reported the photochemical decomposition of DOI in which each mole of DOI was converted to about one mole of I^- without change in IO_3^- and total dissolved iodine. The disappearance of DOI and the formation of I^- were directly proportional to irradiation. They also discussed DOI as the source for the photochemical CH_3I production in seawater observed by Moore and Zafriou (1994), as described above.

Particulate iodine

Particulate iodine is obtained from detritus – nonbiological organisms such as dead organisms and cell debris. Wong *et al.* (1976) determined the dependence of iodine concentration in particulate form on the depth of seawater at 13 stations in the Atlantic Ocean from 75°N to 55°S, and obtained the following features: (i) a typical depth profile of particulate iodine showed the sharp maximum (20–127 ng-I·kg⁻¹-seawater) in the surface waters; (ii) below the euphotic zone, the concentration drops sharply to about 1–2 ng-I·kg⁻¹-seawater and remains approximately constant at greater depths; (iii) the distribution of particulate iodine is similar to that of particulate organic carbon, phosphorous, and nitrogen, suggesting a biogenic origin. These results suggest the production and recycling of particulate iodine in the surface waters. A simple box model of surface and deep waters suggests that 97% of particulate iodine is recycled within the euphotic zone.

Iodine in/near the sediments

Iodine in sediments is supplied from dead organisms and decayed compounds. When detritus in the sediments is decomposed, iodide is liberated under anoxic conditions and diffused in pore water (the concentration is very high). The iodide is partly diffused in seawater, thus higher concentration of iodide was observed in and near the bottom water and the concentration decreased with the distance from the water-sediment interface. The iodide produced on the bottom is very slowly oxidized to iodate in the deep

water during diffusion (Tsunogai, 1971; Price and Calvert, 1977). Smith *et al.* (1990) showed that when oxygen in the bottom water is lower (1 ml·oxygen·l⁻¹) near-bottom waters were enriched with all nutrients (ammonia, nitrate, nitrite, orthophosphate, and reactive silicate) and reduced forms of arsenic and iodine, indicating inputs of those elements from the anoxic sediments to the overlying waters. Lower dissolved iodate and higher dissolved iodide were obtained under oxygen-depleted conditions. Above the water column, a slight thermocline was observed from the variations of temperature and dissolved oxygen. Similar pattern a little higher iodide is also obtained (near bottom waters in Figure 9.4 and Nakayama *et al.*, 1989).

Summary Points

1. Iodine is one of the most abundant micronutrients and most biologically essential micronutrients in seawater.
2. Total dissolved iodine concentration is almost constant at 0.4–0.5 μM in most stations; thus, the ocean is a huge reservoir of iodine.
3. Iodine in seawater exists mainly as IO_3^- and I^- with minor DOI, where the distribution varies with depth and geographical location.
4. Although IO_3^- is most abundant throughout seawater, IO_3^- is converted to I^- by biological processes in surface water and also to I^- in and near the sediments under anoxic conditions.
5. The trace levels of volatile organic iodine, such as CH_3I and CH_2I_2 , are produced in surface seawater by both biological processes and light.
6. I_2 may also be produced in surface water.
7. Volatile organic iodine, such as CH_3I and CH_2I_2 , and I_2 are evaporated into the air, are transferred and deposited onto soils and fresh water accompanying chemical reactions in the air, and are then incorporated into plants and animals.
8. This is one of the main pathways in the iodine cycle through which iodine enters the human food chain.
9. Iodine in seawater is also incorporated into microalgae (phytoplankton) and macroalgae (seaweed).
10. In the future, it is likely that more information about the iodine cycle in seawater will be obtained, together with progress in iodine speciation.

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Geochemical Cycling of Iodine Species in Soils

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Abstract

Iodine is an important element in studies of environmental protection and human health, global-scale hydrologic processes, and nuclear nonproliferation. Biogeochemical cycling of iodine in soils is complex, because iodine occurs in multiple oxidation states and as inorganic and organic species that may be hydrophilic, atmophilic, and biophilic. In this study, we applied new analytical techniques to study the content and speciation of stable iodine in representative surface soils, and sorption and transport behavior of iodine species (iodide, iodate, and 4-iodoaniline) in sediments collected at numerous nuclear facilities in the United States, where anthropogenic ^{129}I from prior nuclear fuel processing activities poses an environmental risk. The surface soil samples were chosen for their geographic locations (e.g., near the ocean or nuclear facilities) and for their differing physico-chemical characteristics (organic matter, texture, etc.). Extracted solutions were analyzed by IC and ICP-MS methods to determine iodine concentrations and to examine iodine speciation (iodide, iodate, and organic iodine). In natural soils, iodine is mostly (nearly 90% of total iodine) present as organic species, while inorganic iodine becomes important (up to 50%) only in sediments with low organic matter. Results from laboratory column studies, aimed at examining transport of different iodine species, showed much greater retardation of 4-iodoaniline than iodide or iodate. Careful attention must be given to potential interconversion among species when interpreting the biogeochemical behavior of iodine in the environment. In addition to speciation, input concentration and residence time effects will influence the biogeochemical cycling of anthropogenic ^{129}I deposited on surface soils.

Abbreviations

CBD Citrate-bicarbonate-dithionite
DOE Department of Energy

HPLC High-performance liquid chromatography
IC Ion chromatography
ICP-MS Inductively coupled plasma-mass spectrometry
NIST National Institute of Standards and Technology
rpm Revolutions per minute
ORR Oak Ridge Reservation
SRM Standard Reference Material
SRS Savannah River Site
TMAH Tetramethyl ammonium hydroxide

Introduction

Iodine is an essential micronutrient in animals and humans, necessary for the production of thyroid hormones and for the proper functioning of the thyroid gland, and deficiencies can lead to severe metabolic disorders. As reported by the World Health Organization in 1999, iodine deficiency is a significant public health problem in 130 countries, with one-third of the world's population estimated to be at risk. Furthermore, because radioactive iodine is concentrated in the human thyroid (VanMiddlesworth *et al.*, 2000), an uncontrolled release of radioactive iodine could constitute a direct threat to human populations.

Iodine has one stable isotope, ^{127}I , and 25 radioactive isotopes that include 10 fission products with very short half-lives ranging from minutes to a few hours. With a very long half-life (1.57×10^7 years), high-abundance fission yield, and presumably high mobility in the environment, ^{129}I has been recognized as one of the most important radionuclides in studies of environmental protection and human health, global-scale hydrologic processes, and nuclear nonproliferation. Nuclear fuel reprocessing facilities constitute the major source of ^{129}I released to the

environment (Table 10.1). Until 1998, a total of 2360 kg of ^{129}I was discharged in the marine environment by two European facilities at La Hague in France and Sellafield in England, an amount that is 50 times the total release from nuclear weapon tests (Raisbeck and Yiou, 1999; Hou *et al.*, 2000; Fréchet and Calmet, 2003). From 1944 through 1972, the plutonium-production operation at the Hanford Site in Washington released about 266 kg of ^{129}I into the air (Raisbeck and Yiou, 1999). In comparison, the operation of production reactors from 1953 to about 1990 at the Savannah River Site (SRS) in South Carolina released about 32 kg of ^{129}I into the air. By comparison, the nuclear accident at Chernobyl released about 1–2 kg ^{129}I (Raisbeck and Yiou, 1999). The proposed geological repository for storing high-level nuclear waste at Yucca Mountain of Nevada will contain as much as 13,300 kg ^{129}I based on the storage of 70,000 tons of nuclear waste.

As the only naturally occurring radioactive isotope of iodine, ^{129}I is produced by cosmic-ray interactions with xenon in the upper atmosphere and by spontaneous fission of uranium-238 in the geosphere (Table 10.1). Anthropogenic inputs of ^{129}I have overwhelmed the natural inventory, increasing the hydrospheric $^{129}\text{I}/^{127}\text{I}$ ratio from 1.5×10^{-12} during the pre-nuclear era to up to 10^{-10} – 10^{-4} (Moran *et al.*, 1998; Fréchet and Calmet, 2003; Kekli *et al.*, 2003). This marked change in the ratio creates an opportunity for the application of anthropogenic ^{129}I as an environmental tracer, originally proposed by Edwards (1962), to examine a variety of processes including ocean circulation, biogeochemical cycling, and regional hydrologic (e.g., atmosphere, surface water, and groundwater) processes (Raisbeck *et al.*, 1995; Schink *et al.*, 1995; Moran *et al.*, 1999; Oktay *et al.*, 2001).

However, the biogeochemical behavior of iodine needs to be well understood before $^{129}\text{I}/^{127}\text{I}$ ratios can be used as an environmental tracer or geochronometer (Santschi and Schwehr, 2004). Iodine “notoriously” exhibits complex biogeochemical behavior, and occurs as various species with their associated hydrophilic, amphiphilic, and biophilic

characteristics (Fuge and Johnson, 1986). With oxidation states ranging from -1 to $+7$, the predominant states in aqueous systems are -1 (iodide, I^-) and $+5$ (iodate, IO_3^-) (Figure 10.1). In reducing environments, aqueous iodine usually occurs as the mobile monovalent anion, I^- . Under more oxidizing conditions, iodine is present as the more reactive IO_3^- , which can lead to retarded transport through interaction with clays and organic matter.

The coexistence of various inorganic and organic iodine species, in different proportions, has been reported in various environments (Liss *et al.*, 1973; Couture and Seitz, 1983; Yuita, 1992, 1994; Yamada *et al.*, 1999; Muramatsu and Ohmono, 1988; Baker *et al.*, 2001). Organically bound iodine can be a significant fraction of total iodine in aqueous systems and in the atmosphere. For example, methyl iodide is an important gaseous form of iodine in the marine atmosphere and in releases from nuclear fuel reprocessing facilities, while dissolved organo-I compounds comprise up to 50% of total iodine in aqueous samples from estuaries, rivers, and rain (Santschi and Schwehr, 2004).

The research objectives of this study are to evaluate the distribution of various iodine species in some soils and sediments in the United States, and to understand the extent and rate of interaction of iodine species (i.e., iodide, iodate, and organoiodine in the form of 4-iodoaniline) with sediment from nuclear facilities in the US Department of Energy (DOE) complex, where major releases of radionuclides, including ^{129}I , have occurred (NRC, 2000). In this study, we infer important aspects of the environmental behavior of ^{129}I , based on the similarities and differences between this radionuclide and stable iodine. Salient insights and future work regarding geochemical cycling of iodine in soils are then summarized.

Table 10.1 Major source of ^{129}I in the environment

Source	^{129}I	
	kg	TBq
Natural hydrosphere	100	0.65
Natural atmosphere	0.0005	0.000003
Atmospheric testing	50	0.32
Chernobyl	1–2	0.01
Savannah River Site	32	0.21
Hanford Reservation	266	1.7
NTS underground nuclear testing	10	0.065
Yucca Mountain repository	13,300	87
Spent fuel reprocessing (Europe)	2360	15

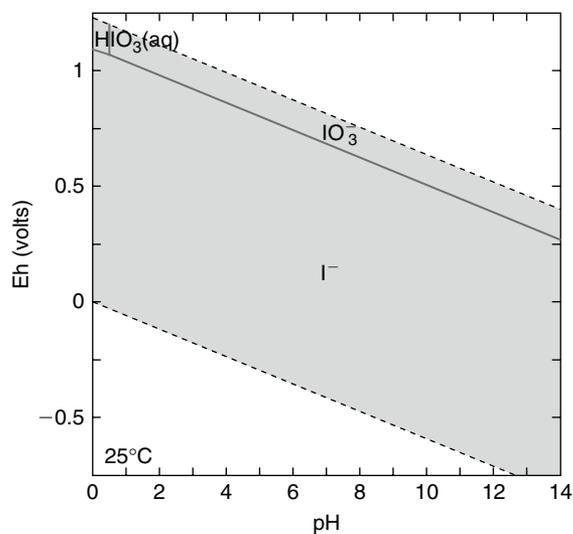


Figure 10.1 Eh-pH diagram, drawn at 25°C and activity of 10^{-11}M , for iodine in a typical $\text{Na}^+\text{K}^+\text{HCO}_3^-$ type water. Diagram produced using the “thermo.com.V8.R6 +” database in the Geochemist’s Workbench (version 6.0).

Materials and Methods

Materials

We obtained a total of 26 soil and sediment samples from across the US (Table 10.2). Among them, 14 surface soils were procured from the North American Proficiency Test Program of the Soil Science Society of America, a repository of various representative US soils used by soil, plant, and water testing laboratories for inter-laboratory sample exchanges and a statistical evaluation of analytical data. These 14 soils were chosen to represent a wide range in texture, physico-chemical properties, and distance from the oceans; factors which may affect the global cycling of iodine. Two standard soil samples from the National Institute of Standards and Technology (NIST), for which total iodine contents have been analyzed by various approaches and research groups, were also obtained for method comparison. In addition, a total of 10 uncontaminated surface and sediment samples were obtained from various nuclear facilities of the US DOE where radionuclide contamination, including from ^{129}I , could be an environmental problem (Table 10.2). These locations also represent a wide range in geologic and climatologic conditions (NRC, 2000). For example, SRS in South Carolina has a humid subtropical climate with annual rainfall of 91–112 cm, and geology representative of the Atlantic Coastal Plain. In contrast, the Hanford Site in Washington is located in an arid region with an average annual rainfall of only 16 cm and a stratigraphy consisting of bedded alluvial plain sediments with sands and gravels. Three SRS sediment samples were collected at different depths, providing an opportunity to examine the iodine content and speciation, as well as sorption and transport of iodine species, in a vertical sediment core.

All sample properties were measured according to standard procedures (Klute, 1986; Sparks, 1996), and are presented in Table 10.2. The wide range in sample types provide an opportunity to examine the correlation of total iodine content with soil properties (e.g., organic matter, clay mineralogy, soil pH, and texture), and the influence of these properties on the transport behavior of iodine species.

Extraction of iodine species from solid samples

Chemical extractions using KCl and/or tetramethyl ammonium hydroxide (TMAH; either sequential or single-step) were conducted to investigate iodine speciation (inorganic and organic) in these soil and sediment samples. Extraction of inorganic iodine from soils was performed by adding 10 ml 0.005 M KCl to approximately 2 g of soil sample. The mixture was shaken for 1 h on a table shaker, and centrifuged at 5000 rpm for 30 min to remove particle sizes larger than 0.45 μm . The supernatant was decanted for analysis and an extraction with 0.005 M

KCl was repeated; both supernatants were analyzed by ion chromatography. This extraction scheme was selected based on an evaluation of the extraction efficiency determined by spiking iodine species into a NIST Standard Reference Material (SRM) 2709 soil (San Joaquin), using three different extractants (deionized water, 0.05 M KCl, and 0.5 M KCl) and different iodine species.

Extraction of iodine contained in organic (humic and fulvic) components of the soils was performed by shaking 2 g soil samples in 20 ml of 5% TMAH, using a table shaker, for 4 h. The mixture was then centrifuged, and the supernatant analyzed using ion chromatography and inductively coupled plasma mass spectrometry (ICP-MS). Using the NIST SRM soils with well-known total iodine contents, we also evaluated extraction variables, such as the temperature (TMAH extraction under either room temperature or 80°C), on quantitative iodine extraction.

Column transport studies of iodine species

Either glass (Kontes in Vineland, NJ; 2.5 cm inner diameter, 15.0 cm long) or stainless steel columns (Alltech in Deerfield, IL; 2.5 cm inner diameter, 7.0 cm long) were incrementally packed with the air-dried soil (<2 mm) to obtain a uniform bulk density. The packed columns were slowly wetted from the bottom with an electrolyte solution of 5 mM CaCl_2 to establish saturation. Approximately 100 column pore volumes of electrolyte solution were pumped through the column prior to the transport study.

The methods employed for the miscible displacement studies were similar to those used in previous experiments (Hu and Brusseau, 1998). We connected a high-performance liquid chromatography (HPLC) pump (Model 301 from Alltech Associates Inc., Deerfield, IL) to the column, and placed a three-way valve in-line to facilitate switching between treatment solutions. Several iodine species (iodide, iodate, and 4-iodoaniline) were used to study transport behavior. We also examined the transport of tritium and bromide, commonly used conservative tracers, so that we could compare their transport behavior with iodine species. For transport experiments of 4-iodoaniline, which is used as a representative refractory organic iodine species, the solution was allowed contact only with glass or stainless steel, to avoid potential interaction of organoiodine with plastics in the column system. Column effluents were collected with an automated fraction collector (Retriever 500, ISCO Inc., Lincoln, NE) for chemical analysis, as described below.

Analyses of iodine species

We used a Dionex Corp. (Sunnyvale, CA) ion chromatography (IC) DX-600 system to analyze I^- and IO_3^- . The system includes a GP50 gradient pump, an ED50A electrochemical detector, and an AS50 autosampler with a thermal compartment for temperature control. To measure low

Table 10.2 Information and physico-chemical properties of samples used in this study^a

Note	Sample name	Location	Texture	pH (1:1 0.01 M CaCl ₂)	OM (%)	CEC (meq/ 100g)	Particle size			Carbon (g/kg)		Aluminum (g/kg)	
							Sand (%)	Silt (%)	Clay (%)	CBD ext.	Oxalate ext.	CBD ext.	Oxalate ext.
Surface soil	Ashdos	Washoe County, NV	Sandy loam	6.20	1.78	16.1	61.0	25.4	13.9	3.92	0.60	0.46	0.71
	Bodenburg	Alaska	Silt loam	4.91	5.86	17.6	34.6	59.9	5.5	6.88	3.86	1.16	1.03
	Brazos	Texas	Sand	<u>5.70^h</u>	0.64	1.5	86.7	10.0	4.0	1.00	0.22	0.20	0.10
	Columbia	Columbia Basin, WA	Silt loam	7.43	1.10	12.5	46.0	43.8	11.0	4.66	1.12	0.36	0.58
	Lakeland	Florida	Sand	<u>5.20</u>	1.40	2.7	92.3	3.2	3.6	0.42	0.33	0.83	0.57
	McKinley	Sanger, Fresno County, CA	Sand	<u>7.05</u>	1.80	6.4	85.2	8.9	5.0	3.16	0.76	0.27	0.19
	Michael	Elba, Winona County, MN	Sandy loam	<u>6.20</u>	1.75	8.0	57.7	31.0	11.3	4.50	1.08	0.55	0.36
	Modesto	Modesto, Stanislaus County, CA	Loamy sand	<u>5.02</u>	0.60	2.9	83.0	12.9	4.0	2.58	0.65	0.34	0.21
	Newark	Delaware	Sandy loam	5.00	1.70	5.2	55.0	32.6	12.0	5.56	0.64	1.21	0.55
	San Joaquin soil ^a	San Joaquin				2.04							
	Montana ^a	Montana				3.40							
	Owngate	Santa Fe, NM	Clay loam	7.50	2.30	29.4	35.0	39.2	25.7	3.82	0.21	0.48	0.37
	Pasco	Pasco, Franklin County, WA	Sand	7.78	0.03	4.8	93.2	5.1	1.7	1.94	0.45	0.17	0.21
	Providence	Clinton, East Feliciana Parish, Louisiana	Silt loam	4.61	1.90	7.0	28.0	58.2	13.2	7.06	1.75	1.33	0.55
	Stockton	Holt, San Joaquin County, CA	Loam	4.52	28.10	74.6	44.5	32.0	23.2				
	Wisconsin	Plover, Portage County, WI	Sandy loam	5.52	8.70	26.3	67.0	21.0	12.0	10.1	5.86	1.42	1.25
DOE facility samples	Hanford sediment ^b	Richland, WA	Sand	7.48	0.06	7.37	89.0	7.9	3.1	3.26	0.94	0.28	0.46
	INL basalt ^c	Idaho Falls, ID	Loamy sand	7.46	0.04	1.67	77.4	21.1	1.5	3.12	12.90	0.15	0.39
	LLNL alluvium ^d	Livermore, CA	Sandy clay loam	7.10	0.03	26.3	42.0	25.6	32.4	9.78	0.94	0.14	0.37
	LLNL aquifer ^d	Livermore, CA	Sandy loam	7.75	0.17	21.1	64.8	22.3	12.9	2.04	7.35	0.38	1.40
	NTS alluvium ^e	Mercury, NV	Sandy loam	7.53	0.06	11.8	69.9	19.7	10.3	7.68	0.08	0.36	0.43
	NTS tuff ^e	Mercury, NV	Sand	7.80	0.14	4.54	89.1	8.8	2.1	1.48	0.12	0.20	0.12
	ORR sediment ^f	Oak Ridge, TN	Sandy loam		0.12	9.41	7.7	70.9	21.4	10.8	1.844	4.60	0.80
	SRS aquifer ^g	Aiken, SC	Sand	5.60	0.01	0.064	97.2	0.9	1.9	7.30	0.04	0.42	0.0081
	SRS subsoil ^g	Aiken, SC	Sandy loam	4.20	0.07	1.78	76.7	3.5	19.8	15.9	0.20	2.54	0.37
	SRS surface soil ^g	Aiken, SC	Sand	3.84	2.33	1.29	87.6	2.9	9.5	2.50	0.32	0.97	0.38

^aSan Joaquin soil is the NIST (National Institute of Standards and Technology) SRM (Standard Reference Material) 2709 which was collected 13–46 cm below the surface of an agricultural field, and ground to <74 μm. Montana soil is the NIST SRM 2712 which was collected from the top 15.2 cm of an agricultural field, and ground to <74 μm. NIST SRMs 2709 and 2711 have a NIST-noncertified (yet, verified from many researchers) total iodine value of 5 and 3 μg/g, respectively. OM: organic matter; CEC: cation exchange capacity; CBD: citrate-bicarbonate-dithionite; oxalate: ammonium oxalate.

^bComposite of core samples 9–12 m below surface in the S-SX tank farm of Hanford Site.

^cCrushed basalt core from Well S-4 at 0.3 m below surface at Box Canyon near Idaho National Laboratory (INL).

^dThe alluvium and aquifer samples were collected at 0.7 and 14.5 m below surface, respectively, at Site 300 of Lawrence Livermore National Laboratory (LLNL).

^eThe alluvium sample was from ~300 m below surface in the U-1a tunnel complex of Nevada Test Site (NTS), and the tuff sample was a crushed tuff collected at ~300 m below surface of Yucca Mountain at NTS.

^fComposite of core samples 2–3 m below surface in Y-12 National Security Complex of Oak Ridge Reservation (ORR).

^gAquifer, subsurface (composite of sediments collected over 3-m span 12 m below surface), and surface soil of Savannah River Site (SRS).

^hUnderlined pH values were measured from 1:2 (soil:solution) of 0.01 M CaCl₂ solution.

(sub-ppb) concentrations of iodide, we used the ED50A in pulsed amperometric mode, with a silver working electrode and a Ag/AgCl reference electrode, after separation using IonPac AG11 and IonPac AS11 columns. Iodide separation was achieved with 50 mM nitric acid eluent under an isocratic flow of 1.5 ml/min. Under this analytical condition, there is no peak response for iodate. Quantitative conversion of iodate to iodide was achieved by adding 0.1 M NaHSO₃ in a volume ratio of 1:10 to the liquid sample, and the solution was mixed by shaking and standing for several minutes to reduce iodate to iodide; the resulting sample was analyzed for iodide by amperometry. We used a 25 μl injection loop size for sample analyses and Dionex PeakNet 6.2 software for system control, data collection, and processing.

Measurement of total iodine or 4-iodoaniline was carried out using a quadrupole ICP-MS system (Hewlett Packard 4500, Agilent Technologies, Palo Alto, CA or X-7 Series ICP-MS, Thermo Electron Corporation, West Palm Beach, FL). The ICP-MS was operated at a forward power of 1400 W with argon flow rates of 16, 1.0, and 0.9 l/min, respectively for plasma, auxiliary gas, and carrier gas flows. The column effluent sample was spiked with 10 μg/l internal standard elements for iodine-127 analysis. A rinse solution of 10% methanol was used between samples to mitigate potential memory effects. For ICP-MS iodine analysis, we tested several internal standards (Y-89, Rh-103, In-115, Tb-159, and Bi-209) to examine potential matrix effects introduced from soil extractants. Rh-103 stood out as having the most consistent response for all samples types, and was therefore used in the iodine data reduction.

Results and Discussion

Analyses of inorganic iodine species by chromatographic amperometry

The method of amperometric detection, after chromatographic separation of ions, provides a fast (with a run time less than 3 min) and sensitive way (with a method detection limit of 0.6 μg/l; comparable to 0.1 μg/l by ICP-MS for total iodine analysis) to determine iodide concentration. Minimal memory effect was observed, as evidenced by a measured apparent iodide concentration of only 0.005% for the first noniodide-containing sample injected immediately after a high-concentration (100 mg/l) iodide standard.

Conversion of iodate to iodide using NaHSO₃ to measure the iodate concentration is shown to be complete (about 100%). **Figure 10.2** presents the example chromatogram showing no response of the amperometric method for iodate, and conversion of iodate to be detected as iodide. In this study, this approach is used for detailed studies of quantifying the most common inorganic iodine species (iodate and iodide).

In addition to providing the insights on speciation of inorganic iodine, the amperometric method is also very useful in elucidating iodate–iodide interconversion. *Hu et al. (2005)* found that a portion of iodate, after being in contact with several soils (Hanford and SRS sediments) and clay minerals (kaolinite, illite, and montmorillonite), was converted to iodide, and this abiotic reduction was probably mediated by structural iron (Fe) present in the clay minerals.

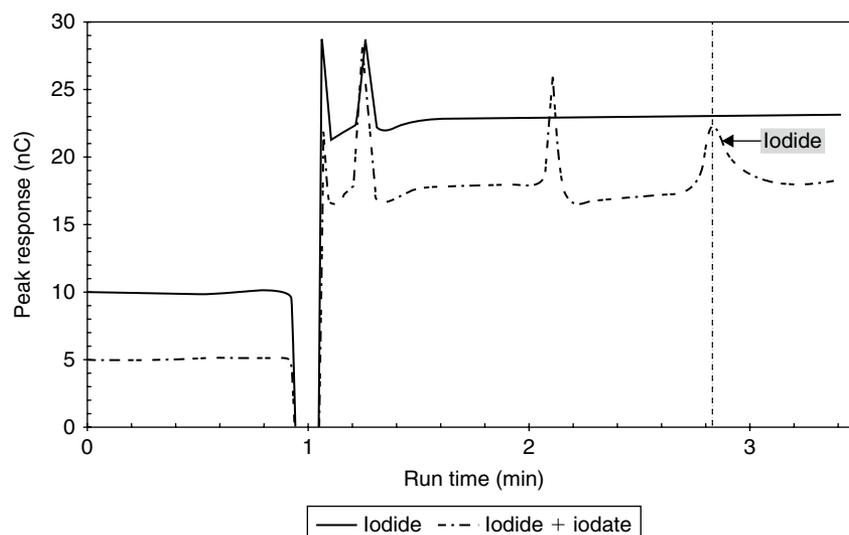


Figure 10.2 Chromatograms for amperometric detection of iodide and/or iodate in a 0.1 ppm iodate sample. We adjusted the position of the baseline in the plot, but the magnitude of the peak signal response in the y-axis has not been altered.

Iodine content and speciation in US soils and sediments

We evaluated the efficiency of KCl solution in extracting the iodine species by spiking a known amount of iodide, iodate, or 4-iodoaniline in NIST SRM 2709, which has a reported total iodine content of 5 µg/g. The first test involved using different concentrations of KCl in extracting iodide spiked at 1 ml of 0.91 mg/l into 2 g of SRM 2709. The spiked soils were mixed and left for 3 days. **Table 10.3** presents the results of different ionic strength solutions in extracting iodide. Most (>60%) iodide was extracted in the first pass of the extraction, though the second extraction still contributed to an appreciable (up to 20%) amount of iodide. Among the three ion-strength tests, the lowest one (deionized water) has the best extraction recovery. Therefore, a concentration of 0.005 M KCl solution, with two passes, was selected to extract soil samples; this concentration was suitable for minimizing disaggregation of clay particles and direct sample measurement of extractants using ICP-MS.

We further assessed the extraction recovery of different iodine species in a KCl extractant (**Table 10.4**). Two concentrations (either 0.091 or 0.91 mg/l) of iodine spike species were used. In the mixed iodine treatment, a mixture of iodide, iodate, and 4-iodoaniline (each with a concentration of either 0.091 or 0.91 mg/l) was spiked into SRM 2709. It appears, for iodide and iodate, the lower spike concentration produced a recovery of >100%; this is likely related to the larger analytical uncertainty at lower

concentration and the contribution of extracted inorganic iodine from the soil itself. This interpretation is supported by mass balance calculations, which include the measured inorganic iodine species in SRM 2709 of about 9%. For treatments with 4-iodoaniline, a higher spike level exhibits higher recovery. Overall, iodine species can be extracted with the KCl extractant at a recovery of >70% (**Table 10.4**). This study served to assess whether newly spiked organic iodine can be extracted by the KCl solution, and whether organically bound iodine species are obtained from TMAH extractions.

Finally, we studied the extraction efficiency using 5% TMAH to measure soil iodine contents from two SRM

Table 10.3 Extraction evaluation of various extractants for iodide in NIST SRM 2709

Extractant	Duplicate	Extraction efficiency (%)			
		Extraction pass	Each pass	Total	Avg. ± std. dev.
Deionized water	A	1st	81.8	97.2	93.4 ± 3.4
		2nd	15.4		
	B	1st	74.4	90.6	
		2nd	16.3		
	C	1st	85.9	92.3	
		2nd	6.34		
0.05 M KCl	A	1st	62.5	77.9	90.9 ± 11.2
		2nd	15.5		
	B	1st	75.1	97.2	
		2nd	22.1		
	C	1st	87.2	97.6	
		2nd	10.4		
0.5 M KCl	A	1st	68.3	80.3	76.2 ± 6.0
		2nd	12.0		
	B	1st	68.1	69.3	
		2nd	1.1		
	C	1st	70.9	79.1	
		2nd	8.27		

Table 10.4 Extraction evaluation of 0.05 M KCl for various iodine species in NIST SRM 2709

Spiked iodine	Duplicate	Extraction pass	Extraction efficiency (%)		
			Each pass	Total	Avg. ± std. dev.
0.091 ppm iodide	A	1st	125	138	108 ± 27.5
		2nd	13.2		
	B	1st	70.6	83.7	
		2nd	73.1		
	C	1st	95.1	104	
		2nd	8.99		
0.91 ppm iodide	A	1st	43.9	63.9	73.0 ± 8.4
		2nd	20.0		
	B	1st	55.3	80.4	
		2nd	25.2		
	C	1st	66.3	74.4	
		2nd	8.14		
0.091 ppm iodate	A	1st	64.2	125	121 ± 5.7
		2nd	60.9		
	B	1st	60.0	117	
		2nd	57.0		
0.91 ppm iodate	A	1st	60.7	89.7	78.9 ± 15.3
		2nd	29.0		
	B	1st	42.9	68.0	
		2nd	25.1		
0.091 ppm 4-iodoaniline	A	1st	63.4	94.4	69.5 ± 10.7
		2nd	30.9		
	B	1st	61.6	94.1	
		2nd	32.5		
0.91 ppm 4-iodoaniline	A	1st	52.4	62.0	78.9 ± 15.3
		2nd	9.56		
	B	1st	64.7	77.1	
		2nd	12.4		
0.091 ppm mixed iodine	A	1st	53.9	61.4	63.0 ± 2.3
		2nd	7.49		
	B	1st	48.1	64.1	
		2nd	16.5		
0.91 ppm mixed iodine	A	1st	69.7	90.8	92.1 ± 1.9
		2nd	21.0		
	B	1st	66.1	93.5	
		2nd	27.3		

soils with much longer contact time and well-known total iodine contents. Iodine extracted with TMAH at room temperature is operationally identified as a labile organic species (after subtracting inorganic iodine), and under elevated temperature (80°C), as total iodine. Tests with SRM soils at elevated temperature produced a complete recovery of $103.9 \pm 5.1\%$ for SRM 2709 and $93.8 \pm 9.4\%$ for SRM 2711, respectively. Note that 5% TMAH solution also extracts the inorganic iodine (Table 10.5). TMAH-extractable iodine is nearly identical to the combined iodine content from KCl extraction followed by TMAH extraction. Alkaline TMAH was first reported by Takaku *et al.* (1995) to be a favorable matrix for iodine analysis by ICP-MS. Using TMAH for quantitative iodine extraction of soil samples was first proposed by Yamada *et al.* (1996), and employed in several studies (Rädlinger and Heumann, 1998; Yamada *et al.*, 1999; Tagami *et al.*, 2006).

The measured total iodine contents in 15 (except for Stockton soil, which is a peat sample with an unusually high concentration of organic matter of 28.1%) US surface soils range between 0.46 and 5.42 mg/kg on a dry weight basis for the soils (Table 10.6). For surface soils (usually sampled to 15 cm depth) on a worldwide basis, the average iodine content is about 5 mg/kg (cf. Whitehead, 1984). The iodine contents in 132 surface soils from widely differing soil types in the UK was found to range from 0.5 to 98.2 mg/kg, with a mean of 9.2 mg/kg. The high iodine values are from soils that are close to the coast in the UK where there is relatively high rainfall, and from areas with a substantial proportion of grassland with high soil organic matter. Muramatsu *et al.* (2004) reported a range of 0.2–150 mg/kg iodine in more than 50 soil samples in Japan; the high iodine concentrations are likely caused by the direct influence of the marine atmosphere, high

rainfall (about 2 m per year), and high adsorption capacity for iodine of the Japanese Andosol soils.

In addition to measurement of the total iodine content, assessment of iodine speciation in soils is a major focus of this study. In the natural soil samples that we analyzed, iodine is mostly (nearly 90% of total iodine) present as organic species, among them appreciable amounts (about 50% for most soils) are nonlabile, i.e., extractable with 5% TMAH under elevated temperature (Table 10.6). In contrast, inorganic iodine becomes important (up to 50%) in sediments with low organic matter, however, organic species are still the dominant form of iodine.

Inorganic labile iodine, extractable from a low ion-strength salt, comprised less than 10% of the total iodine in surface soils. This is consistent with the reports that approximately 80% of 183 UK soils contained less than 10% cold-water extractable iodine (cf. Fuge and Johnson, 1986). For some soil samples, we also evaluated the proportion of iodide and iodate in inorganic iodine (Table 10.7), and it seems that either may be dominant. We are not aware of other reports on the distribution of iodide and iodate in soil iodine. Yamada *et al.* (1999) assumed that iodide is more soluble than iodate, and used the first

Table 10.5 Extraction evaluation of KCl and/or TMAH in NIST SRM 2709

Sample name	TMAH only (mg/kg)	KCl + TMAH (mg/kg)
Ashdos	0.38	0.39
Brazos	0.32	0.30
McKinley	0.18	0.18
Michael	0.74	0.79
Newark	2.17	2.04
Providence	3.84	3.01
NIST SRM 2711	0.65	0.76
NIST SRM 2709	0.96	1.03
Hanford sediment	0.11	0.14
INL basalt	0.02	0.03
LLNL alluvium	0.16	0.12
NTS tuff	0.11	0.13
ORR sediment	1.23	1.15
SRS aquifer	0.08	0.13
SRS subsoil	1.36	1.17
SRS surface soil	0.63	0.74

Table 10.6 Iodine speciation in soils and sediments

Sample name	Total iodine (g/kg)	Inorganic iodine (g/kg)		Labile organic iodine (g/kg)		Nonlabile organic iodine (%)
	(g/kg)	(g/kg)	(%)	(g/kg)	(%)	(%)
Ashdos	0.898	0.045	5.06	0.344	38.3	56.6
Bodenburg	0.702	0.029	4.20	0.343	48.8	47.0
Brazos	0.449	0.036	7.97	0.269	59.8	32.2
Columbia	1.08	0.079	7.36	0.190	17.6	75.0
Lakeland	2.63	0.210	8.00	1.39	52.9	39.1
McKinley	0.460	0.026	5.59	0.155	33.7	60.7
Michael	1.32	0.080	6.07	0.710	53.8	40.1
Modesto	0.393	0.022	5.60	0.040	10.3	84.1
Newark	2.93	0.132	4.50	1.91	65.2	30.3
Owngate	0.897	0.118	13.2	0.329	36.7	50.1
Pasco	0.477	0.039	8.23	0.439	92.0	-0.20
Providence	5.23	0.166	3.17	2.85	54.5	42.4
Stockton	33.0	0.812	2.46	27.4	83.0	14.5
Wisconsin	1.58	0.027	1.71	1.11	70.3	27.9
San Joaquin soil	5.42	0.478	8.82	0.556	10.3	80.9
Montana	3.01	0.286	9.51	0.473	15.7	74.8
Hanford sediment	0.272	0.031	11.4	0.107	39.3	49.3
INL basalt	0.021	0.012	59.9	0.018	87.0	
LLNL alluvium	0.721	0.006	0.79	0.110	15.2	84.0
NTS tuff	0.029	0.012	42.4	0.016	55.2	2.40
ORR sediment	1.71	0.035	2.04	1.12	65.4	32.6
SRS aquifer	0.172	0.050	29.1	0.077	44.8	26.2
SRS subsoil	2.07	0.088	4.24	1.08	52.0	43.8
SRS surface soil	1.02	0.010	0.98	0.735	71.9	27.1

two repeated extractions with 0.1 M KCl as the iodide content and the subsequent two extractions as the iodate content. They reported that iodide and iodate contributed about equal amounts (about 5 mg/kg) in a red-yellow mountain soil, while iodate (about 6 mg/kg) was the only inorganic iodine found in another Andosol virgin soil.

To assess the correlation of iron and aluminum oxides with iodine content, we used a selective extraction to target the different fractions of Fe and Al in the soil samples. Citrate-bicarbonate-dithionite (CBD) extractable Fe contains crystalline iron oxide minerals, such as hematite, goethite, lepidocrocite, and ferrihydrite, while ammonium oxalate (NH₄-Ox) extraction targets noncrystalline ("free") Fe oxides, including ferrihydrite and ferrihydrite-like minerals (Loeppert and Inskeep, 1996). The CBD and NH₄-Ox extractions also remove some crystalline and noncrystalline aluminum oxide phases, respectively.

We performed regression analyses of various iodine components with different physico-chemical properties of surface soils (Table 10.8). The correlation is highest for organic matter and cation exchange capacity, followed by

CBD-extractable Al, clay content, and CBD-extractable Fe. It is commonly reported that the content of organic matter has a dominant influence on total iodine content (Whitehead, 1973; Fuge and Johnson, 1986). As reported in Fuge and Johnson (1986), among a total of 213 soil samples, it was found that soils rich in organic matter were generally enriched in iodine ($r = 0.57$). Surface soil samples (0–20 cm) showed a good correlation between organic matter and total iodine ($r = 0.70$). The good correlation between iodine and organic matter in the surface soils is likely related to the supply (atmospheric input) of iodine and its retainability. However, in 154 samples collected in successive 10-cm increments from 18 soil profiles in the UK, the total iodine content was closely correlated with oxalate-soluble Al, but not with oxalate-soluble Fe or organic matter (Whitehead, 1978). However, in the five most acidic soils, with pH below 4.8, the iodine content was more closely correlated with Fe than with Al. The lack of correlation of iodine content with organic matter in the soil-profile study was ascribed to the short time scale for organic matter turnover, as these soils were either under grass or cultivated annually. Overall, this study confirms the dominant influence of organic matter, clays, and sesquioxides in retaining iodine in soils.

Table 10.7 Inorganic iodine speciation in some soils

Sample	KCl-extractable iodide		KCl-extractable iodate	
	(mg/kg)	(%)	(mg/kg)	(%)
Bodenburg	0.005	0.71	0.024	3.49
Columbia	0.058	5.4	0.021	1.97
Lakeland	0.103	3.92	0.107	4.07
Modesto	0.022	5.6	0.00	0.00
Owngate	0.030	3.34	0.088	9.8
Pasco	0.014	2.94	0.025	5.29
Wisconsin	0.025	1.58	0.002	0.13
Stockton	0.054	0.16	0.758	2.30

Physico-chemical properties affecting sorption and transport of iodine species

We studied the transport of iodine species, along with tracers of bromide and tritium, in various sediments from both DOE nuclear operation facilities and from the Wisconsin soil, which has a high (8.7%) organic matter content. In all of the samples bromide, which is negatively charged like iodide and iodate, exhibited ideal

Table 10.8 Correlation of iodine species with soil properties

Iodine species	Regression	pH (1:1 DI water)	CEC OM (%)	CEC (meq/100 g)	Particle size			Iron (g/kg)		Aluminum (g/kg)	
					Sand (%)	Silt (%)	Clay (%)	CBD ext.	Oxalate ext.	CBD ext.	Oxalate ext.
Inorganic iodine	Slope	-0.072	0.024	0.009	-0.002	0.001	0.016	-0.003	-0.011	0.050	0.001
	Intercept	0.591	0.068	-0.007	0.285	0.094	-0.034	0.090	0.092	0.044	0.077
	Correlation	-0.428	0.762	0.834	-0.278	0.119	0.555	-0.123	-0.294	0.368	0.004
Labile organic iodine	Slope	18.8	-1.3	-2.4	7.7	1.5	-2.7	0.3	0.7	-0.1	0.4
	Intercept	-2.51	0.93	0.33	-0.08	0.04	0.52	0.12	0.08	1.36	0.63
	Correlation	-0.429	0.946	0.878	-0.257	0.108	0.522	0.382	0.155	0.744	0.257
Nonlabile organic iodine	Slope	4.225	0.686	0.136	2.310	0.385	-0.094	0.338	0.601	0.131	0.451
	Intercept	0.228	-0.022	0.016	0.017	-0.021	-0.035	-0.135	-0.038	-0.336	2.935
	Correlation	-0.510	0.615	0.779	-0.416	0.286	0.562	0.304	0.023	0.573	0.187
Total iodine	Slope	23.6	-0.5	-2.2	10.3	2.0	-2.8	0.7	1.4	0.0	1.0
	Intercept	-3.10	1.09	0.39	-0.11	0.06	0.63	0.18	0.07	2.12	0.95
	Correlation	-0.445	0.931	0.870	-0.283	0.135	0.533	0.345	0.089	0.692	0.230

breakthrough with symmetrical behavior and negligible tailing (examples are shown in **Figures 10.3–10.4**) and conservative (not retarded; with the retardation factor of 1) transport (**Table 10.9**). A slight retardation of tritium was observed in the columns packed with Wisconsin soil and Oak Ridge sediment (**Table 10.9**). Tritium sorption of similar magnitude, postulated to occur from the interaction of tritium with clay lattice hydroxyls via hydroxyl exchange (**Stewart and Baker, 1973**), has been reported in numerous publications.

Iodide sorption onto many types of geologic media has been reported to be extremely small (**Whitehead, 1974; Kaplan et al., 2000**). We also observed very limited sorption of I^- during transport in SRS surface soil, SRS aquifer, and Hanford sediment samples (**Hu et al., 2005**). However,

soil properties play an important role in the sorption of I^- . There was significantly retarded transport of I^- , compared to bromide/tritium, in the SRS subsoil sample. This sample contained an appreciable amount of iron and aluminum oxide minerals (**Table 10.2**), which possesses positively charged surfaces that contribute to anionic I^- sorption. The appreciable number of positively charged surfaces is confirmed by anion exchange capacity (AEC) measurements; the SRS subsoil has a high AEC (4.04 meq/100g sample) – more than 10 times higher than the other two SRS samples. Similarly, sorption of I^- has been observed (**Yoshida et al., 1992, 1998**) in Japanese soils that contain substantial amounts of imogolite, ferrihydrite, and allophane, which create a relatively high number of positively charged surfaces.

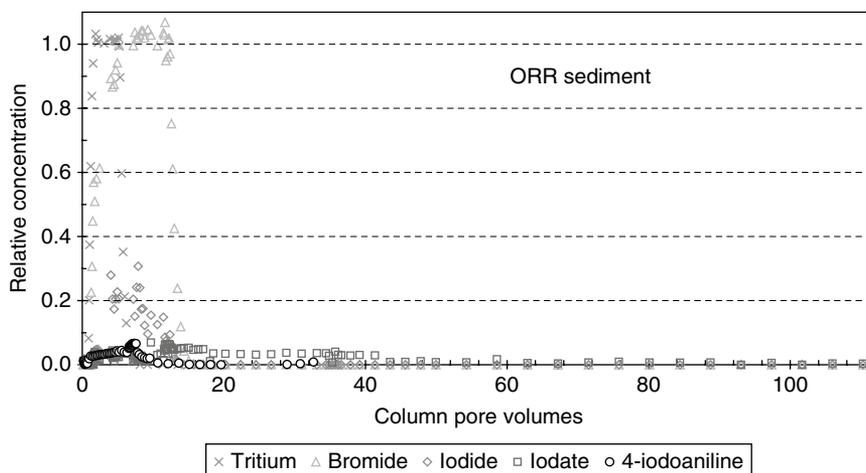


Figure 10.3 Breakthrough curves of tritium and iodine species in a column homogeneously packed with Oak Ridge Reservation (ORR) sediment. The input concentration and pulse, which was varied for different solutes, are presented in **Table 10.9**.

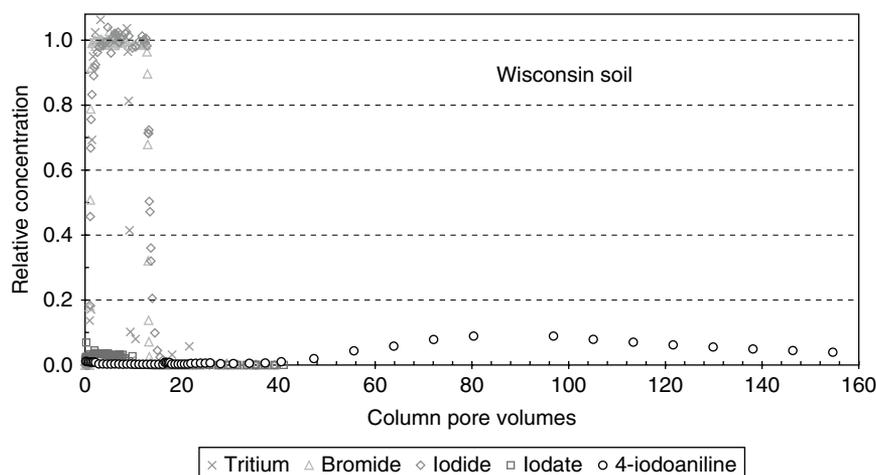


Figure 10.4 Breakthrough curves of tritium and iodine species in a column homogeneously packed with Wisconsin soil. The input concentration and pulse are presented in **Table 10.9**.

Table 10.9 Experimental parameters and mass recovery for the transport of iodine species

Sample	Solute	Input conc.	Input pulse	Mass recovery	R_f
ORR sediment	Tritium	30kBq/l	4.60	1.01	1.17
	Bromide	1×10^{-4} M	11.2	1.00	2.48
	Iodide	1×10^{-4} M	11.2	0.20	>100 ^a
	Iodate	1×10^{-4} M	11.2	0.16	>100 ^a
	4-iodoaniline	4×10^{-5} M	6.61	0.06	>500 ^a
Wisconsin soil	Tritium	30kBq/l	7.87	1.06	1.36
	Bromide	1×10^{-4} M	12.3	0.95	1.05
	Iodide	1×10^{-4} M	12.3	1.00	1.19
	Iodate	1×10^{-4} M	12.3	0.02	>50 ^a
	4-iodoaniline	1×10^{-5} M	16.5	0.44	>90 ^a

^aTo obtain the extent of sorption for solutes with large sorption, an alternate approach, such as a batch sorption study, is more appropriate than the column approach.

Iodate commonly exhibits more retarded transport than iodide because it interacts more strongly with both clays and organic matter (Couture and Seitz, 1983; Ticknor and Cho, 1990; Shepard and Thibault, 1992; Yoshida *et al.*, 1998). As discussed by Kaplan *et al.* (2000), the cause for the difference in I^- and IO_3^- sorptive behavior is not known, but is presumably the result of the harder base nature of IO_3^- , as compared to I^- , which would favor hard-hard interactions with the hard acid sites on the mineral surfaces. The disparate sorption behavior of I^- and IO_3^- was observed in our study, which consistently showed greater sorption for IO_3^- than for I^- . Even in the Hanford sediment, which has a very low AEC, noticeable sorption of IO_3^- took place (Hu *et al.*, 2005). For the Wisconsin soil with high organic matter, sorption of IO_3^- is much stronger than for I^- (Figure 10.4 and Table 10.9).

Compared to I^- and IO_3^- , studies on the transport of organoiodine compounds are much more scarce, given the challenges associated with the analysis of various organoiodine compounds. In this work, we used 4-iodoaniline as a representative nonvolatile organoiodine species. As expected, we found that transport of 4-iodoaniline is quite different from that of the inorganic forms, I^- and IO_3^- , and seems to be related to the amount of organic matter in the sample. This finding is consistent with the hydrophobicity of organoiodine and its affinity for hydrophobic organic matter. In SRS surface soil with 2.33% organic matter, transport of 4-iodoaniline is delayed by more than 20 times that of I^- . In contrast to SRS surface soil results, 4-iodoaniline migrates without retardation, similar to bromide/tritium, in the SRS subsoil, while IO_3^- and I^- are significantly retarded. This is also true for the SRS aquifer material, though the magnitude of sorption for IO_3^- and I^- is less than for SRS subsoil. Both SRS subsoil and aquifer samples have extremely low organic matter content, but high amounts of iron and/or aluminum oxides that exhibit anion-sorption capacity. This example, showing disparate transport behavior for the different iodine species

in three types of samples in a vertical cross-section, highlights the need to consider sediment properties when predicting the geochemical behavior and transport of iodine. Furthermore, inorganic iodine could be fixed onto macromolecular humic substances, as reported by Tikhomirov *et al.* (1980) and Rädlinger and Heumann (2000), and this fixation can change the physico-chemical characteristics and associated fate and transport behavior of iodine.

The retardation factor (R) and mass recovery, calculated by moment analyses of the breakthrough curves for the transport of iodine species in Oak Ridge Reservation (ORR) sediment and Wisconsin soil, are listed in Table 10.9. The effluent mass recovery for all iodine species in ORR sediment is very low (<20%), with the remainder of the released iodine irreversibly retained in the sample components such as iron/aluminum oxides and organic matter; the ORR sediment has a relatively high amount of both of these components. In the Wisconsin soil with a very high amount of organic matter, mass recovery for iodate and 4-iodoaniline, which is more sorptive than iodide, is also very low. In these cases, geochemical cycling of iodine could be very slow, due to the soil/sediment characteristics and iodine speciation.

Other factors affecting iodine cycling

In addition to iodine speciation, the input concentration and contact time have an effect on iodine sorption and transport behavior. Because the sorption of both I^- (especially) and IO_3^- are related to the presence and magnitude of soil components that possess positively charged surfaces, and since positively charged surface sites are limited, the extent of sorption will probably be affected by the input concentration. Positively charged adsorption sites may exist on the edges of 2:1 clays (such as smectite and illite), on Al- and Fe-oxide surfaces, and on 1:1 clays (such as kaolinite). The number of sorption sites in variable-charge minerals is influenced by ionic strength, solution

pH, and counterion valence (Seaman *et al.*, 1996). Using an input concentration of 10^{-5} – 10^{-4} M, Hu *et al.* (2005) studied the sorption and transport of I^- and IO_3^- in SRS and Hanford sediments. The trend of I^- and IO_3^- sorption with respect to the initial concentration is consistent with the limited number of positively charged sites for sorption. Greater I^- sorption at a much lower (10^{-12} M) concentration for three subsurface sediments from the Hanford Site was reported by Kaplan *et al.* (2000).

Ideally, one would use a concentration that is comparable to real-world situations, i.e., 10^{-8} M for stable iodine in the hydrosphere (Fuge and Johnson, 1986), and a few orders-of-magnitude lower for anthropogenic ^{129}I , as observed at SRS (Beals and Hayes, 1995). However, for trace concentrations of I^- , usually introduced as radioactive ^{125}I in laboratory experiments, in a system open to air, the I^- can be oxidized to IO_3^- (Couture and Seitz, 1983; Fuhrmann *et al.*, 1998), which complicates data interpretation considering the different sorption behavior of I^- and IO_3^- . It is not improbable that sorption of iodide, observed in some studies, is actually the result of conversion to IO_3^- and subsequent sorption.

The effect of residence (contact) time also needs to be considered; this is more critical for anthropogenic ^{129}I which has only been participating in iodine cycling over the past 50 years. Because of the longer interaction times (thousands of years) and the higher concentrations of ^{127}I compared to ^{129}I , ^{127}I preferentially occupies the thermodynamically favorable sorption sites. Without newly-generated sorption places, which could lead to irreversible ^{129}I adsorption, virtually no irreversible adsorption and immobilization of ^{129}I can take place (Ernst *et al.*, 2003). The relative portion of reversible binding sites is substantially higher for ^{129}I than for ^{127}I on the time scale of anthropogenic ^{129}I fallout. Therefore, trace ^{129}I possesses a higher mobility than stable ^{127}I .

Alvarado-Quiroz *et al.* (2002) measured total iodine and ^{129}I in Ontario, Canada, where low-level radioactive waste has been stored in trenches above a sandy aquifer and drained into an organic-rich swamp. The results indicated that the K_d distribution coefficients for ^{127}I and ^{129}I were 1.3 and 1.6 l/kg for the sandy aquifer, and 486 and 92.8 l/kg for the swamp sediment. Incremental leaching experiments on the geologic materials produced consistent results showing ^{127}I being more strongly sorbed than ^{129}I . This was postulated to be the result of kinetically controlled sorption mechanisms and the differing residence times for stable vs. radio-iodine in the hydrologic regime.

The input concentration and extent of sorption, as well as sorption kinetics, are seen to be correlated during iodine sorption and transport (Hu *et al.*, 2005). In experiments on iodine transport in SRS surface soils at varying initial concentrations and residence times, iodate sorption was stronger, both at lower concentration and at longer residence time. The first-order desorption rate coefficients

(T^{-1}), k_2 (which specifies the degree of disequilibrium in systems where disequilibrium increases as k_2 decreases), are much higher for organic than inorganic iodines. For the same iodine species, the value of k_2 decreases as input concentration decreases. Overall, anthropogenic ^{129}I , with its lower concentration and short contact time with soils will likely take a prominent role in the geochemical cycle; the presence of different iodine species further compounds this effect.

Summary Points

The following can be summarized regarding the current understanding and suggested future work on geochemical cycling of iodine in soils:

- Amperometric detection of iodide after chromatographic separation has proven a sensitive method to study stable iodine, while accelerator mass spectrometry is the method of choice for analyzing ^{129}I . However, further development of sensitive and specific analytical methods for different iodine species in complex matrices is warranted. Multiple and integrated approaches are needed to tackle complex issues surrounding iodine speciation.
- Various iodine species in different proportions exist in soil and sediments. Organically bound iodine, with limited solubility and mobility, commonly comprises the major proportion of total iodine; it is well-correlated with total organic matter, sesquioxides, and clay content.
- Different iodine species exhibit very different sorption and transport behavior in geologic samples. Sorption of iodate is consistently greater than that of iodide, while the transport of organoiodine (exemplified by 4-iodoaniline in this study) is quite limited, and related to the amount of organic matter in the sample. The physical and chemical processes affecting iodine transport include iodate reduction, irreversible retention or mass loss, and rate-limited and nonlinear sorption of iodine.
- Interconversion of iodine species, both abiotically and biologically, may be a very important process and deserves more attention (Councill *et al.*, 1997; Amachi *et al.*, 2003; Hu *et al.*, 2005).
- Examination of iodine speciation, with due attention to potential interconversion among species, is essential when interpreting the environmental behavior of iodine. Conflicting reports of iodine cycling in the environment could be due to the lack of understanding of iodine speciation and their disparate environmental behavior.
- With a shorter contact time than stable iodine, anthropogenic ^{129}I will likely have a higher mobility when in an inorganic form. However, ^{129}I will experience a similar speciation process as stable iodine and will eventually be retained strongly, with organoiodine as the dominant species.

Acknowledgments

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Transfers of Iodine in the Soil–Plant–Air System: Solid–Liquid Partitioning, Migration, Plant Uptake and Volatilization

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Abstract

Human exposure to soil iodine depends on the partitioning of the iodine into the mobile (liquid and gaseous) soil phases. From the liquid phase, iodine can be transported to surface- and groundwaters, plant roots and, consequently, the human diet. From the gaseous phase, iodine can be transported to the atmosphere where human exposure may occur due to inhalation. The literature suggests that the vast majority of soil iodine is strongly bound by the soil solid phase, and hence is considered immobile. However, partitioning into the liquid and gaseous phases clearly does occur, primarily under anoxic conditions due to the presence of poorly sorbed iodine species. Under such conditions, iodine leaching, plant uptake (for plants with roots that can withstand anoxic conditions, e.g., rice) and volatile emissions to the atmosphere are likely to be increased, increasing the potential for human exposure to iodine.

Abbreviations

E_h	Redox potential
I^-	Iodide
I_2	Elemental Iodine
IAEA	International Atomic Energy Agency
IO_3^-	Iodate
K_d	Soil solid–liquid partitioning coefficient
TF	Soil–plant transfer factor

Introduction

Iodine is an essential trace element for animals, including humans, but is considered nonessential for plants (Brady and Weil, 1996). It is ubiquitous in the biosphere (Sheppard *et al.*, 1994), and is capable of transferring between all three soil phases (solid, liquid and gaseous). Thus, its mobility away from the soil body in the liquid and gaseous phases has important implications in

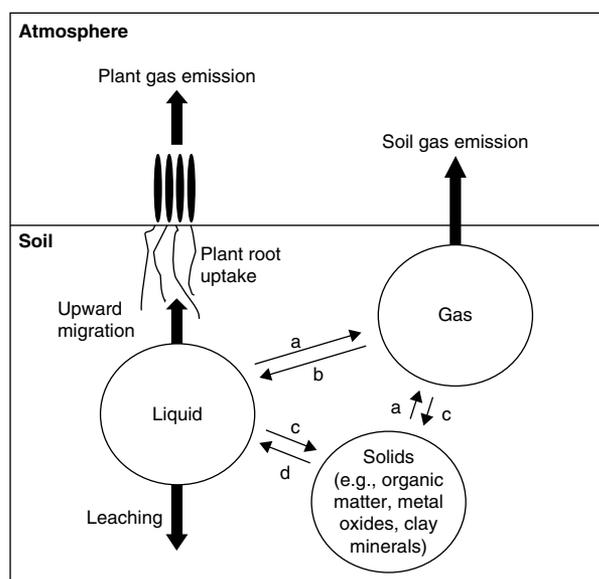


Figure 11.1 Schematic of potential transfers of iodine in the soil–plant–air system. Transfers, illustrated by arrows, between the solid, liquid and gas soil phases are: (a) volatilization; (b) dissolution; (c) adsorption; and (d) desorption and dissolution.

terms of human exposure. Its leaching into surface- and groundwaters, uptake by crop roots and volatilization into the atmosphere are all processes by which human exposure is increased, either through dietary intake or inhalation. The soil–plant–air system processes controlling the behavior of iodine are summarized in [Figure 11.1](#) and discussed in detail in this chapter. [Table 11.1](#) shows important iodine isotopes that are discussed in this chapter.

Iodine in Soils

Sources

The weathering of iodine-containing rock material leads to the enrichment of soils with stable iodine. [Fuge and](#)

Table 11.1 Important iodine isotopes discussed in this chapter

Isotope	Radioactive?	Environmental significance?	Potential sources in soil
¹²⁷ I	No, stable	Yes	Atmospheric deposition (marine-derived) – (Fuge and Johnson, 1986) Rock weathering – (Schmitz and Aumann, 1995; Muramatsu and Wedepohl, 1998)
¹²⁹ I	Yes, 15.7 million year half-life	Yes	Atmospheric deposition (derived from 1960s atomic weapons testing and emissions from nuclear fuel reprocessing and waste solidification facilities) – (Robens and Aumann, 1988) Radioactive waste materials – (Nirex, 2003)
¹²⁵ I	Yes, 60-day half-life	No	Not generally of concern in soils due to short half-life, but serves as very useful surrogate for ¹²⁷ I and ¹²⁹ I in laboratory experiments

Notes: Of the other 21 iodine isotopes, all are radioactive and have half-lives measurable in minutes or days. Consequently, they are of little environmental significance and are rarely used as surrogates for ¹²⁷I or ¹²⁹I in laboratory soil-plant studies.

Johnson (1986) reported average values of 0.24 mg·kg⁻¹ for igneous rock, 5–200 mg·kg⁻¹ for recent sediments, 2.7 mg·kg⁻¹ for carbonates, 2.3 mg·kg⁻¹ for shales, and 0.8 mg·kg⁻¹ for sandstones. Soils become further enriched due to the deposition of marine-derived iodine onto the soil surface. Krupp and Aumann (1999) reported total annual deposition of stable iodine on the surface of German soils as between 2.3 (±0.6) × 10⁻⁴ and 7.8 (±2.2) × 10⁻⁴ g·m⁻² for the year 1994, and between 1.2 (±0.4) × 10⁻³ and 2.2 (±0.6) × 10⁻³ g·m⁻² for the year 1995. They further compared deposition on the soil in relation to distance from the ocean and found that, despite higher iodine loadings in rainfall near the coast, deposition loads on the soil were no greater. They attributed this to the lower rainfall at the coastal sites. For the same 2 years, Schnell and Aumann (1999) reported the iodine concentrations for the top 15 cm of 12 German soils as ranging from 0.4 to 6.5 mg·kg⁻¹. Again, these authors did not observe a relationship between soil concentration and distance from the coast.

Concentrations

Yuita (1994) reported that most Japanese soils (except paddy soils) are rich in iodine due to the wet, mild climate – conditions that are conducive to the deposition of iodine derived from the surrounding ocean. For example, in forest and upland soils of Japan, Yuita and Kihou (2005) noted that iodine concentrations were highest in the oxic soil surface (0–0.3 m) layer, and ranged from 42 to 71 mg·kg⁻¹. They further report that iodine concentrations fell markedly below the surface layer, particularly in the anoxic zone below the water table (at 2.5 m depth) where a concentration of around 0.1 mg·kg⁻¹ was found. This highlights the importance of soil redox chemistry in controlling iodine behavior. In rice paddy soil, the same workers found iodine concentrations of around 12 mg·kg⁻¹ in the oxic soil horizon (0.6–0.9 m depth) due to the accumulation of leached iodine from the anoxic (flooded) surface horizon above. This upper layer was shown to have an iodine content of 2.8 mg·kg⁻¹.

The British Geological Survey soil iodine database (Johnson, 2003) gives a wide range of mean iodine concentrations (0.5–36.9 mg·kg⁻¹) for United Kingdom topsoils sampled from 80 sites between 1973 and 1999. The overall mean value was 8.8 mg·kg⁻¹ (standard deviation 6.9 mg·kg⁻¹). The same database gives an overall mean value for German topsoils of 2.2 mg·kg⁻¹ (standard deviation 1.3 mg·kg⁻¹), which agrees with the values given for such soils by Schnell and Aumann (1999), and those for topsoils in neighboring Austria (Gerzabek *et al.*, 1999). For Japanese topsoils, the database gives an overall mean value of 14.2 mg·kg⁻¹ (standard deviation 16.2 mg·kg⁻¹), agreeing somewhat with the values of Yuita and Kihou (2005). For Russian topsoils, the database gives an overall mean value of 3.8 mg·kg⁻¹ (standard deviation 2.8 mg·kg⁻¹). For the Gandak Basin region of Bihar, India, Ghose *et al.* (2003) reported soil iodine concentrations of 3.65–12.59 mg·kg⁻¹.

Behavior of Iodine in the Soil-Plant-Air System

In common with other elements found in soils, a combination of physical, chemical, and biological processes controls the behavior of iodine. Iodine may distribute (or partition) between the solid, liquid, and gas components of a soil. The extent of this partitioning is critical since it, in turn, determines the environmental outcome of iodine. For example, a high degree of iodine sorption onto the solid phase of a soil suggests that it is likely to be immobile. In contrast, an affinity for the liquid or gaseous phases (low sorption) suggests a significant degree of mobility away from the soil body, e.g., uptake by plant roots, leaching into surface waters, or gaseous diffusion into the atmosphere. Under conditions such as these, human exposure to

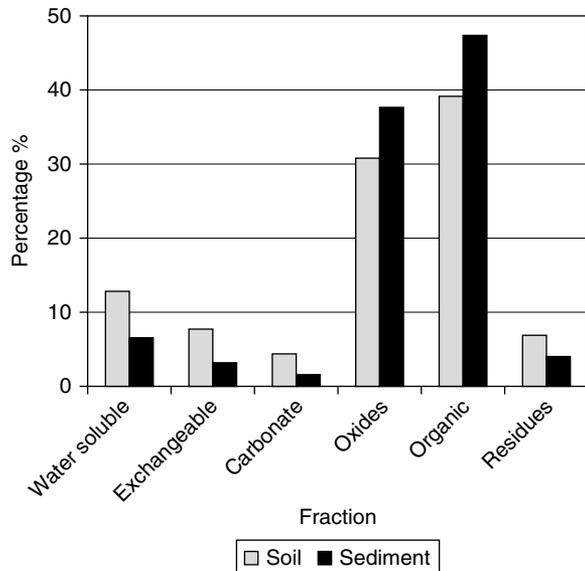


Figure 11.2 Distribution of ^{129}I in different fractions of Chernobyl soil and Irish Sea sediment. From left to right, the fractions generally increase in the strength with which they bind iodine. Source: Hou *et al.*, (2003) reproduced with permission from Elsevier.

iodine is likely to be increased. In this section, literature pertaining to the soil processes controlling iodine sorption and partitioning are reviewed.

Sorption of iodine onto soil solids

A substantial amount of iodine present in a soil is likely to be adsorbed onto the soil solid phase. Hou *et al.* (2003) studied the fate of radioactive ^{129}I deposited on soils from the Chernobyl area and sediments from the Irish Sea, following the Chernobyl nuclear reactor accident. They found that around 75% of the soil ^{129}I , and around 90% of the sediment ^{129}I , was strongly bound to solid phases (metal oxide, organic matter and residual fractions). The data of Hou *et al.* (2003) are summarized in Figure 11.2. Many other workers have also reported the importance of organic matter in binding iodine (Whitehead, 1973; Bors *et al.*, 1988; Yamada *et al.*, 2002; Bostock *et al.*, 2003), believed to be primarily due to the formation of covalent bonds between iodine and humic macromolecules (Mercier *et al.*, 2000; Reiller *et al.*, 2006). However, such sorption may relate only to well-degraded organic material (i.e., humic material) since Muramatsu *et al.* (1996) found that adding nonhumified organic substrates, such as straw and glucose, to the soil did not enhance iodine adsorption. Very much related to the turnover and sorption potential of organic materials in the soil are microbial processes. Studies have shown that a reduction in the microbial biomass of a soil (e.g., by autoclaving) can lead to a sometimes marked reduction in the sorption of iodine (Bunzl and Schimmack, 1998; Bird and Schwartz, 1996; Muramatsu

and Yoshida, 1999). In addition, however, microbes can themselves accumulate, or immobilize, iodine. Ban-nai *et al.* (2006) found that several strains of filamentous fungi could accumulate, in their hyphae, up to 40% of the ^{125}I present in a liquid medium. Inorganic solid phases (clay minerals) have also been shown to adsorb iodine via anion exchange processes and, possibly, covalent bonding. For example, Whitehead (1974) found that freshly precipitated hydrated ferric oxide substantially sorbed iodine at $\text{pH} < 5.5$. Dai *et al.* (2004a) found that adsorption of iodine correlated positively with the amounts of both iron and aluminum oxides in 20 Chinese soils. Similarly, Hakimi (1996) reported that laterite clay materials with varying contents of iron oxides and hydroxides could be used to sorb 90–97% of the ^{125}I in liquid hospital wastes, and could therefore be utilized in the cleanup of such wastes.

Effect of Time on Sorption Importantly, the adsorption processes are also influenced by time. Schmitz and Aumann (1995) used a sequential extraction procedure to compare the partitioning behavior of radioactive ^{129}I (which reached the soil relatively recently via the deposition of emissions from a nuclear fuel reprocessing plant) with that of stable, naturally occurring ^{127}I , and found that only a small fraction of the ^{127}I (2.5–4%) but a large fraction of the ^{129}I (38–49%) was water soluble. Although the influence of time over increasing iodine sorption has been observed through relatively short time periods (Ashworth and Shaw, 2006a), the data of Schmitz and Aumann (1995) suggest that time-dependent sorption continues to occur over extended periods – a process often termed “aging.” This occurs as an element becomes increasingly associated with, and tightly bound by, surfaces such as metal oxides and humic matter, and suggests that the environmental mobility of recently added iodine is likely to be greater due to a lower degree of sorption.

Partitioning of iodine into the liquid phase of the soil

The proportion of total soil iodine partitioning into the liquid phase of a soil is often considered to be low due to high levels of adsorption onto solid-phase components. For example, Johnson (1980) analyzed 183 soil samples from the United Kingdom and found that water-extractable values could be $< 0.1 \text{ mg} \cdot \text{kg}^{-1}$, representing $< 0.1\%$ of total soil iodine. However, the full range of values for this set of soil samples extended to $13.6 \text{ mg} \cdot \text{kg}^{-1}$ in the liquid phase, equivalent to around 25% of total iodine. Similarly, Hou *et al.* (2003) found that readily available (the most environmentally mobile) ^{129}I (water-soluble and exchangeable fractions) accounted for around 20% of total ^{129}I in Chernobyl soil and around 10% of total ^{129}I in Irish Sea sediment (Figure 11.2). Therefore, appreciable quantities of iodine

may partition into the liquid phase. This partitioning is dependent on a number of geochemical processes.

Effects of Reduction–Oxidation (Redox) Potential Yuita *et al.* (2005, 2006) determined concentrations of iodine naturally present in soil solutions of forest, upland, and paddy field soils of Japan. Despite seasonal fluctuations of iodine concentration in precipitation at the various sites, these workers found no seasonal variation in soil solution concentration of the forest and upland soils at any depth. However, at shallow depths in the paddy field soil, flood-irrigation conditions over the summer months induced a marked increase in iodine solution concentration (to around $50 \mu\text{g}\cdot\text{l}^{-1}$) when compared to pre-flood concentration (below $3 \mu\text{g}\cdot\text{l}^{-1}$). This was attributed to the low redox potentials or E_h (~ -150 – 200 mV) brought about by flooding of the soil. Flooding limits the diffusion of atmospheric oxygen into the soil pore space, leading ultimately to a “reduced” (low E_h) soil (Sposito, 1989). This process is known to chemically reduce elements, such as iodine, leading to the production of different iodine species. For example, iodate (IO_3^-) may become chemically reduced to iodide (I^-) as E_h falls. Since different species have different solid–liquid partitioning behavior, the process of soil reduction has important implications for the subsequent mobility of iodine. Other workers have also demonstrated the control of E_h on iodine partitioning behavior. Muramatsu *et al.* (1996) showed that high desorption of radioactive ^{125}I from experimental soil samples occurred when soil E_h fell below around -100 mV. Similarly, Sheppard and Hawkins (1995) found lower soil adsorption of added ^{125}I under anoxic conditions than under oxic conditions, and Muramatsu and Yoshida (1999) noted that the microbially-driven lowering of soil E_h (i.e., inducement of anoxic conditions) produced a desorption of iodine from the soil solids into the liquid phase. Yuita (1994) reported that the iodine dissolution ratio (from soil solids) increased 1000-fold under anoxic conditions when compared to oxic conditions. More specifically, Yuita *et al.* (2005) found a clear negative relationship between the E_h of a paddy field soil and the iodine concentration in soil solution, with concentrations rapidly increasing when E_h fell below around 150 mV in the summer months following irrigation flooding of the soil (Figure 11.3). A similar relationship was observed in laboratory-based experiments by Ashworth and Shaw (2006a).

Iodine Speciation in the Liquid Phase of Soil Yamaguchi *et al.* (2006) further investigated iodine sorption behavior in relation to E_h by determining the speciation of the element in Japanese paddy field soils subject to oxic and anoxic conditions brought about by irrigation management. These workers observed the disappearance of added IO_3^- from anoxic soils as I^- concentrations in soil solution increased, i.e., the transformation from oxic to anoxic

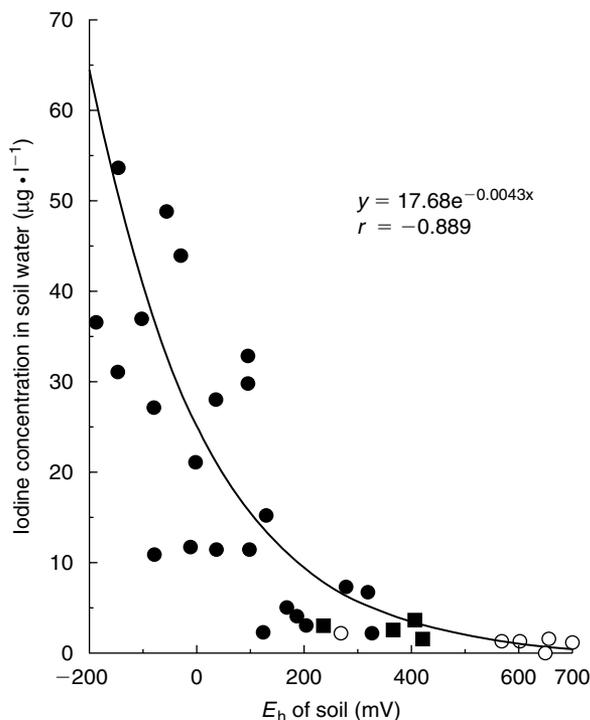


Figure 11.3 Relationship between iodine concentration in soil water and redox potential (E_h) of paddy field soil. Closed round symbols represent flooded conditions during summer irrigation; closed square symbols represent flooded conditions during the winter period; open round symbols represent nonflooded conditions after drainage. Source: Yuita *et al.*, (2005) reproduced with permission from Blackwell Publishing.

iodine species. Out of the two major inorganic iodine species likely to exist in soil solution (IO_3^- and I^-), Fukui *et al.* (1996) reported that the oxic IO_3^- form is likely to become more strongly adsorbed to soil than the anoxic, I^- form. Yamada *et al.* (1996) determined the speciation of iodine in water extracts of soil and concluded that of the three species determinable by their technique (IO_3^- , I^- , and organically bound iodine), the I^- ion was predominant. However, Sheppard *et al.* (1995) found that nonsorbed iodine in both an organic and a carbonated sandy soil was primarily associated with dissolved organic matter. The importance of dissolved organic matter in binding iodine in the soil solution was also reported by Muramatsu *et al.* (1996) and Yamada *et al.* (1996). Yuita (1992) reported that for Japanese soils, the oxic conditions of nonflooded soils led to an iodine speciation distribution in the soil solution that was dominated by IO_3^- (almost 90% of soluble iodine). In contrast, anoxic, flooded conditions were reported to result in a speciation dominated by I^- (again, almost 90% of soluble iodine).

However, some workers have found the speciation of iodine in soil solution to be somewhat more complex,

Table 11.2 Mean concentrations and percentage speciation of iodine in soil solution samples from varying depths of Japanese paddy field soils under both summer and winter flood conditions

Depth of soil sample (m)	Summer irrigation period				Winter irrigation period			
	Total I ($\mu\text{g}\cdot\text{l}^{-1}$)	Species (%)			Total I ($\mu\text{g}\cdot\text{l}^{-1}$)	Species (%)		
		IO_3^-	I^-	I_2		IO_3^-	I^-	I_2
0.2	26.6	47	52	1.0	2.4	75	24	1.0
0.5	30.5	42	58	0.3	1.8	78	21	1.1
1.0	10.6	42	58	0.4	1.7	78	22	0.3
2.0	–	–	–	–	2.8	71	28	0.7

Note: The three iodine (I) species are iodate (IO_3^-), iodide (I^-), and elemental iodine (I_2). Source: Data taken from Yuita *et al.*, (2005) reproduced with permission from Elsevier.

with both IO_3^- and I^- present (in varying proportions) under both oxic and anoxic conditions. Yuita *et al.* (2005) found that the iodine present at relatively low soil solution concentrations (around $1.7\text{--}2.8\mu\text{g}\cdot\text{l}^{-1}$) under oxic conditions was around three-quarters IO_3^- and one-quarter I^- . Under anoxic conditions, iodine concentrations in the soil solution increased to around $10.6\text{--}30.5\mu\text{g}\cdot\text{l}^{-1}$ and the proportion present as the I^- species to just over one-half (52–58%) with much of the remainder as IO_3^- (Table 11.2). In addition to the shift in distribution of the IO_3^- and I^- species due to anoxia, these data illustrate that the concentration of both species increases under anoxic conditions. Thus, in addition to the potential for chemical reduction of IO_3^- to I^- , anoxia apparently induces the release of both species, but particularly I^- , from the solid phase, thereby increasing total iodine concentration in the soil solution. It is possible that this release into the solution phase under anoxic conditions occurs due to geochemical processes that take place within soil as redox status changes. For example, the pH of acidic soils (the soil used by Yuita *et al.* (2005) had a pH of around 6) is known to increase to around neutrality as a result of anoxia (Rowell, 1994). This process, in turn, leads to a decrease in the net, negative, pH-dependent charge associated with soil solids. Thus, sorbed anionic (negatively charged) species may be electrostatically repelled into the solution phase.

Iodine Solid-Liquid Partitioning Coefficients (K_d Values) in Soil A useful approach for determining the partitioning of an element between the solid and the liquid soil phases is the partition coefficient, or K_d value, which is calculated as

$$K_d (\text{l}\cdot\text{kg}^{-1}) = \frac{\text{Dry soil solid phase iodine concentration} (\text{mg}\cdot\text{kg}^{-1})}{\text{Soil liquid phase iodine concentration} (\text{mg}\cdot\text{l}^{-1})}$$

Thus, a high K_d value indicates a high degree of sorption onto the solid phase, and a low K_d value a high propensity

for the liquid phase. Ashworth and Shaw (2006a) used a mini-column approach to compare K_d values of ^{125}I in a sandy loam soil maintained at either nonsaturated or saturated moisture status. Over the first two weeks of the experiment, K_d values were below $11\cdot\text{kg}^{-1}$ for both treatments, indicating a relatively low degree of adsorption onto the soil. After a 49-day period, K_d values in the saturated treatment equilibrated at over $21\cdot\text{kg}^{-1}$, and, in the nonsaturated treatment, at almost $81\cdot\text{kg}^{-1}$. Clearly therefore, the time-dependent sorption of the iodine in the drier soil was mitigated to some extent in the saturated treatment. Using field lysimeter experiments, Sheppard and Motycka (1997) also found lower iodine (^{125}I) K_d values in flooded soil (around $0.61\cdot\text{kg}^{-1}$) than in drained soils (around $61\cdot\text{kg}^{-1}$). Bird and Schwartz (1996) reported average K_d values ranging from 0.1 to $0.51\cdot\text{kg}^{-1}$ for a sandy sediment under oxic conditions and with an equilibration period of 48 h. Fukui *et al.* (1996) found average values of $2.2\text{--}4.01\cdot\text{kg}^{-1}$ for I^- and $4.2\text{--}201\cdot\text{kg}^{-1}$ for IO_3^- for a fine sand using a 14-day equilibration period. Bors *et al.* (1991) found K_d values of between 5 and $551\cdot\text{kg}^{-1}$ for a podzol (84% sand) using an equilibration period of at least 8 days. Compendia values for iodine K_d are also available. For example, in reviewing a range of literature sources, Sheppard *et al.* (2002) reported a geometric mean K_d value for sandy soils of $81\cdot\text{kg}^{-1}$. However, the overall range of literature K_d values found for sandy soils by these workers was large ($0.23\text{--}6951\cdot\text{kg}^{-1}$). IAEA (1994) reported values for a number of different soil types. These range from 1.3×10^{-2} to 8.5×10^1 for sandy soil, 8.2×10^{-2} to 2.4×10^2 for loam soil, 8.2×10^{-2} to 3.3×10^1 for clay soil, and 5.0×10^1 to 1.5×10^3 for organic soil. For each soil type, the iodine K_d values reported by IAEA (1994) are generally low compared to those of the other 32 elements listed in the document, suggesting that iodine may have a relatively high potential for liquid-phase migration through soil and uptake by the plant roots. Additionally, the K_d data suggest that such processes would potentially be most significant under anoxic soil conditions.

Migration of liquid-phase iodine through soil

Field Observations Numerous observations have led to the inference that anoxic conditions induce the migration of iodine through soil due to increased liquid-phase concentrations. Yuita *et al.* (2006) found that drainage of saturated, anoxic paddy field soil led to reduction in iodine concentration of surface soil due to leaching of soluble iodine with the drainage waters. Muramatsu *et al.* (1996) also found that the presence of iodine in soil solution under anoxic conditions led to a leaching of the element from the soil. Paddy soil was therefore found to have lower total iodine concentrations than forest and upland field soils. In agreement, Yuita (1994) concluded that the generally low iodine concentrations observed in Japanese paddy soils are likely to be a result of this dissolution and consequent leaching of iodine from the soil. Yamaguchi *et al.* (2006) further suggested that iodine leached in this way was in the form of the I^- ion.

Effects of Oxic–Anoxic Boundaries Ashworth *et al.* (2003) showed that iodine migration occurs more significant in anoxic, rather than oxic, soil. These workers found that ^{125}I added in the water table migrated to soil columns upward (due to an advective flux caused by evapotranspiration at the soil surface) through the saturated, anoxic soil zone at the base of the columns. Its migration was arrested at the boundary between the anoxic soil and the nonsaturated, oxic soil region above it. These findings were confirmed by Ashworth and Shaw (2006b) in similar experiments, but with a fluctuating water table depth. Under these conditions, the increased extent of saturated, anoxic soil gave rise to a similarly increased extent of ^{125}I migration. A comparison of the migration of ^{125}I for both fixed and fluctuating water tables is shown in Figure 11.4. Similarly, Thomson *et al.* (1995) demonstrated an accumulation of stable iodine occurring immediately above the anoxic/oxic boundary in deep-sea sediment cores. Sheppard *et al.* (1989) found that iodine released at the base of a

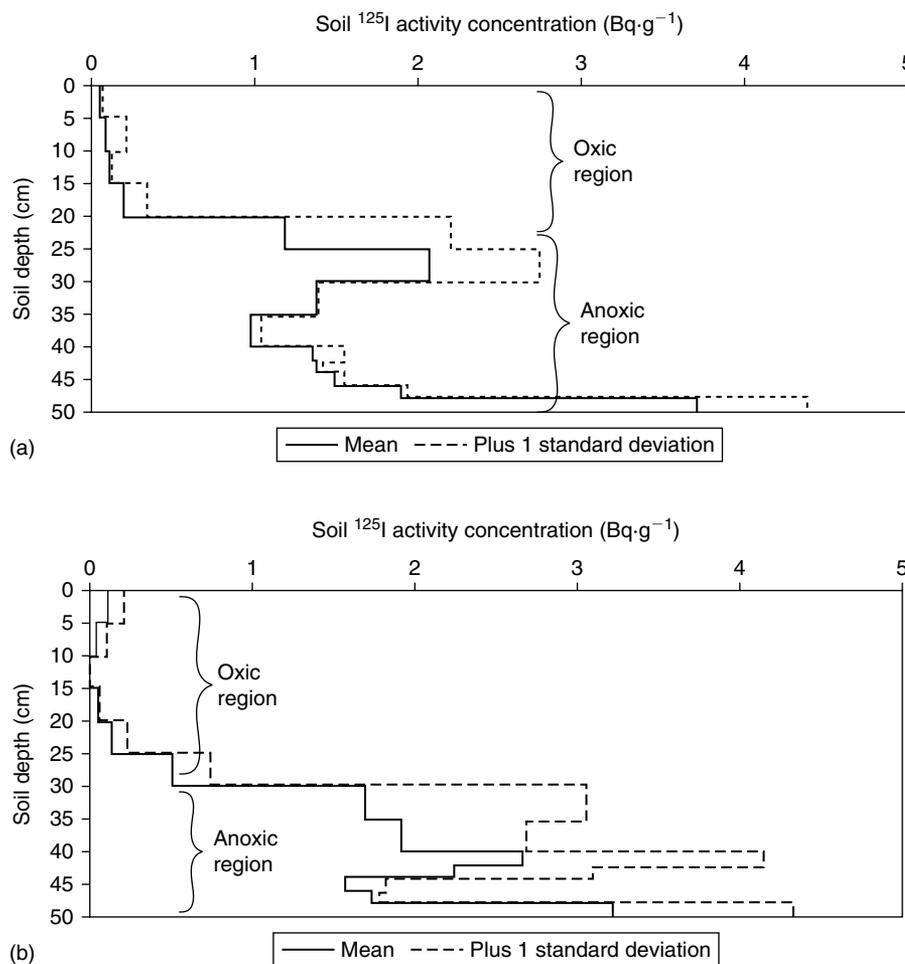


Figure 11.4 Upward soil migration of ^{125}I from a contaminated water table over a 6-month period. (a) The water table height was manipulated, in 0.5 cm increments, from 45 cm depth up to 30 cm depth (over the initial 3 months) and back down to 45 cm depth (over the latter 3 months). (b) The water table remained at 45 cm depth for the duration of the experiment. Source: Ashworth and Shaw (2006b) reproduced with permission from Elsevier.

Canadian peat bog migrated vertically over a distance of 1 m within a month and subsequently migrated no further. They also found that iodine K_d values generally decreased with depth. Their results imply that soil redox status may have played an important role in governing the extent of iodine migration. However, E_h was not reported in their paper.

Migration in Oxic Soils Overall, literature data regarding the migration of iodine suggest that the presence of anoxic conditions promotes elevated iodine concentration in the soil solution from where the element can migrate with the water flux. It is further evident that a boundary between anoxic and oxic soil (e.g., between groundwater-saturated soil at depth and surface soil above) represents a barrier to iodine migration. Accumulations of iodine observed in such regions suggest that iodine migration in oxic soil is very limited. This is confirmed by the findings of Kashparov *et al.* (2005) who undertook field studies to determine the downward migration of ^{125}I applied to the arable layer of a range of Russian soils (Figure 11.5). Under the oxic soil conditions of their experiments, less than 4% of the total applied iodine had leached down to below the 20 cm arable layer over a 269-day period. They attributed this to the high degree of adsorption of the iodine onto solid soil surfaces in the oxic, upper layer. Nevertheless, Ashworth *et al.* (2003) and Ashworth and Shaw (2006b) did note the upward movement of small amounts of iodine through the oxic soil zone in their experiments, indicating that even the relatively low amounts of iodine present in the liquid phase of oxic soils (Yuita *et al.*, 2006) can also lead to migration (Figure 11.4).

Overall, iodine clearly has the potential to migrate through soils in the liquid phase. However, it is likely that

appreciable migration only occurs under anoxic conditions, indicating that iodine deposited onto the soil surface (e.g., aerially deposited) is unlikely to migrate deeper to any great extent. However, iodine potentially present below the water table (e.g., from rock weathering) is likely to exhibit relatively high mobility. It may, therefore, be transported to surface- and groundwaters, potentially increasing its contribution to the human diet through drinking water intake.

Iodine uptake by plants

Plant root uptake of iodine from the liquid phase of soils is an important process, since it is a means by which iodine may subsequently enter food chains and be released to the atmosphere following conversion into volatile forms (see following section). Iodine is not categorized as an essential element for plant growth (Brady and Weil, 1996). Furthermore, at high concentrations in soil, iodine has been shown to adversely affect the growth of pak choi (Dai *et al.*, 2004b), spinach (Dai *et al.*, 2004b; Zhu *et al.*, 2003), and rice (Mackowiak and Grossl, 1999), indicating that its uptake has the potential to cause toxic effects in certain plants (Pel and Schuttelkopf, 1995).

Relationship between Soil Iodine Concentration and Plant Uptake Despite an apparently positive relationship between soil and plant iodine concentrations (Sheppard and Motycka, 1997; Weng *et al.*, 2003; Dai *et al.*, 2006), Yuita (1994) suggested that iodine uptake by plants is generally low. Although this worker found relatively high (compared to other parts of the world) soil iodine concentrations (average $43 \text{ mg} \cdot \text{kg}^{-1}$) across Japan, they also found that plant concentrations were only slightly higher

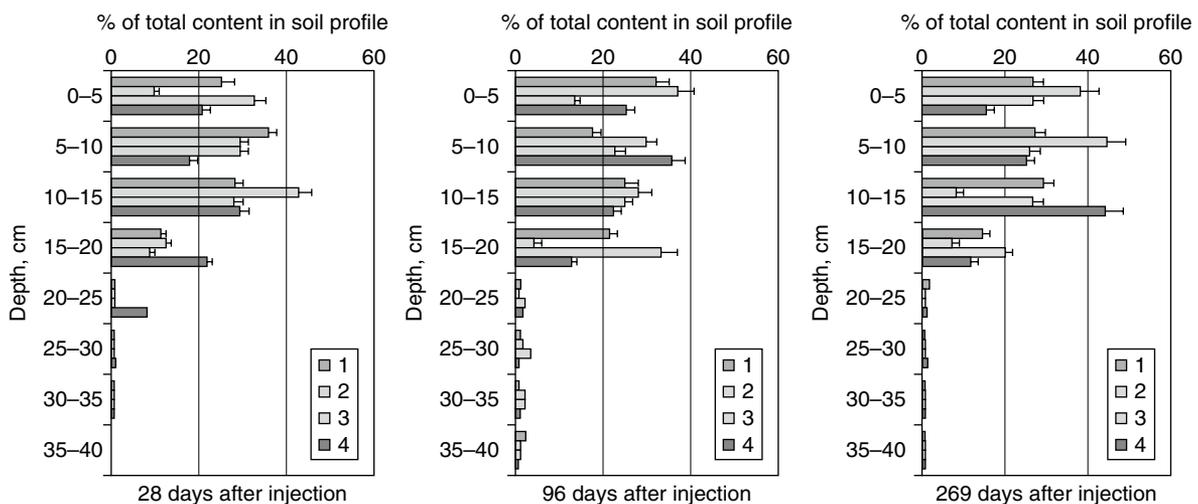


Figure 11.5 Vertical distribution of ^{125}I in soil profiles at various times after incorporation into the arable layer (0–20 cm). 1 – podzoluvisol, 2 – greyzem, 3 – meadow chernozem, and 4 – typical chernozem. Source: Kashparov *et al.*, (2005) reproduced with permission from Elsevier.

than other parts of the world. It was therefore hypothesized that the element largely occurs as an insoluble form that cannot easily be taken up by plants. This seems consistent with the observations that iodine in oxic soil regions is more strongly adsorbed, since the roots of most plants are only able to survive in oxic soils. In studying perennial ryegrass uptake of iodine, Ashworth *et al.* (2003) found that less than 0.1% of the total ^{125}I in soil column systems was associated with aboveground biomass of ryegrass, even after a 12-month period. In addition, Ashworth and Shaw (2006b) detected essentially zero transfer of ^{125}I from soil to aboveground perennial ryegrass biomass over 6 months. In both these experiments, the lack of transfer was primarily due to a limited overlap between the plant roots in the upper oxic region of the soil columns and the lower anoxic region where the majority of the ^{125}I was present.

Iodine Soil–Plant Transfer Factors The most convenient way to assess plant uptake of iodine is to consider the transfer factor (TF), since it inherently takes into account the relationship between soil and plant elemental concentrations. This is determined according to the formula

$$\text{TF (unitless)} = \frac{\text{Dry plant iodine concentration (mg} \cdot \text{kg}^{-1}\text{)}}{\text{Dry soil iodine concentration (mg} \cdot \text{kg}^{-1}\text{)}}$$

Thus, high TFs indicate a high degree of transfer from the soil to the plant. Elements with a conservative, nonsorbing nature in soils, and which generally follow the water flux, exhibit exceptionally high TFs. For example, (radio)chlorine TFs (from soil to perennial ryegrass) of up to 785 were recorded by Ashworth and Shaw (2006b). In contrast, iodine TFs tend to be low. The IAEA (1994) compendium of TFs “expected” values of 3.4×10^{-3} for grass (95% confidence range of 3.4×10^{-4} to 3.4×10^{-2}) and 2×10^{-2} for an unspecified crop. Dai *et al.* (2004b) reported that iodine TFs increased with increasing soil concentration for a range of vegetables. At the highest soil addition of iodine in their work ($5 \text{ mg} \cdot \text{kg}^{-1}$ concentration of iodine in soil), *fresh weight* TFs from soil to edible parts of the vegetables ranged from around 0.1 to 10. These can be approximately converted to dry weight (so as to be comparable to other values that are usually expressed on a dry weight basis) by dividing by 10 (plant material is generally around 90% moisture) to give a range from 0.01 to 1. Their values followed the increasing order: carrot root = onion stem < celery shoot < water spinach shoot < pak choi leaf < spinach leaf.

Sheppard *et al.* (1993) reported iodine soil–plant TFs ranging from 0.024 to 0.19 for corn, beets, and cabbage. Kashparov *et al.* (2005) found ^{125}I TFs of 0.01–0.03 for radish roots and lettuce leaves, 0.003–0.004 for bean pods and 0.001 for wheat grains in podzoluvisol soil. Greyzem

and typical and meadow chernozem soils gave TFs around an order of magnitude lower for the same crops, suggesting that these soils adsorbed the iodine more strongly and limited bioavailability. Shinonaga *et al.* (2001) calculated soil to grain TFs for cereal grains cultivated at 38 locations in Austria and found values of 0.0005–0.02. These values correlated negatively with iodine concentration of the soils in which the cereals were grown, as well as with the amount of clay in the soils, again suggesting that soil characteristics (e.g., clay minerals) can increase soil adsorption, and reduce plant availability, of the element. Schmitz and Aumann (1994) found soil to pasture grass TFs for ^{127}I of $1.4 \pm 0.4 \times 10^{-1}$ and for ^{129}I of $9.0 \pm 2.8 \times 10^{-1}$. The difference between the two isotopes is consistent with the authors’ data for water extractability of these isotopes, which was higher for ^{129}I .

Overall, TFs for iodine are low, indicating that the element is not readily transferred from the soil to plants. A probable reason for this is the strong soil adsorption of the element in the oxic region of soils where plant roots predominate. Therefore, the contribution to the human diet of iodine derived from crop plants is likely to be limited. However, plants with roots that are able to survive in anoxic soils, where iodine tends to be partitioned into the liquid phase to a greater extent, would therefore be expected to exhibit greater TFs. Indeed, Sheppard and Motycka (1997) noted that rice, the roots of which can tolerate anoxic soil, exhibited greater transfer of iodine from anoxic soil to the plant than under oxic conditions (TFs of 0.25 and 0.17, respectively). Cultures where rice is a staple food may therefore be exposed to the greatest plant-derived iodine loadings.

Partitioning of iodine into the gaseous phase (volatilization)

Conversion of Iodine into Volatile Forms Although the partitioning of iodine into the gaseous phase of the soil is thought to be influenced by factors such as organic and mineral adsorbing phases, pH, speciation, temperature and E_h , the conversion of iodine into the volatile form is considered to be driven by microbial processes. Amachi *et al.* (2005) showed that I^- -oxidizing bacteria (which are distributed widely in the environment and oxidize I^- to molecular iodine) also produce volatile organic iodine, which they identified as diiodomethane and chloroiodomethane. The importance of microbes in iodine volatilization was also illustrated by Amachi *et al.* (2004) who found that, from seawater, only organic iodine volatilized. They noted that 1–2% of total iodine was volatilized as methyl iodide by isolated strains of bacteria. Volatilization did not occur when samples were autoclaved or passed through $0.22 \mu\text{m}$ filters, indicating that the bacteria were required to induce volatilization. In addition to bacteria, Ban-nai *et al.* (2006) reported that filamentous

fungi strains volatilized “considerable” amounts (up to 3.4%) of ^{125}I in a liquid medium. Amachi *et al.* (2003) amended soil with I^- ions and found that iodine was emitted mainly as methyl I^- , a process that was sometimes enhanced by the addition of glucose to stimulate microbial activity. The same workers also amended soils with ^{125}I , and again found that microbes played a significant role in iodine volatilization, since emissions were enhanced in the presence of yeast, but inhibited by autoclaving of the soil. Furthermore, antibiotics that inhibited bacterial growth strongly reduced iodine emissions, while a fungal inhibitor had little effect. The authors concluded that iodine-volatilizing bacteria that are ubiquitous in the soil are responsible for the formation of organic, volatile methyl iodide.

Volatile Iodine Species Sheppard *et al.* (1994) gave the different forms of volatile iodine as molecular iodine, I^- and IO_3^- (as hydrides, hydrogen iodide, or hydrogen IO_3^-), and methyl iodide. Taghipour and Evans (2001) reported that organic iodides dominate the airborne speciation of iodine. More specifically, Tessier *et al.* (2002) suggested that volatilization occurred due to the methylation of inorganic iodine forms and further identified up to eight volatile iodine species as alkyl iodides in European estuarine waters. Methyl iodide accounted for the majority, 40%, of the various species. Muramatsu and Yoshida (1995) also reported methyl iodide as the primary iodine species volatilized from a rice flooded soil system, and estimated the methyl iodide emission mass for rice paddies worldwide as around $2 \times 10^{10} \text{ g} \cdot \text{year}^{-1}$. Redeker *et al.* (2000) studied the influences on the emission of methyl iodide from rice paddies and calculated that up to 5% of atmospheric methyl iodide arises from rice fields. Methyl iodide is evidently the dominant iodine species leaving the soil–plant system in the gaseous phase.

Indirect Measures of Volatile Iodine Losses from Soil A number of studies have determined relatively large losses of iodine from soil systems, often based on a deficit in a system mass balance (i.e., the actual volatilization loss may not have been measured directly). Prister *et al.* (1977) found 55–60% loss of soil iodine over 10 days in the absence of irrigation. When soils were irrigated, the loss was around 20% over a 6-day period. Sheppard and Thibault (1991) reported a 21–26% iodine loss from spiked soil cores after a 4-year period. A 9% loss of iodine spiked near the surface of a sandy soil after 1 year was reported by Sheppard *et al.* (1987). The potential importance of iodine volatilization in determining the fate of environmental iodine is illustrated by the work of Sheppard *et al.* (1994). These workers undertook modeling simulations of iodine transport in soil–air systems and found that when the volatilization process was represented in the model, soil iodine concentrations were five times lower and air iodine concentrations were 25 times greater than when the process was not represented.

Direct Measures of Volatile Losses of Iodine from Soil

Generally, workers who measure volatilization directly tend to report much lower values, particularly in organic soils where iodine becomes strongly adsorbed onto the solid organic phase. Sheppard *et al.* (1994) determined ^{125}I volatilization from peat soil in laboratory experiments and found a loss of just 0.07% over 66 days. Bostock *et al.* (2003) studied the soil–plant volatilization of ^{125}I from coniferous forest soil over a 22-day period and measured a total loss of 0.011%. The rate of emission of iodine was shown to decline over time and follow a double exponential model, i.e., declining very steeply initially, followed by a more gradual decline (Figure 11.6a). These authors further studied volatilization losses over the initial 48 h period following addition of the iodine to the coniferous forest soil and to a grassland soil (Figure 11.6b, c, respectively). In these cases, total volatile losses were measured as 0.011 and 0.004%, respectively, and emission rates declined over time according to a single exponential model. The authors attributed the low volatilization losses to the strong association they observed, in both soils, between iodine and the organic substances in the soil solid phase. Overall, the authors concluded that volatilization is not a significant pathway for the transport of ^{125}I in soil–plant systems.

Volatilization from Plants In addition to volatilization of iodine from the soil, plants can also convert the element into a volatile form that is emitted from the plant pores. Muramatsu and Yoshida (1995) reported that iodine emissions were highly stimulated by the presence of oats on nonflooded soil and, particularly, by the presence of rice on flooded soil where around 10% of the added ^{125}I was volatilized. Importantly, the authors reported that iodine emission from the shoots of the rice plants was significantly higher than that from the flooded soil surface alone. Muramatsu and Yoshida (1999) reported the volatilization of microbially generated methyl iodide gas from rice plants as a process by which iodine concentrations of soils may be reduced. Redeker and Cicerone (2004) showed that methyl iodide emissions from rice paddy soils are determined by the growth stage of the rice plant, with secondary influences of air temperature, soil iodine concentration, and soil moisture content. Muramatsu *et al.* (1995) also stressed the importance of rice plant growth in controlling iodine volatilization, with emissions markedly decreasing in the late cultivation period of the rice plants.

The process giving rise to enhanced methylation, and hence emissions, from rice-planted and flooded soil was postulated by Muramatsu and Yoshida (1995). This process entails the desorption of iodine from the solid phase of the soil due to low E_h and consequent predominance of I^- in the soil solution. I^- in the rhizosphere then becomes biomethylated by the effect of enzymes produced by soil microorganisms or roots (e.g., methyl halide transferase (Wuosmaa and Hager, 1990)). The methyl iodide produced

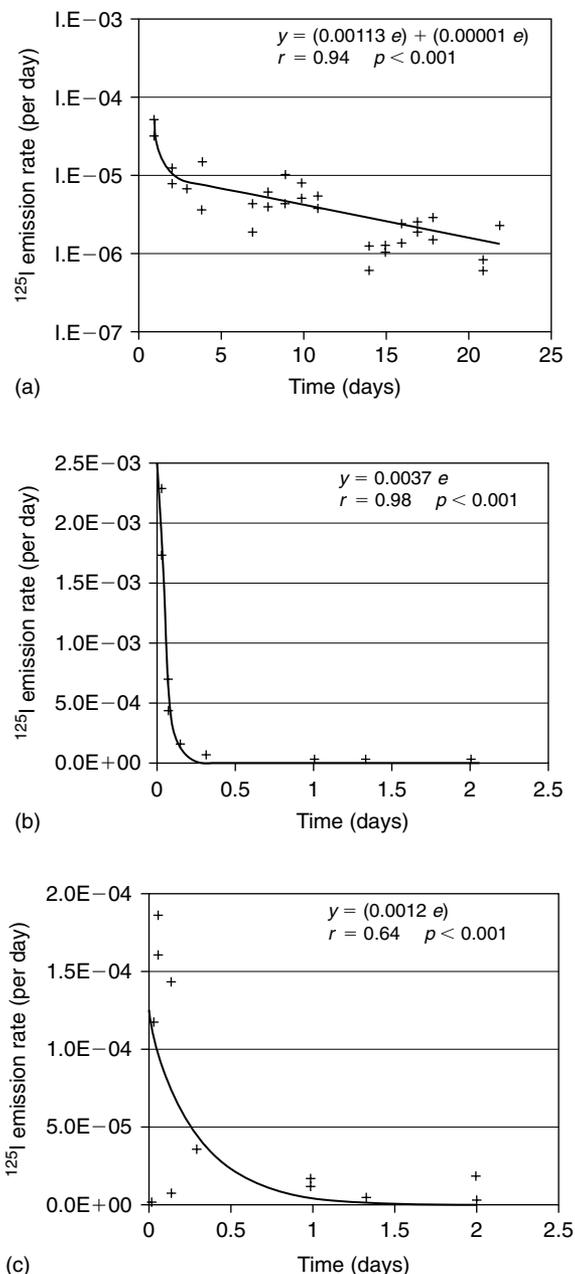


Figure 11.6 Volatile ^{125}I emission rates from soil. (a) Coniferous forest soils over a 22-day period. (b) Coniferous forest soils over a 48-h period. (c) Grassland soils over a 48-h period. Sample points are measured values. Lines are fits of the exponential equations shown. Source: Bostock *et al.*, (2003) reproduced with permission from Elsevier.

may then be transferred to the atmosphere by diffusion either through the soil pore space or, perhaps more significantly, through the intercellular gas space and aerenchym system in the plants. Alternatively, these authors propose that methyl iodide may be produced within the plant shoots from I^- taken up through the roots.

A mechanism exists by which soil iodine can become volatile and transfer to the aboveground atmosphere. A

pathway for human exposure thereby exists via inhalation. However, the low levels of iodine, which have actually been measured as soil–plant emissions, may significantly limit this exposure, particularly once this iodine-bearing air is diluted in the wider atmosphere.

Summary Points

- Iodine, an essential element for animals, including humans, is ubiquitous in the biosphere.
- Iodine partitioning between all three soil phases (solid, liquid, gas) leads to potential transfers away from the soil body, e.g., by leaching, plant uptake, and volatilization.
- The vast majority of soil iodine is most likely adsorbed to the soil solid phase, although appreciable amounts may be released into the liquid and gaseous phases, particularly under anoxic soil conditions.
- Under anoxic (low E_h) soil conditions, e.g., after flooding, the release of iodine into the liquid phase increases due to reduced sorption induced by changes in soil geochemistry and iodine speciation.
- Mobility of iodine under anoxic soil conditions is markedly greater than under oxic conditions. Accumulations of iodine at the boundary between anoxic and oxic soil regions are likely to occur.
- Mobility of iodine in oxic soils is low, leading to limited plant root uptake.
- Plants with roots able to tolerate anoxic conditions (e.g., rice) tend to take up greater quantities of iodine from the soil.
- Volatilization of iodine, primarily as methyl iodide, occurs from both soil and plants. Again, the presence of anoxic soil conditions is likely to enhance this process.
- Although the majority of soil iodine remains adsorbed to the soil solid phase, its release into the liquid and gaseous phases does occur, enabling potential transfer pathways that are likely to increase human exposure to iodine.

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Iodine in the Ecosystem: An Overview

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Abstract

Iodine may have originally emerged as a substrate for thyroid hormone production due to its role as an indicator of resource availability in the ecosystem and the corresponding need to modulate growth, reproduction, metabolic rate and lifespan. The reliability of iodine as a surrogate for resource availability may have declined due to changes in the modern diet, and the prehistoric link of iodine to thyroid function may have become less adaptive due to evolutionary displacement. Deciphering this teleologic link may lead to a better understanding of those modern diseases involving iodine and thyroid biology.

The Customization of Life Histories

Many factors regulate customization of life histories, including genetics, epigenetics and hormonal pathways. Compelling evidence suggests that epigenetic processes such as gene silencing, methylation and genetic imprinting endow phenotype plasticity to organisms in search of higher fitness (Gluckman and Hanson, 2004; Barker *et al.*, 2002; Cooney *et al.*, 2002; Curhan *et al.*, 1996; Weaver *et al.*, 2004). Developmental plasticity enables a range of phenotypes to develop from a single genotype in response to environmental cues (Gluckman and Hanson, 2004). Specific regulatory molecules, such as growth factors and thyroid hormones, permit organisms to express phenotypes optimized for a particular environment (Table 12.1).

Organisms exhibit tremendous plasticity in their ability to modulate behavior, depending on the perceived availability of resources in the environment. This determination may occur through analysis of bottom-up data such as caloric intake or top-down data such as ambient temperature. Integration of these various sources of data then allows for the development of fitness strategies tailored to

Table 12.1 Factors regulating customization of life histories

<i>Factor</i>	<i>Method</i>
Genetics	Protein expression
Epigenetics	Gene silencing, methylation, imprinting
Hormonal pathways	Growth factors, thyroid hormone

Note: Many factors regulate customization of life histories, including genetics, epigenetics and hormonal pathways.

Table 12.2 Relationship of energy environment to life-history strategy

<i>Energy environment</i>	<i>Life-history strategy</i>
High energy	Aggressive
Low energy	Conservative

Notes: Environments with an abundance of available energy promote life-history strategies that engender greater innovation and a more aggressive allocation of biological resources to exploit times of opportunity. Conversely, lower energy environments favor more conservative strategies.

life history. Environments with an abundance of available energy promote life-history strategies that engender greater innovation and a more aggressive allocation of biological resources to exploit times of opportunity. Conversely, lower energy environments favor more conservative strategies. Thyroid hormones play particularly important roles in modulating life-history strategy based on resource availability (Table 12.2).

Numerous nutritional factors influence the production of thyroid hormone (Danforth *et al.*, 1979; Azizi, 1978; Burman *et al.*, 1979; Serog *et al.*, 1982); among the most important is dietary intake of iodine (Kopp, 2004). Given the adaptive role that thyroid hormone plays in determining an appropriate life-history strategy, its dependence on dietary iodine for production remains curious.

Iodine and Life in the Ocean

Life on Earth likely began in the ocean and the earliest species likely emerged near the ocean surface, where light energy was able to undergo conversion to forms that could drive biochemical reactions. Kelps, order *Laminariales*, class Phaeophyceae, are one of many types of algae that populate the ocean surface (Colin *et al.*, 2003). One of the richest sources of iodine (Shilo and Hirsch, 1986; Miller, 1998; Anonymous, 1975), kelps are characterized by variable lifecycles that may include a microscopic filamentous gametophyte and a macroscopic sporophyte, the latter of which accumulates iodine at 30000 times its concentration in the ocean and up to 1% of its dry weight (Colin *et al.*, 2003; Küpper *et al.*, 1998).

The kelp forest represents one of the foundations of the marine ecosystem and ecosystem (Steinberg *et al.*, 1995; Sala and Graham, 2002), supporting substantial biodiversity, trophic structures and ecological interactions. Upward-acting resource limitations apparently constrain consuming species (Hairston *et al.*, 1960; Matson and Hunter, 1992; Carpenter *et al.*, 1985). Given their importance in the emergence of marine ecosystems, and their relatively early appearance during evolution dating to at least the Tertiary period more than 20 million years ago (Berta and Morgan, 1986; Estes and Steinberg, 1988; Druehl *et al.*, 1997), kelp forests may have had a strong influence in shaping the biology of later-appearing organisms in the Darwinian cascade.

Little is known about the adaptive functions of iodine in kelps and other marine plants or the mechanism involved in its concentration (Colin *et al.*, 2003). Iodine may play a role in the production of volatile hydrocarbons, thus creating defense metabolites that scavenge activated oxygen species and biocides (Ohsawa *et al.*, 2001; Wever *et al.*, 1991; Pedersen *et al.*, 1996). Algae may also concentrate iodine to protect themselves from protozoans and bacteria (Hartmann, 1990).

While marine algae may use iodine for self-defense, herbivores and higher-order predators further down the ecosystem may use dietary iodine as a surrogate sensor for overall availability of ecosystem resources. The tuning of the life-history strategy of each successive species in the ecosystem based on iodine intake could iterate evolution. Because of the absence of potential for internal modulation, iodine would serve as a more effective signal detection mechanism than caloric intake, which could undergo dampening or distortion based on use (Table 12.3).

Iodine and Thyroid Function

Thyroid function is sensitive to the integration of numerous inputs that relate to environmental resource availability.

Table 12.3 Potential roles for iodine in the ecosystem

Role	Organism
Production of volatile hydrocarbons	Kelps
Direct antimicrobial effect	Kelps
Surrogate for resource availability	All

Note: While marine algae may use iodine for self-defense, herbivores and higher-order predators further down the ecosystem may use dietary iodine as a surrogate sensor for overall availability of ecosystem resources.

Table 12.4 Associations with low thyroid hormone levels

Reduced serum glucose	Reduced growth hormone
Reduced serum insulin	Shorter juvenile period
Decreased metabolic rate	Decreased fertility
Smaller adult stature	Longer lifespan

Note: Thyroid hormone may play a role in lifespan expansion in response to direct cues of energy availability.

Lower ambient temperature, an indirect surrogate for lower energy availability, negatively regulates thyroid hormone production (Wingfield *et al.*, 1996) and hypothyroid states are associated with lower core body temperature (Schonholz and Osborn, 1949). Thyroid function becomes enhanced at higher temperatures not only in warm-blooded species, but also in fish, snakes, amphibians, turtles and lizards (Turner and Tipton, 1972; Maker, 1964).

Thyroid hormone may play a role in lifespan expansion in response to direct cues of energy availability. Reduced serum glucose and insulin levels, a direct cue of energy availability, are associated with low thyroid function (Weindruch and Sohal, 1997; Masoro, 1995). Metabolic rates decline, while triiodothyronine and thyroxine levels are lower in calorie-restricted animals (Passadore *et al.*, 2004; Picard *et al.*, 2004). Downregulation of triiodothyronine during caloric deprivation is observed in both endothermic and ectothermic vertebrates (Eales, 1988). Low thyroid hormone levels are independently associated with smaller adult stature, reduced growth hormone and metabolic rate, a shorter juvenile period, decreased fertility, and longer lifespan (Miller, 1999; Brown-Borg *et al.*, 1996; Ooka *et al.*, 1983; Meites, 1993; Sohal and Weindruch, 1996; Chattopadhyay *et al.*, 2003) (Table 12.4).

Given the high energetic cost of developmental transition, high thyroid hormone levels may indicate sufficient energy to fuel this phenotypic change. In many lower species, thyroid hormones determine the timing of developmental metamorphosis (Spangenberg, 1974; Wright and Yonsum, 1977; Dickhoff and Darling, 1983). Among salmonids, thyroxine levels appear to regulate the smoltification changes that precede the migration from fresh water to salt water, an energy-intensive process (Dickhoff *et al.*, 1978; Nishikawa *et al.*, 1979). Exogenous administration

of l-thyroxine shortens intervals between broods and accelerates oocyte development in the next batch (Lam and Loy, 1985), consistent with hyperthyroid states. The functional link between thyroid and ovarian function, as well as diseases that involve overlap of these systems such as struma ovarii, is reviewed elsewhere (Gerhard *et al.*, 1991).

Decoupling: Iodine in the Modern Era

While dietary iodine intake may have once served as a useful proxy for nutritional availability, this linkage has apparently become decoupled in the modern era. Independent from any association with resource availability, iodine deficiency may arise from decline in both soil reservoirs and the terrestrial ecosystem which is caused due to both glaciation and the leaching effects of groundwater.

Although excess iodine intake has been reported in certain Asian cultures (Kim *et al.*, 1998), relative iodine deficiency exists in many parts of the world. Many nations have instituted iodization of salt to appropriately compensate for the problem (Aquaron *et al.*, 2002; Zimmermann and Delange, 2004). Goiters and thyrotoxicosis are diagnosed at higher rates in regions where iodine supply has increased rapidly due to supplementation compared to those areas with high baseline iodine intake (Horst *et al.*, 1967; Als *et al.*, 1995). However, high consumption of dietary iodine has also been implicated in subclinical hypothyroidism in Asian countries with a high intake of seaweed (de Smet *et al.*, 1990; Konno *et al.*, 1994). Induction of hypothyroidism by excessive iodine administration may occur via a reduction of organic binding of iodine by the thyroid gland (Baker, 2004), but the details of the relationship between iodine input and thyroid hormone output remain unresolved (Okamura *et al.*, 1994). In addition, rich sources of iodine such as amiodarone and iodinated contrast can cause hyperthyroidism (Seminara and Daniels, 1998; Kulstad and Carlson, 2004; Kulstad, 2004), but acute administration of these agents can also induce transient hypothyroidism (Gartner and Weissel, 2004; Lomenick *et al.*, 2004).

Increasing biological demand in conjunction with increasing thyroid hormone requirements (Kopp, 2004) may also stem from other modern dietary shifts (Thilly *et al.*, 1992). The human diet has undergone substantial changes in composition in the modern age. In particular, the emergence of a high-carbohydrate diet, combined with possible depletion of iodine in the food supply, may have induced an imbalance of iodine intake relative to energy consumption (Kopp, 2004). This discrepancy between sensed iodine concentrations and sensed energy levels may contribute to physiological malfunction and the pathogenesis of many modern ailments. In fact, relative iodine imbalance and endemic subclinical hypothyroidism may have played a role in the trend toward earlier puberty in humans during

Table 12.5 Potential new strategies to treat hypothyroidism

Insulin sensitizers
Exercise
Weight loss

Note: Insulin sensitizers, exercise, and weight loss may represent new opportunities to treat hypothyroidism in lieu of or as an adjunct to iodine supplementation therapy.

the last century (Herman-Giddens *et al.*, 1997; Whincup *et al.*, 2001) in conjunction with other factors. Iodine imbalance and endemic subclinical hypothyroidism may have also contributed to the extension of the human lifespan (Oeppen and Vaupel, 2002).

Iodine: Its Implications for Disease and Therapy

Although the high caloric intake associated with the modern obesity epidemic presumably serves as a signal of resource abundance, the increasing prevalence of hypothyroidism suggests that the body may also simultaneously perceive a situation of resource scarcity due to the lack of concordance between prevailing iodine concentrations and energy levels in the environment. This contradiction in interpretations may be contributing to numerous metabolic and endocrine dysfunctions. Emerging modern pandemics, such as obesity and syndrome X, may well illustrate this phenomenon, given the link between insulin resistance and hypothyroidism (Dessein *et al.*, 2004; Reutrakul *et al.*, 2004). Although the belief remains widely held that hypothyroidism causes weight gain by slowing the metabolic rate, the possibility remains that weight gain induces hypothyroidism by worsening the imbalance between energy and iodine. Insulin sensitizers, exercise, and weight loss may represent new opportunities to treat hypothyroidism in lieu of or as an adjunct to iodine supplementation therapy (Table 12.5).

Iodine or iodine modulators may also have potential uses as modulators of the endocrine axis, thus treating diseases such as syndrome X, infertility, and growth retardation. Local modulation of the female reproductive tract by iodine may explain the increased fertility of patients who undergo hysterosalpinography with oil-based iodinated contrast (Weisman, 1952). Links among syndrome X, insulin resistance, ovarian cysts, and polycystic ovarian syndrome have begun to emerge (Doelle, 2004). Given the association between ovarian cysts (Kulstad and Carlson, 2004; Kulstad, 2004) and hypothyroidism (Takeuchi *et al.*, 2004; Singh *et al.*, 2005), a broader investigation of co-morbid dysfunctions involving the ovary and thyroid appear warranted. Increased production of thyroid hormone in the presence of high iodine levels leads to increased catecholamine production, which in turn may produce age-related sympathetic bias, a process that appears

Table 12.6 Diseases which may benefit from use of iodine and iodine modulators

Syndrome X
 Infertility
 Growth retardation
 Polycystic ovarian syndrome
 Diseases of sympathetic bias

Notes: Iodine or iodine modulators may have potential uses as modulators of the endocrine axis, thus treating diseases such as syndrome X, infertility, and growth retardation. Increased production of thyroid hormone in the presence of high iodine levels leads to increased catecholamine production, which in turn may produce age-related sympathetic bias, a process that appears to drive many of the systemic dysfunctions associated with aging.

to drive many of the systemic dysfunctions associated with aging (Lee *et al.*, 2004) (Table 12.6).

Summary Points

- Iodine may function as an indicator of resource availability in the ecosystem.
- Iodine may have originally emerged as a substrate for thyroid hormone production due to the corresponding need to modulate growth, reproduction, metabolic rate, and lifespan.
- Changes in the modern day diet may have decreased the reliability of iodine as a surrogate for resource availability.
- The prehistoric link of iodine to thyroid function may have become less adaptive due to evolutionary displacement.
- Modern disease may involve iodine and thyroid biology to a greater extent than we now suspect.

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The Nature of Iodine in Drinking Water

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Abstract

Iodine is sparse in the terrestrial but abundant in the marine environment; marine microorganisms assimilate iodine, which is concentrated 30 000 times in some marine algae. Marine organisms form sediments shown to be rich in both iodine and organic matter. These sediments occur in the terrestrial subsurface environment because of geological events and reclamation of bays and wetlands. The subsurface environment constitutes the aquifer source rock, which is important for groundwater composition, and iodine and organic matter, mainly humic substances, may leach from marine deposits. Aquatic humic substances have the capacity to form complexes with trace elements; hence, iodine in drinking water was found in these substances. This may reduce the bioavailability of iodine. Also, iodine in drinking water suggests coexisting humic substances from a marine source rock. Some humic substances have goitrogenic properties, which may be counteracted by the coexisting iodine. It can be speculated that the complex interaction of iodine and humic substances accounts for the inconsistency in the relationship between iodine in drinking water and endemic goiter.

Abbreviation

HPLC High-pressure liquid chromatography
GAC Granular-activated carbon

Introduction

The interdisciplinary nature of the field of iodine in drinking water is demonstrated by the fact that it is one of the four halogens (chemistry) bound and processed as organohalogens in natural waters (geochemistry), found in organic components of drinking water (hydrochemistry), and ingested as a dietary component with a fraction absorbed from the gut (nutrition) with implications for health (health science). This makes the iodine content of drinking water interesting to a number of scientific areas,

requiring an interest in different fields to understand the complexity. Finally, it is a beautiful example of how man depends on nature.

Early Investigations of Iodine in Drinking Water

Iodine was first prepared in 1811 by the French chemist Courtois, who was engaged in the production of potassium nitrate from niter beds for Napoleon's armies. He thought of it as an impurity in the soda ash derived from seaweed which he could isolate as black powder by adding sulfuric acid to the liquor. The black precipitate released a violet vapor on heating (Courtois, 1813), which was recognized as a new element by Gay-Lussac; he gave the Greek name for the color of the vapor, *ioeides*, and described a number of its compounds (Lussac, 1814).

Around 1850 Chatin estimated dietary iodine intake by measuring iodine in dietary components, including iodine in drinking water in different parts of France. He found that the iodine content of drinking water decreased by a factor 10 from Paris to Lyon and again to areas in the Alps. This was parallel to the differences in goiter and cretinism when comparing nongoitrous, noncretinistic Paris with goitrous but noncretinistic Lyon with the goitrous and cretinistic areas in the Alps. Thus, Chatin suggested a causal relationship between the lack of iodine in the environment, including iodine in drinking water, and endemic goiter and cretinism. Shortly after this, Marchand took one step further and suggested an association between the low content of iodine in drinking water and goiter and cretinism, a topic for more investigation in the following years (Fellenberg, 1923; Adlercreutz, 1928).

Fellenberg (1923) investigated different areas and found 5–65 times higher iodine content of drinking water in a nongoitrous area compared to goitrous areas. Since then, the iodine content of drinking water has been associated with goiter in detail in a number of areas, as illustrated in Table 13.1.

Table 13.1 Examples of the association between iodine in drinking water and goiter prevalence

District	Iodine content of drinking water ($\mu\text{g}/\text{l}$)	Correlation to goiter prevalence	References
Australia	0.3–10	-0.95 ^a	Hales <i>et al.</i> , (1969)
E. Germany	1.6–11.6	-0.94 ^a	Felgentäger <i>et al.</i> , (1983)
Sri Lanka	<5–> 101	-0.64 ^b	Balasuriya <i>et al.</i> , (1992)
England	1.2–53	-0.53 ^b	Young <i>et al.</i> (1936), Murray <i>et al.</i> , (1948)
Spain	<1–20	-0.51 ^b	Vivanco <i>et al.</i> , (1971)
Poland	0.75–5.5	-0.43 ^c	Bobek <i>et al.</i> , (1991)
W. Germany	0.2–21.0	0 ^d	Mertz <i>et al.</i> , (1973)
Colombia	1.4–22	0.33 ^d	Gaitan <i>et al.</i> , (1978)

^a $p < 0.01$.^b $p < 0.001$.^c $p < 0.05$.^dNot significant.

Fellenberg (1923) also found seasonal differences in the iodine content of drinking water in mountainous areas. In Australia this was documented to be related to goiter (Hales *et al.*, 1969).

Fellenberg paid attention to the origin of the iodine and also estimated the amount of organic matter in drinking water. He found the iodine content of groundwater to be higher when the fraction of organic material was large (Fellenberg, 1923).

Vinogradov (1939) investigated the sea bottom and confirmed the finding that muds rich in organic matter had a high content of iodine. From his line of observations he concluded that the iodine content of sediments depended on the organic matter content.

Kononov (1959) found that rivers draining Tertiary marine sediments had higher iodine contents than rivers draining other areas. This was attributed to iodine leached from marine sediments.

Humic Substances in Marine Sediments and Groundwater

Marine sediments are rich in organic matter that may be released into groundwater (Pettersson *et al.*, 1994; Weber *et al.*, 2001). The elution of such macromolecules in drinking water derived from a marine source rock is illustrated in Figure 13.1 (Andersen *et al.*, 2002). High-pressure liquid chromatography (HPLC) size exclusion of organic matter with absorbency recorded at 280nm was performed on preconcentrated water from the Skagen aquifer in northern Denmark. A broad monomodal elution pattern with subtle

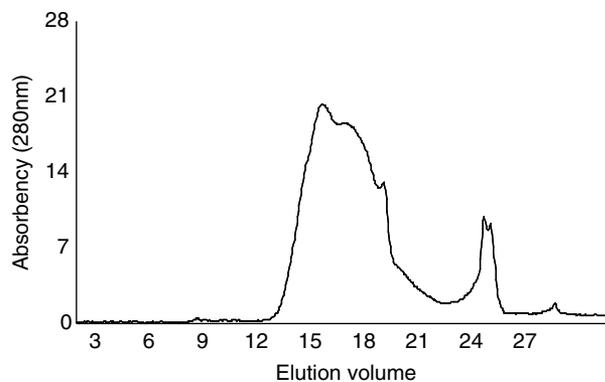


Figure 13.1 HPLC size exclusion performed on preconcentrated Skagen drinking water at pH 7.0. Absorbance at 280nm was recorded. Source: Andersen *et al.*, (2002), reproduced with permission.

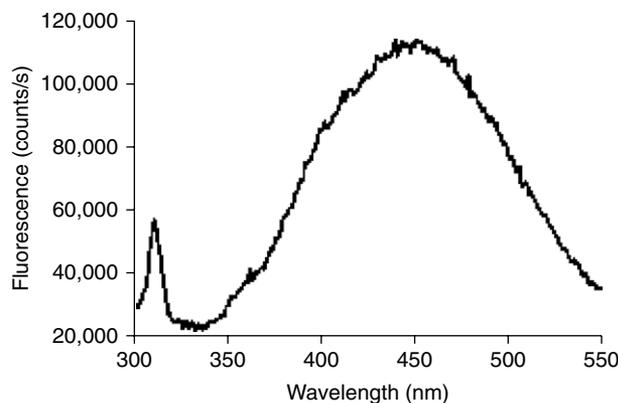


Figure 13.2 Emission fluorescence spectrum of the fraction containing bulk organic matter in tap water from the Skagen aquifer, excited with monochromatic light at 280nm, while an emission spectrum from 330 to 500nm was recorded. The 310nm Raman scatter is accompanied by a single broad band peak around 460nm characteristic of humic substances. Source: Andersen *et al.*, (2002), reproduced with permission.

shoulders and subpeaks was found, typical for humic substances (Andersen *et al.*, 2002).

The major part of groundwater organic matter consists of humic substances (Paxeús *et al.*, 1985; Vartiainen *et al.*, 1987; Wassenaar *et al.*, 1990; Malcolm, 1990; Grøn, 1993; Pettersson *et al.*, 1994; Shin and Lim, 1996; Calace *et al.*, 1999; Weber *et al.*, 2001).

Humic substances are heterogeneous mixtures of naturally occurring molecules produced by the decomposition of plant and animal tissues (Thurman, 1985; MacCarthy and Suffet, 1989; Shin and Lim, 1996; Calace *et al.*, 1999; Nissinen *et al.*, 2001; Weber *et al.*, 2001). They represent an intermediate step in the degradation pathway from higher organic material to kerogen and eventually coal (Wassenaar *et al.*, 1990; Siskin and Katritzky, 1991). Thus, the humic substances in drinking water are highly inhomogeneous, as illustrated in the fluorescence emission spectrum of humic substances in water from the Skagen aquifer shown in Figure 13.2.

Apart from the narrow Raman scatter of water at 310 nm, the fluorescence spectrum showed a single broad band peak at 460 nm, and should be perceived as an average fluorescent signature of the humic substances in accordance with their composite nature.

Higher plants may chelate trace elements and this ability carries through to humic substances (Paxeús *et al.*, 1985; Huljev, 1986; Perdue, 1989; Alberts *et al.*, 1992; Chin *et al.*, 1994; Shin and Lim, 1996; Calace *et al.*, 1999). Hence, as humic substances may be present in significant concentrations even in deep groundwaters (Dellis and Moulin, 1989; Wassenaar *et al.*, 1990; Pettersson *et al.*, 1994; Shin and Lim, 1996; Calace *et al.*, 1999) they may have an effect on the speciation of several trace elements in water (Thurman and Malcolm, 1981; Huljev, 1986; Thanabalasingam and Pickering, 1986; Dellis and Moulin, 1989).

Thus, aquatic humic substances are interesting because of their capacity to complex trace elements (Thurman and Malcolm, 1981; Huljev, 1986; Thanabalasingam and Pickering, 1986; Perdue, 1989; Dellis and Moulin, 1989; Chin *et al.*, 1994).

Aspects of Iodine Chemistry

Iodine is a nonmetallic element, the fourth member of the halogen family, i.e., Group VII of the Periodic Table, which also includes fluorine, chlorine, bromine, and astatine. Iodine has the atomic number 53, an atomic mass of 127, and only one stable isotope in the Earth's crust, ^{127}I , though 23 isotopes have been recorded.

Iodine has a solid black, crystalline appearance with a slight metallic lustre at ordinary temperatures. At high pressures iodine crystals assume the electrical characteristics of a metal. The first ionization potential for iodine is not much greater than those for some metals (Bailar *et al.*, 1973). Thus, iodine has metal-like capacities, and it reacts with humic substances (Christiansen, 1990).

Iodine in Marine Sediments and Water

Iodine is abundant in the marine environment and its content in seawater is approximately $50\ \mu\text{g/l}$ (Shishkina and Pavlova, 1965; Fuge and Johnson, 1986; Pedersen *et al.*, 1999). The marine microorganisms (algae, seaweed, and some invertebrates) assimilate iodine, which is concentrated 30 000 times in some marine algae (Vinogradov, 1939; Tong and Chaikoff, 1955; Fuge and Johnson, 1986). These organisms are built into marine sediments that have been shown to be rich in iodine (Shishkina and Pavlova, 1965; Price and Calvert, 1977; Harvey, 1980; Francois, 1987; Fuge and Johnson, 1986; Fuge, 1996).

The ability of marine organisms to further assimilate iodine was illustrated in a simple experiment by Vinogradov (1939). He added 19 mg iodine to a sample of fresh mud and sea water. After 8 months in the dark 17 mg

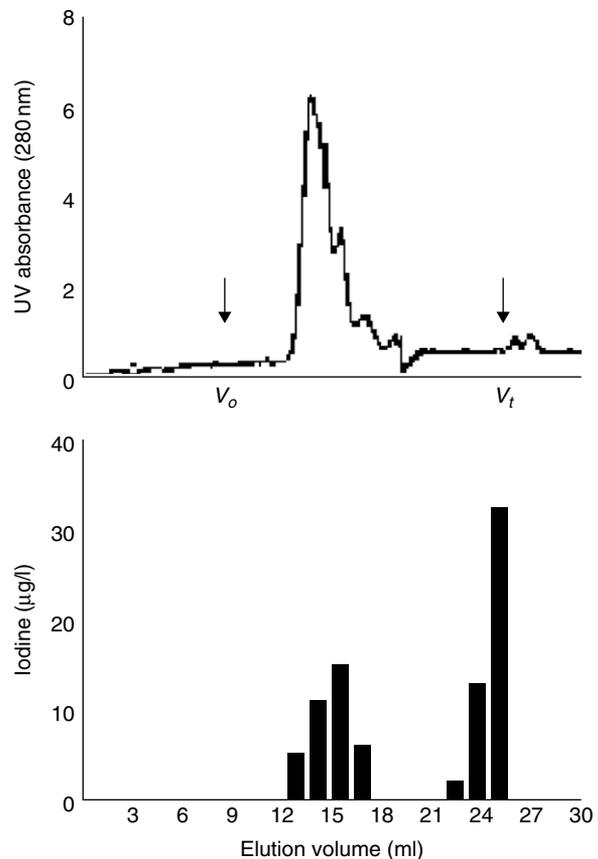


Figure 13.3 HPLC size exclusion elution pattern of Skagen tap water (20 ml containing $2.8\ \mu\text{g}$ iodine) preincubated for 24 h with potassium iodide (1 ml containing $3.0\ \mu\text{g}$ iodide). The iodide added eluted corresponding to V_{total} . Source: Andersen *et al.*, (2002) reproduced with permission.

(90% of the iodine) was absorbed by the mud. Enzymatic contributions to this binding of iodine have been demonstrated in more recent *in vitro* studies (Christensen and Carlsen, 1991; Huang and Lu, 1991; Carlsen *et al.*, 1992), which may explain why a further iodine enrichment of iodine containing humic substances was not recorded in a 24 h incubation study, shown in Figure 13.3.

This supports the enzymatic binding of iodine rather than simple hydrophobic interaction with humic substances.

The iodine in the detritus of marine organisms, i.e., humic substances, forms sediments in the decay process, adding to the concentration of iodine in muds.

Also, the chemical composition of iodine in waters of petroliferous areas has a number of features pointing to a marine origin, and the high iodine accumulation in the bed waters is a general geochemical phenomenon related to the history of petroleum formation (Vinogradov, 1939).

The iodine in sediments may be formed either by the detritus of sea organisms and algae (fixed and retained in the sea muds) or by the process of iodine accumulation in muds. The burial of sediments occurs under the influence of geological events, such as tectonic movements or glacial processes.

Geological Influences on Aquifers

During the Jurassic period large parts of the Northern Hemisphere were covered by sea. The coastal areas consisted of flood and delta plains, which were covered with vegetation. In the middle of this period, the tectonic uplift resulted in parts of the basin being elevated above sea level. Later, the land was again inundated by a rise in the sea level.

During the Tertiary period continental movements resulted in the opening of the North Atlantic. Scandinavia rose to become a pronounced highland, where rivers transported clay and sand out to the sea. These deposits created floody plains and delta plains that led to the Danish area being above the sea level.

During the Quaternary period the climate alternated between cold and warm; the period was characterized by changes between ice ages and interglacial stages. The sea level changed accordingly, and was very low during ice ages, with glaciers in northern Europe. During the interglacial stages the ice disappeared and the land and the sea were similar to that today.

During glacial periods, ice depressed the Earth's crust by up to several hundred meters. When the ice melted, the land rose again, after a delay. During this delay the sea flooded the deglaciated terrain. This was followed by an uplift, exposing large areas of sea floor.

Denmark was in the center of these geological events, as illustrated in Table 13.2.

As suggested by Chatin in the middle of the 19th century and subsequently detailed by others (Fellenberg, 1923; Hales *et al.*, 1969; Felgentäger *et al.*, 1983), drinking water in mountainous areas has a low iodine content; this content in general increases with proximity to the sea, which may relate to flooding by sea leaving subsurface marine deposits during uplift, deposition of sediments, or retraction of sea.

Table 13.2 Glacial evolution of northern Europe during the past 1.6 million years, the Quaternary period

Ice age	Interglacial stage	Cover of Denmark
Menapian		Ice
	Cromerian	?
Elsterian		Ice
	Holstenian	Sea
Saalian		Ice
	Eemian	Sea
Weichselian		None (west), ice (east)
	Holocenean	
	Deglaciation	Sea
	Continental period	None
	Flandrian transgression	Sea
	Uplift of northern Denmark	None

Geology: The Danish Example

Ice covered Central and Northern Europe during several geological events. During the last ice age, Denmark was a tundra plain which was repeatedly invaded by glaciers with subsequent deglaciation, and flooding of the land. This was followed by land rise with exposure of the sea floor leading to the presence of marine sediments above sea level. Toward the end, that is during the Weichsel period (70 000–10 000 years before present), the northernmost part of Jutland was covered by the sea. After deglaciation, the eastern land was submerged due to a rapid rise in Littorina sea level approximately 6000 years ago.

Marine deposits have been exposed through subsequent land rise that has reached 60 m in northern Denmark, where about one-third of today's area consists of marine plains. In eastern Denmark large areas of bays, heaths, and wetlands reclaimed add to recent marine deposits in subsurface Denmark (Grøn, 1989; Larsen, 2002). Thus, two different mechanisms contribute to regional differences in aquifers in Denmark.

Iodine in Drinking Water: The Danish Example

The subsurface environment constitutes the source rock of an aquifer, which is important for groundwater composition (Wassenaar *et al.*, 1990; Grøn, 1993; Grøn *et al.*, 1996); most drinking water in Denmark is local groundwater (Villumsen, 1991). As shown in Figure 13.4 iodine in drinking water differs between East and West Denmark

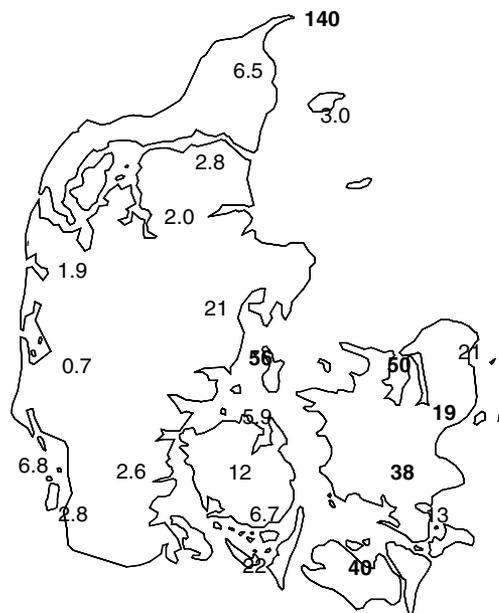


Figure 13.4 Map showing the iodine content ($\mu\text{g/l}$) of tap water collected at 22 waterworks in Denmark. Tap water from the six sites indicated were analyzed further (Figure 13.7). Source: Andersen *et al.*, (2002) reproduced with permission.

(Andersen *et al.*, 2002), which was in accordance with both geological data (Larsen, 2002) and some previous studies on the presence of iodine in tap water in Denmark (Table 13.3).

The finding of stable iodine content of drinking water over time suggests that the effect of iodine on man has the potential to carry through to populations in these areas, as has been found in a large population-based study (Laurberg *et al.*, 2006).

Table 13.3 Average iodine content of drinking water in Denmark ($\mu\text{g/l}$)

West Denmark	East Denmark	References
Low ^a	30	Gram <i>et al.</i> (1932)
5.0	25	Pinndal <i>et al.</i> (1982)
5.0	24	Pedersen <i>et al.</i> (1999)
5.5	19	Rasmussen <i>et al.</i> (2000)
5.0	23	Andersen <i>et al.</i> (2002)

^aFrom one area (Varde): below detection limit.

Iodine in Humic Substances

The technical literature describes coexisting organic matter and halogens widespread in the nature (Asplund and Grimvall, 1991). Investigations have shown that they are present in most aquifers in Denmark (Grøn, 1989, 1991, 1993).

A study by Grøn *et al.* (1996) demonstrated the presence of fulvic acids, a fraction of small humic substances, released from 4500-year old marine sediments, in Skagen natural waters. In HPLC size exclusion, iodine eluted corresponding to these humic substances, despite altering ionic strength (Figure 13.5) and pH (Figure 13.6) (Andersen *et al.*, 2002).

This could be a local phenomenon related to the humic substances from the Skagen aquifer. Thus, tap water samples were collected from six locations that geographically covered most of Denmark, i.e., from Skagen in the furthest northwest to the waterworks of Nakskov on the island of Lolland in the southeast, to Copenhagen on the eastern shore of Zealand, and including Nykøbing and Ringsted in northern and central parts of Zealand, as well as the island of Samsøe in Kattegat (Figure 13.4). Size

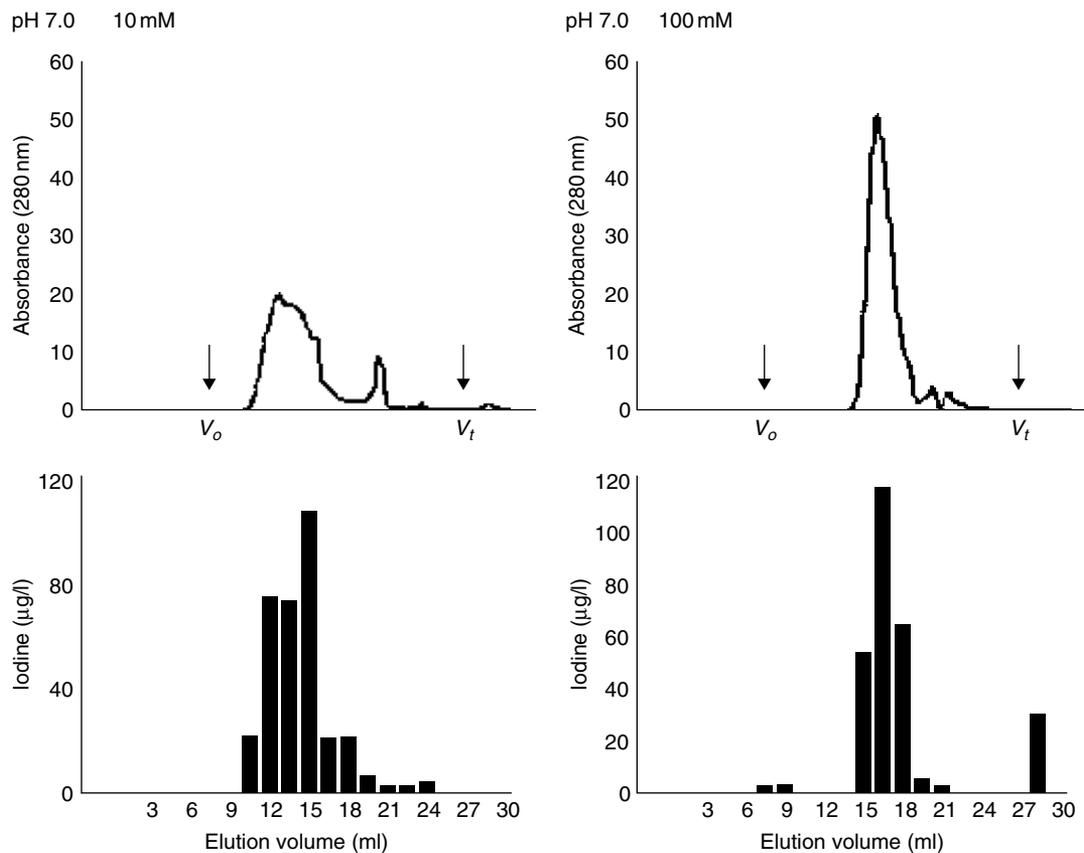


Figure 13.5 HPLC size exclusion performed on pre-concentrated Skagen tap water at pH 7.0. Absorbance at 280nm was recorded and iodine measured in fractions of 1.5ml. Iodine eluted in humic substances. Increasing ionic strength from 10 to 100mM caused a change in the elution of humic substances (upper panel) accompanied by an identical change in the elution of most of the iodine in tap water (lower panel). Source: Andersen *et al.*, (2002) reproduced with permission.

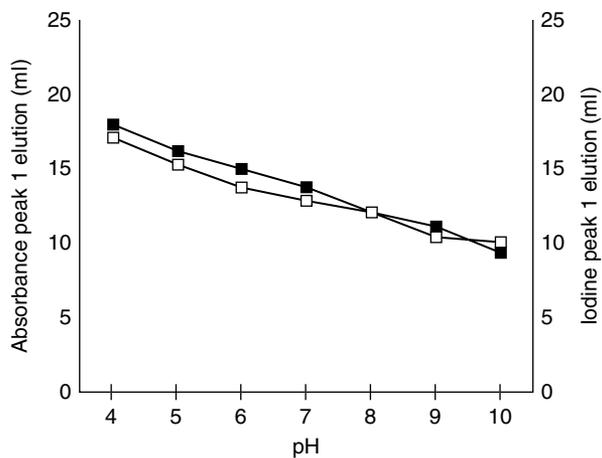


Figure 13.6 HPLC size exclusion of Skagen tap water was performed at different pH values. Humic substances (closed boxes) and iodine (open boxes) eluted simultaneously. Changing pH caused a parallel change in the elution of iodine and humic substances. Source: Andersen *et al.*, (2002) reproduced with permission.

exclusion chromatography of these water samples showed minor differences in the traces but similar-size organic matter content that contained iodine at all locations (Figure 13.7) (Andersen *et al.*, 2002).

The notion that iodine in drinking water suggests coexisting humic substances from a marine subsurface environment rich in iodine (Francois, 1987; Balasuriya *et al.*, 1992; Grøn *et al.*, 1996) was supported by the finding that tap water iodine concentration was associated with the absorbency of humic substances (Spearman's ρ 0.85, $p = 0.03$), illustrated in Figure 13.8 (Andersen *et al.*, 2002).

Iodine and Processing of Drinking Water

Raw water is processed at the waterworks before it is distributed to consumers. The content of organic matter may cause coloring and taste and smell unpleasantness for consumers (Huck *et al.*, 1991; Cancho *et al.*, 1999). Also, distribution systems are vulnerable to bacterial growth facilitated by humic substances (Huck *et al.*, 1991, 1992). Therefore, the treatment of raw waters has been intensified at waterworks, particularly in Europe. Raw waters may be treated with aeration, sedimentation and filtration in order to remove particulate and organic matter (Vartiainen *et al.*, 1987; Huck *et al.*, 1991, 1992; Shin and Lim, 1996; Nissinen *et al.*, 2001). The filtration steps differ between waterworks, with sand filters commonly used. Additional chemical coagulation, further filtration steps, adsorption in granular-activated carbon contractors, and chlorination may be used (Huck *et al.*, 1991, 1992; Shin and Lim, 1996). These increasingly efficient water processing steps

aim to reduce the risk of bacterial growth and lower the content of organic matter (Vartiainen *et al.*, 1987; Huck *et al.*, 1992; Shin and Lim, 1996; Nissinen *et al.*, 2001). The filtration step using granular-activated carbon contractors reduces the fraction of humic substances slightly better than the conventional treatment (Nissinen *et al.*, 2001); reduction in iodine content when using extensive water treatment steps on raw groundwater from Skagen, Denmark is shown in Figure 13.9 (Andersen *et al.*, 2007).

The mean iodine content of raw water was 152.7 $\mu\text{g/l}$ ($\pm 4.0 \mu\text{g/l}$), which decreased to 139.7 $\mu\text{g/l}$ ($\pm 5.2 \mu\text{g/l}$) after water treatment involving aeration, sedimentation and chemical coagulation before sedimentation, adsorption in granular-activated carbon (GAC), and tandem contractors, and tandem sand filtration. Some variation in the reduction of iodine content was seen between months, which was in accordance with the variation in the efficiency of biological filtration known to occur, depending on the biological activity in the filter (Huck *et al.*, 1992; Nissinen *et al.*, 2001). Thus, the extensive water treatment reduced the iodine content by a mere 8.5% (Andersen *et al.*, 2007). This indicates that differences in aquifer source rock carry through to drinking water, despite intensified water treatment.

Bioavailability of Iodine in Humic Substances in Drinking Water

The association between iodine deficiency and goiter was established long ago (Fellenberg, 1923; McClendon and Hathaway, 1924; Hercus *et al.*, 1927). The presence of goiter despite iodine sufficiency was also demonstrated early in the 20th century (Lieck, 1927; Scheffer, 1932), but a cause was not unveiled until the 1970s.

In Germany, the lack of relationship between goiter and iodine in drinking water led Mertz *et al.* (1973) to the conclusion that a geologically derived factor could interfere. In Colombia, Gaitan found a positive correlation between goiter prevalence and both iodine in drinking water and urinary iodine excretion, the latter being significant (Gaitan *et al.*, 1978; Gaitan, 1983). This relation was elucidated in a series of investigations concluding that drinking water contained goitrogenic substances (Gaitan, 1990) derived from humic substances in the aquifer (Gaitan, 1983). However, it could be speculated that humic substances also influence the bioavailability of iodine, and thereby affect the occurrence of goiter.

An ecological study showed a strong association between the iodine content of tap water in 41 towns and the iodine excretion estimated from more than four thousand 24 h urine samples collected 30 years apart (Figure 13.10) (Pedersen *et al.*, 1999).

This confirms that iodine in drinking water is stable with time, and that it is, at least to some extent, absorbed.

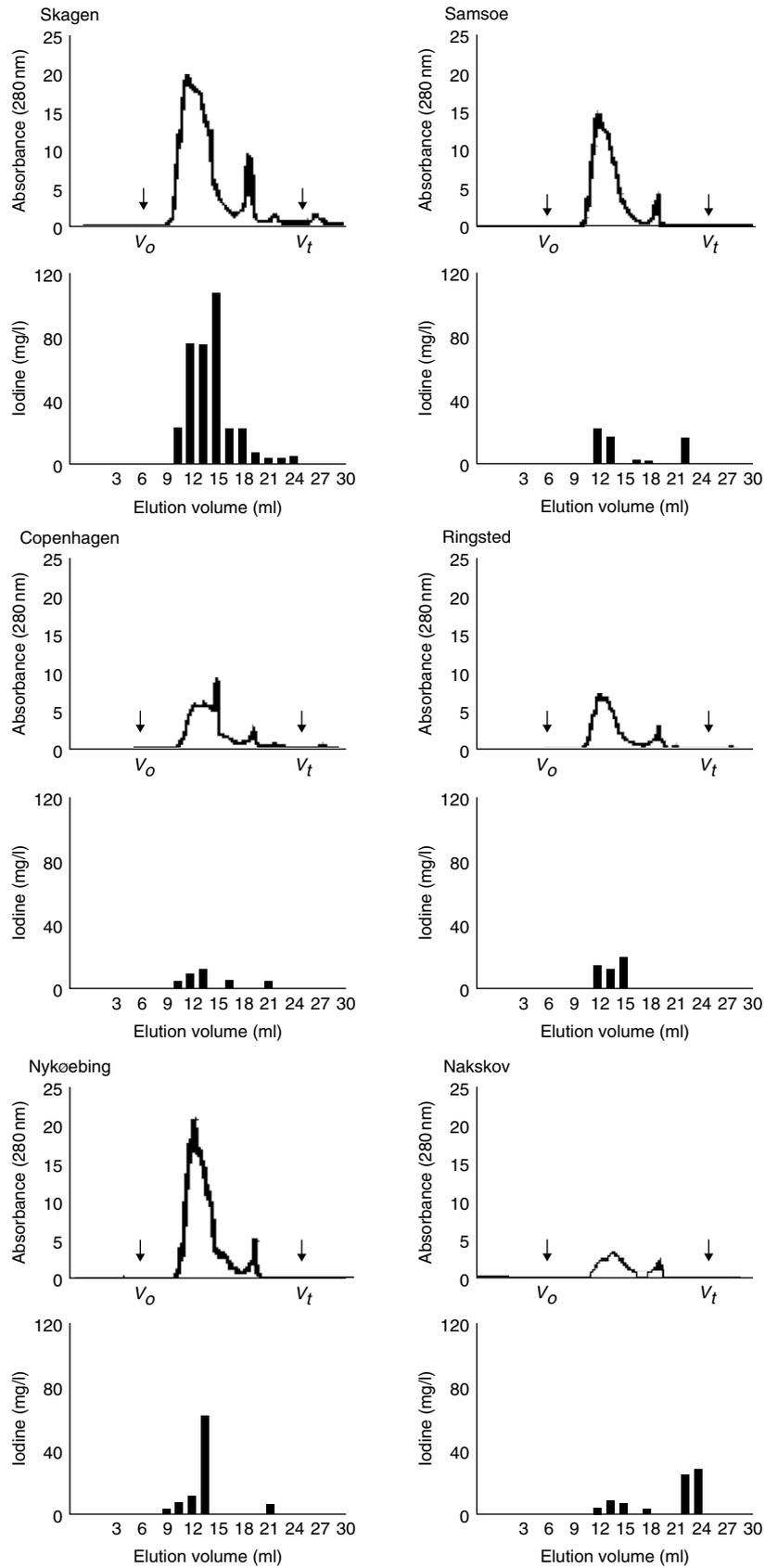


Figure 13.7 HPLC size exclusion traces of tap water from six locations in Denmark and the corresponding iodine content of fractions. A main peak eluted around 14 ml (K_{av} 0.34), corresponding to 50kDa. The quantity of iodine eluted in this peak with the remaining eluting as low molecular weight substances.

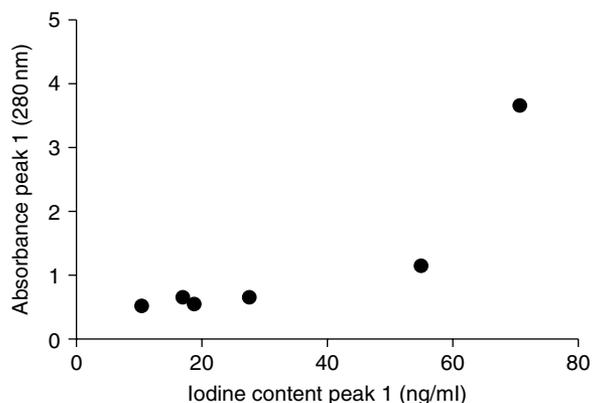


Figure 13.8 The association between the content of iodine and humic substances in tap water from six locations in Denmark. Source: Andersen *et al.*, (2002) reproduced with permission. Each dot represents one tap water sample. Spearman's ρ value was 0.85, $p = 0.03$.

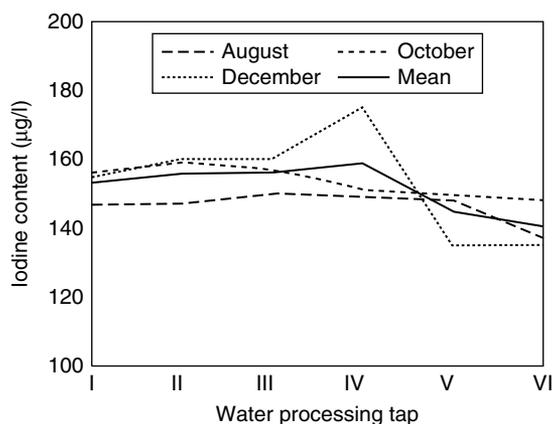


Figure 13.9 Iodine content at each step in processing of groundwater to drinking water at the waterworks in Skagen in August, October and December 1997. Tap I, groundwater; II, aeration; III, flocculation; IV, GAC; V, sand filtration; VI, chlorination.

A recent study of a population in Skagen, Denmark, calculated the available fraction of ingested iodine in humic substances to be around 85% (Andersen *et al.*, 2007). Thus, humic substances may influence the bioavailability of iodine, but this may differ between aquifers in accordance with the large diversity of humic substances.

Goiter and Iodine in Humic Substances in Drinking Water

Humic substances may affect thyroid gland morphology and function when included in the diet (Huang *et al.*, 1994; Seffner, 1995; Gaitan, 1990, 2000). However, the characteristics and concentration of humic substances in groundwater varies between aquifers depending on geology and geochemistry (Thurman, 1985; Grøn *et al.*, 1996), and definitive compositional differences have been shown

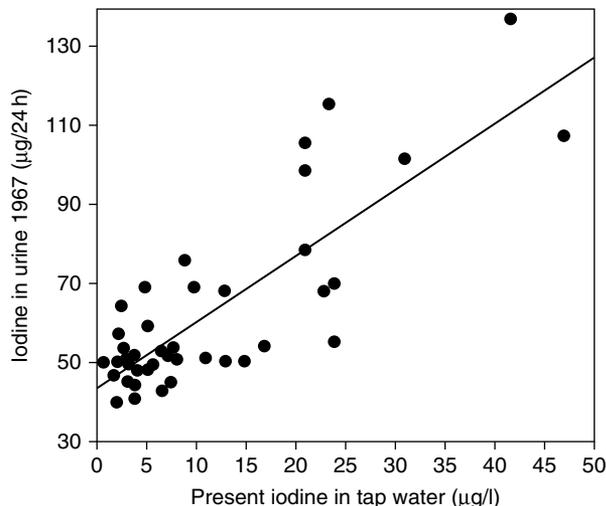


Figure 13.10 Correlation between iodine content of tap water in 41 towns in Denmark and the average 24 h urinary iodine excretion in young men in each town. Source: Pedersen *et al.*, (1999) reproduced with permission.

to exist between different sources (Malcolm, 1990). Also, the goitrogenic properties may differ between sites (Gaitan *et al.*, 1978; Gaitan, 1983). Furthermore, the physiological impact could be influenced by a reduced bioavailability of humic substances (Visser, 1973). Iodine has also been shown to counteract some antithyroid effects (Gaitan, 2000). As iodine and humic substances coexist in drinking water, the complex interaction of the factors mentioned above could explain at least some of the inconsistency in the relationship between iodine in drinking water and endemic goiter demonstrated in Table 13.1. Consequently, each source of iodine-rich waters should be evaluated separately.

Conclusions and Perspectives

The iodine content of drinking water varies considerably with geography but remains stable with time. The presence of iodine in natural waters indicates concurring humic substances derived from a marine source rock.

The association between iodine and goiter is well-established, while the relationship between iodine in drinking water and goiter is less clear-cut. Some humic substances possess goitrogenic properties, but differ widely in composition and hence in goitrogenic capacities, counteracted by the concurring iodine. Thus, the nature of iodine in drinking water hampers the validity of generalization of physiological effects expected from iodine in drinking waters.

Summary Points

- Around 1850 iodine in drinking water was suggested to be associated with the development of goiter and cretinism.

- The organic matter in the sea bottom (i.e., marine sediments) contains iodine which in turn leaches into the groundwater. Groundwater organic matter consists mainly of humic substances which are heterogenous mixtures of naturally occurring molecules which also have the capacity to complex trace elements.
- Iodine is abundant in the marine environment. Marine microorganisms assimilate and concentrate iodine up to 30 000 times.
- Marine organisms are contained in marine sediments, and iodine enters into sediments via the decay process. Iodine may also be deposited in marine sediments via muds.
- Geological events influence terrestrial subsurface iodine distribution. For example, in the glacial periods ice melted, the sea rose and the land was flooded. The subsequent uplift of the deglaciated terrain exposed large areas of sea floor.
- Denmark was characterized by repeated glaciation with depression of land, deglaciation, flooding and subsequent uplift of land. These geological events caused marine subsurface environment to be located above sea level.
- Geohistorically, reclamation and shore accumulation added to the subsurface marine environment. Aquifer source rock determines ground water composition.
- Most drinking water in Denmark is local ground water and drinking water iodine content exhibits regional differences due to geochemical variations.
- Organic matter and halogens are present in most aquifers in Denmark and the majority of organic matter is aquatic humic substances. Iodine-containing humic substances, and free iodine coexist in drinking water in Denmark.
- Organic matter in drinking water may cause coloring and taste- and smell unpleasantness. However, their removal reduces the amount of humic substances, as well as iodine content of drinking water.
- Humic substances may increase the occurrence of goiter, and the inconsistency in the association between goiter rate and iodine intake may be caused by humic substances.
- Humic substances may reduce the bioavailability of iodine in tap water, and cause some inconsistency in the association between iodine content of tap water and iodine excretion.
- The interaction of iodine and humic substances may be complex, and each source of iodine rich waters should be evaluated separately.

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Application of Iodine Water Purification Tablets: Iodine's Efficacy against *Cryptosporidium parvum*

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Abstract

Iodine has been used since the early 1800s in the treatment of water. Several different forms of iodine have been used, from Lugol's solution to globaline tablets to impregnated filters and resins. The use of iodine as a disinfection agent has been widespread; it has been proliferated for uses in austere environments by military soldiers, travelers, victims of emergency conditions (such as posthurricane disasters), and adventure enthusiasts (such as high-altitude hikers) who do not have access to readily potable water supplies. Like all other chemicals used as water disinfectants, the addition of iodine will not physically remove any contaminant of concern. However, it has been reported to be efficient against *Giardia lamblia* (Powers, 1991), with limited success against more resistant microbial pathogens such as *Cryptosporidium parvum*, a protozoan resistant to chemical disinfection (Starke *et al.*, 2005).

Abbreviations

ANOVA	Analysis of variance
CT	Contact time reported in mg-min/l
DBP	Disinfection by-products
HIO	Hypoiodous acid
I ₂	Elemental iodine
ID ₅₀	Infectious dose for 50% of the tested population
IDBP	Iodinated disinfection by-products

Iodine was discovered by Bernard Courtois in 1811 and soon used in the treatment of water. It was the introduction of Lugol's iodine (or Lugol's solution) in 1829 that offered a more readily available form for immediate disinfection of drinking water. Iodine has been used as a disinfectant in military applications since World War I (White, 1999). In 1952, the current form of globaline tablets was first integrated into field use for water treatment by the U.S. Army and the tablets were widely distributed to those operating in austere environments (Powers, 1991). Iodine

tablets are still used in situations where they may be the only barrier of protection against a suite of unknown biological contaminants. These tablets have been adapted for use in many commercial applications, ranging from backpacking and hiking to emergency preparedness kits. It is still common to see instructions to use Lugol's solution during emergency conditions.

There is a wide range of disinfectants used in water treatment to ensure the safety of water at the point of use, as well as throughout the entire purification and distribution process. As with all chemical disinfectants used in the water treatment industry, iodine has several advantages and disadvantages that must be considered. Iodine used for disinfection comes in readily available forms and is simple to use. It does leave an unpleasant taste and odor (not as severe as other chemicals), and care must be used in prescribing this treatment strategy to sensitive populations, such as those with existing thyroid conditions. As with other chemical disinfectants, there is much concern about the formation of disinfection by-products (DBP). These are regulated products that can be deleterious to human health with long-term exposure. Dodd (1997) studied the formation of iodinated disinfection by-products (IDBP) from a variety of potential precursors that might be found in the treatment of recycled wastewater on NASA spacecraft. Acetone is the only compound identified as an IDBP precursor and is reacted to produce iodoacetone and iodoform.

Iodine is still commonly used in liquid and solid forms for use in emergency preparation of drinking water sources. The solid forms are sold commercially by various vendors, and are widely distributed by the U.S. Army for use in austere environments. The U.S. Army iodine water purification tablets release 8 mg/l iodine per tablet (Department of the Army, 2002). Current guidelines specify two tablets per 1-quart canteen of water with a minimum contact time of 35 min to disinfect and prevent giardiasis (Department of the Army, 2002). Among the species of iodine, elemental iodine (I₂) and hypoiodous acid (HIO) are the forms with the greatest biocidal efficacy (White, 1999). At pH 8.0 and 25°C, the concentrations of I₂ and HIO are approximately equal (Snoeyink and Jenkins, 1980).

Table 14.1 Initial examination of the effects of globaline tablets

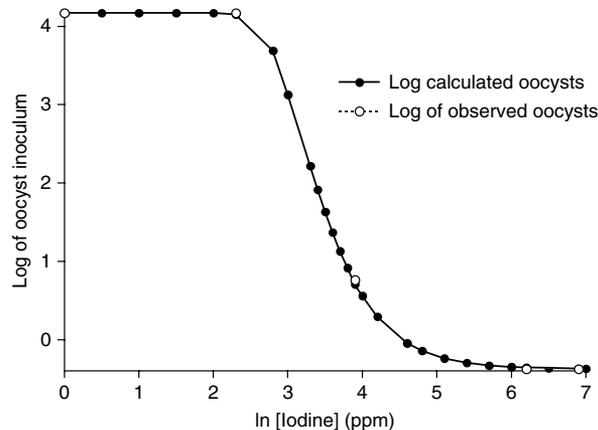
No. of pups/litter	Iodine concentration	No. of pups tested positive	% of pups tested positive
6	Low dose (8 mg/l)	6	100
10	Medium dose (16 mg/l)	10	100
N/A	High dose (32 mg/l)	ND*	ND*

Note: ND, no data. Interference from inert ingredients prevented inoculation.

Several investigators have reported different levels of inactivation for I_2 based on the type of organism being studied. Initial reports indicate that I_2 may have an increased effect upon organisms protected by a membrane such as a spore or cyst (White, 1999 and Gerba *et al.*, 1997a). Iodine has also been reported to inactivate the eggs of common nematodes at concentrations greater than 100 mg/l (Thitasut, 1961). Wilson and Margolin (2001) reported that iodine, as 10% povidone, decreased excystation of oocysts, but did not affect the infectivity of the oocysts in an *in vitro* assay. The biocidal efficacy of iodine against protozoa has been documented for *Giardia muris* (Powers *et al.*, 1994). However, the efficacy of iodine tablets against *Cryptosporidium* species has been reported in a limited number of studies. Using two different challenge waters and excystation to evaluate infectivity, Gerba *et al.* (1997b) reported that iodine tablets inactivated only 10% of the oocysts tested given a 20-min exposure, and approximately 70% given a 240-min exposure time.

A mice infectivity study was conducted by Starke *et al.* (2005) to assess the ability of globaline tablets to inactivate oocysts of *Cryptosporidium*. The *Cryptosporidium parvum* oocysts were obtained from naturally infected, 7- to 14-day-old calves and were used within 1 month of collection. Iodine stock solutions were prepared from Army disinfection tablets and 1.0N standardized solution with phosphate buffer. Disinfection experiments were carried out by combining the iodine stock solution with an oocyst concentration of 10^6 oocysts/10 ml and pH 7.06 and 7.15. A preliminary experiment using iodine tablets showed that there was no effect on *C. parvum* when exposed to two tablets (16 mg of I_2/l) for 35 min (Table 14.1). Higher concentrations could not be tested in this infectivity assay due to interference from the inert ingredients (83.3% by manufacturer standards) in the tablets. Subsequently, aqueous iodine solution was used to achieve the higher concentrations of iodine desired.

Twenty timed-pregnant female CD-1 mice with litters were inoculated with oocysts. For the dose titration curve in the mice pups, infective oocysts were diluted such that the mice received 2 μ l of water containing 0, 1, 10, 100, 1000, or 10000 oocysts. Oocysts from the test samples were diluted in water such that each mouse pup received

**Figure 14.1** Dose-response curve of mice infectivity data.

10000 oocysts to ensure infectivity. Based on a linear regression, a standard curve was prepared by analyzing the log oocyst dose vs. the proportion of mice infected to determine the infectious dose for 50% of the tested population (ID_{50}) to show positive results. An ID_{50} of 79 oocysts was calculated, which was within the previously reported ranges (Jenkins *et al.*, 1997). Of the 20 litters of 10 pups that were given oocysts during the course of this trial, only two litters lost pups after inoculation. It should be noted that pup loss is not unexpected and appears to be a random factor associated with the use of neonatal mice that are handled as part of the experimental process (Figure 14.1).

For the disinfection experiments, two litters of 10 mice were each given 10000 oocysts that had been treated with 1, 10, 50, 100, 500, or 1000 ppm of iodine. One litter of 10 mice each received 10000 oocysts treated with iodinated water. Seven days after infection, the mouse pups were euthanized for the collection of the cecum and colon. The results indicating infectivity after ingestion of the chemically treated solutions for each of the litters are reported in Table 14.2. The number of oocysts inactivated by a dose of iodine plotted against the proportion of mice infected by the oocysts is presented in Figure 14.2. The data indicated a sigmoidal form and was fitted with the Gompertz equation to generate log-calculated oocyst concentration:

$$F(x) = A \exp(-e^{(\beta - \kappa x)})$$

where $A = 1$, $\beta = 5.950 \pm 0.1996$, $\kappa = 1.854 \pm 0.0591$, $F(x)$ is the proportion of mice infected by oocysts that was converted to log number of infectious oocysts, and x is the natural log of the iodine concentration. Based on this nonlinear regression, ~ 30 ppm iodine resulted in 50% of the mice inoculated with a batch of 10^4 oocysts becoming infected, or a reduction of the viable oocyst concentration to ~ 79 oocysts, or at least a two-log reduction of the original inoculum. The model predicts that an iodine concentration of ~ 123 ppm will reduce the number of infectious

Table 14.2 Effects of iodine concentration on the percentage of mouse pups per litter tested positive

Target iodine concentration (mg/l)	Actual iodine (mg/l)	CT (mg-min/l)	No. of pups tested positive	Total pups examined	% pups tested positive
0	0	0.0	10	10	100
1	0.42	14.7	10	10	100
1	0.46	16.1	10	10	100
10	1.0	35.0	10	10	100
10	1.52	53.2	10	10	100
50	11.90	416.5	3	10	30
50	14.18	496.3	2	10	20
100	30.6	1071.0	1	10	10
100	34.4	1204.0	0	10	0
500	228	7980.0	0	10	0
500	260	9100.0	0	10	0
1000	598	20930.0	0	10	0
1000	504	17 640.0	0	10	0
16 ^a	4.3 ^a	150.5	8	9	88.9

Notes: Actual iodine concentration was measured after the 35-min reaction time. Contact time (CT) was calculated as the product of the measured iodine concentration and the 35-min reaction time. Each mouse pup received 10000 treated oocysts.

^aDepicts the use of globaline tablets.

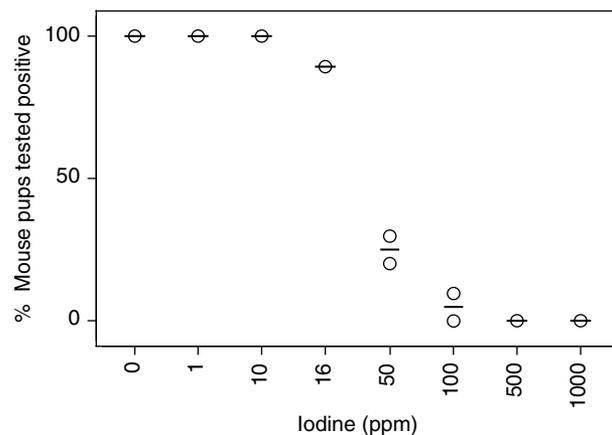


Figure 14.2 ANOVA analysis of mice infectivity data. The circle represents the percentage of mouse pups per litter that tested positive. Group means are indicated by lines.

oocysts to <1, which is less than the range reported to cause human infection (Okhuysen *et al.*, 1999).

The analysis of variance (ANOVA) comparing the percentage of mouse pups per litter which were given oocysts treated with globaline tablets or iodine revealed that the effects of the globaline tablets were not significantly different than oocysts treated with 0, 1, or 10 ppm iodine (Figure 14.2, $p < 0.0005$). Oocysts treated with greater than 50 mg/l of iodine demonstrated reduced infectivity. No reduction in oocyst infectivity was observed for concentrations greater than 100 mg/l, as demonstrated by the analysis of variance procedure. The results of the ANOVA (Figure 14.2) are in good agreement with the regression equation (Figure 14.1)

in the vicinity of 30 mg/l of iodine. At least 29 mg/l with a 35 min contact time (or 1015 mg-min/l) is required to achieve a 2-log (90%) inactivation with iodine.

Despite the relative ineffectiveness for chemical disinfectants including iodine, in order to achieve the required 2-log inactivation of *C. parvum*, there are several applications that may incorporate its use in future designs. These designs are for personal water purification devices that incorporate a physical removal process (filtration) followed by the use of a chemical disinfectant. Iodine seems to be a popular choice, due to its relative stability and proven success against bacterial and viral contaminants. Iodine is used to impregnate the materials used in purification systems, such as a resin, or a physical removal system, such as a straw. The combination of physical and chemical processes does warrant further consideration to improve the achieved level of protection.

Summary Points

- Iodine has been used as a disinfectant in the treatment of water for over 180 years.
- The use of iodine, like all disinfectants, has its advantages and disadvantages. These must be evaluated for populations that may have sensitivity to iodine, such as a thyroid condition.
- Iodine is a relevant disinfectant for those operating under austere or emergency conditions, such as military, adventure hikers and emergency responders. However, the current prescribed concentration and operating contact times may not be adequate to provide protection against *C. parvum*.

- The use of iodine to enhance protection from microbial pathogens should be combined with other purification processes such as filtration.
- *C. parvum* is a virulent microbial pathogen that demonstrates resistance to chemical disinfectants at doses previously believed to be adequate against similar species. A mice infectivity study outlined above indicates an increased dose requirement to achieve desired regulatory inactivation goals.
- A dose of 29 mg/l of iodine with a contact time of 35 min is required to achieve a 2-log (99%) inactivation of *C. parvum* in demand-free water. Increased dose requirements to achieve the same inactivation can be expected in challenge waters with increased concentrations of precursor materials.

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Iodine Speciation in Foodstuffs, Tissues, and Environmental Samples: Iodine Species and Analytical Method

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Abstract

Different species of iodine are involved in the transport of iodine from air, soil and water to food, and from food to the human body. In water, most iodine occurs as iodide and iodate while, in some cases, the concentration of organic iodine may be high. In the air, iodine exists as particle associated iodine, inorganic gaseous iodine (I_2 , HIO), and organic gaseous iodine (CH_3I , CH_2I_2 , etc.). In the body of humans and other mammals, iodine is utilized by the thyroid gland for the biosynthesis of the thyroid hormones T_4 and T_3 . Besides T_3 and T_4 , iodine also exists as MIT, DIT, T_3 , and rT_3 , which are mainly bound with proteins in thyroid and other tissues, but function as free T_3 and T_4 . In milk and urine, most iodine occurs as iodide, but some species of organic iodine were also found. In seaweed, iodine species varies widely with the species of seaweed. In brown seaweed, most of iodine exists as iodide; while in green seaweed, iodine is mainly bound to organic molecules, such as protein and polyphenol. Iodine species in fish are similar to that in the human body. The most commonly used methods of assay are chromatographic techniques, such as anion exchange, size exclusion and reverse-phase chromatography, coupled with ICP-MS detection. This chapter presents the analytical methods for iodine species in water, air, tissues and biological samples, and also in foodstuffs and environmental samples. The bioavailability and toxicity of different iodine species are also discussed.

Abbreviations

CE	Capillary electrophoresis
DIT	Diiodotyrosine
GC	Gas chromatography
HPLC	High-performance liquid chromatography

IC	Ion chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
IIH	Iodine-induced hyperthyroidism
LC	Liquid chromatography
MIT	Monoiodotyrosine
NAA	Neutron activation analysis
RP-HPLC	Reverse-phase high-performance liquid chromatography
rT_3	Reverse triiodothyronine
SEC	Size exclusion chromatography
T_3	Triiodothyronine
T_4	Thyroxine

Introduction

Iodine is an essential element in humans and other mammals, which is used for the synthesis of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4). These hormones play a prominent role in the metabolism of most cells of the organism and in the process of early growth and development of most organs, especially brain (Anderson *et al.*, 2000). Besides T_3 and T_4 , reverse T_3 (rT_3), monoiodotyrosine (MIT), and diiodotyrosine (DIT) are also synthesized and distributed in the body of humans and animals, but only T_3 and T_4 have a biological function. Iodine in the human body mainly comes through dietary and water intake, and inhalation of atmospheric iodine. Due to low concentrations of iodine in the air (10–20 ng/m³), food and water intake form the major source of iodine for adults, while for infants it is milk. The concentration of iodine in foodstuffs is directly related to that in the environment where the foods come from. Iodine deficiency disorders are mainly found in places where the concentration of iodine in the soil and drinking water is very low. In the water, foodstuffs, and

Table 15.1 Iodine species in nature

Name	Chemical formula	Samples
Iodide	I ⁻	Water, plant, animal
Iodate	IO ₃ ⁻	Water
Elemental iodine	I ₂	Air
Periodate	IO ₄ ⁻	Water
Hypiodite	IO ⁻	Air, water
Methyl iodide	CH ₃ I	Air, water
Methyl di-iodide	CH ₂ I ₂	Air, water
Ethyl iodide	C ₂ H ₅ I	Air, water
Propyl iodide	C ₃ H ₇ I	Air, water
Butyl iodide	C ₄ H ₉ I	Air, water
Methyl iodide bromide	CH ₂ BrI	Air, water
Triiodothyronine	T ₃ (Figure 15.6)	Animal, plant, milk
Thyroxine	T ₄ (Figure 15.6)	Animal, plant, milk
Monoiodotyrosine	MIT	Animal, plant, milk
Diiodotyrosine	DIT (Figure 15.6)	Animal, plant, milk
Reverse triiodothyronine	rT ₃ (Figure 15.6)	Animal, plant, milk
Particle-associated iodine	–	Air, water

Note: The name and chemical formulas of the iodine species occurring in nature are shown; the possible sample types in which the species exist are also shown in the third column.

environmental samples, iodine exists in different species, such as iodide, iodate and in association with various organic compounds. Table 15.1 shows the various iodine species in nature. The species of iodine in the water and environmental samples is related to the level of iodine in plants and foodstuffs. The species of iodine in foodstuffs directly affects the bioavailability of iodine to humans and animals. This article reviews the speciation of iodine in water, air, foodstuffs and biological and environmental samples. In addition, the bioavailability and toxicity of iodine species are also discussed.

Speciation of Iodine in Water

Interest in the species of iodine in water has increased in the past decades. Water is considered to be an important source of iodine for humans and animals (Rasmussen *et al.*, 2000); it also supplies iodine to plants.

Distribution of iodine species

The iodine species present in water depend on the nature of the water. In seawater, iodine mainly exists as iodate, iodide, and minor organic iodine (Wong, 1991). The distribution of iodine species in seawater depends on the water chemistry, and varies with depth and geographic location. In anoxic water, most iodine exists as iodide, such as in the Baltic Sea and the Black Sea (Luther *et al.*, 1991; Truesdale *et al.*, 2001; Hou *et al.*, 2001). While in oxygenated/oxic

water the concentration of iodate is high, the concentration in the open sea is <0.01–0.2 μM for iodide and 0.2–0.5 μM for iodate. Iodide maxima are often found in surface water. Below the euphotic zone, iodide decreases to below 0.01 μM. Higher iodide concentrations are normally found in coastal and estuary areas; Figure 15.1 shows the distribution of iodide/iodate in the surface water of the North Sea (Hou *et al.*, 2007).

Organic iodine found in coastal and estuary areas corresponded to 5–40% of total dissolved iodine (Schwehr and Santschi, 2003; Jones and Truesdale, 1984; Cook *et al.*, 2000). A few specific organic iodine compounds, mainly volatile compounds, have been identified, such as CH₃I, CH₂ClI, CH₂I₂, and CH₃CH₂CH₂I (Schall *et al.*, 1997; Moore and Graszko, 1999). Although the concentration of organic iodine in seawater is low, it plays an important role in the global geochemical cycle of iodine, because the transfer of iodine from the iodine-rich ocean to the atmosphere, and then to the terrestrial environment, is thought to occur primarily through the volatilization of organic iodine hydrocarbon in seawater (Campos *et al.*, 1996). These volatile organic iodine species were also supposed to relate to the ozone depletion in the stratosphere (Solomon *et al.*, 1994). In freshwater, iodine also exists as iodide iodate and organic iodine, but the concentration of organic iodine is normally relatively high in freshwater, such as river water, lake water and rain (Gilfedder *et al.*, 2007; Abdel-Moati, 1999; Reifenhauer and Heumann, 1990; Truesdale and Jones, 1996).

Speciation analysis of inorganic iodine

A number of analytical methods have been reported for the speciation analysis of iodine in water. In seawater, iodate can be directly determined by titrimetry, colorimetry, and differential pulse polarography methods (Wong and Cheng, 1998; Luther *et al.*, 1991; Herring and Liss, 1974; Stipanicev and Branica, 1996), and iodide by cathodic stripping voltammetry, chromatography, and inductively coupled plasma atomic emission spectrometry or mass spectrometry (ICP-MS) (Tian and Nicolas, 1995; Brandao *et al.*, 1995; Anderson *et al.*, 1996; Radlinger and Heumann, 1997). Organic iodine was normally given as the difference between total iodine (sum of all forms of inorganic and organic iodine) and total inorganic iodine (IO₃⁻ + I⁻), in which the concentration of total iodine was determined as IO₃⁻ or I⁻ after organic iodine had been decomposed and converted into IO₃⁻ or I⁻ by UV irradiation or/and redox treatment (Luther *et al.*, 1991).

In addition, chromatographic separation combined with detection methods, such as neutron activation analysis (NAA) and ICP-MS, was also reported for chemical speciation analysis of iodine in water (Hou *et al.*, 1999b; Reifenhauer and Heumann, 1990; Schwehr and Santschi, 2003). In

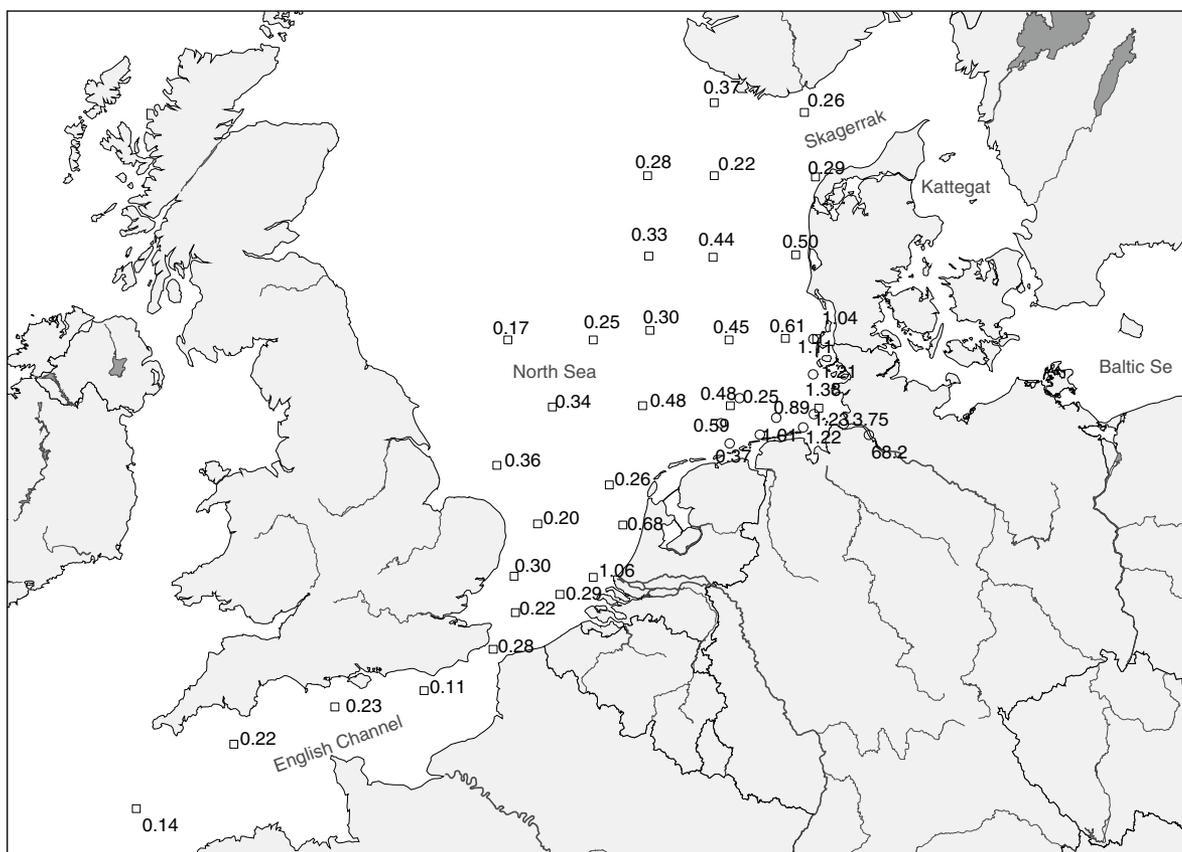


Figure 15.1 Distribution of iodide and iodate in the surface water of the North Sea. The ratios of iodide/iodate in the surface water collected from the North Sea in 2005 are mapped; a high iodide concentration was seen in the coastal area, especially in the German Bight.

this method, iodide and iodate are separated by an anion exchange column. Due to different affinities, iodate passes through the column, while iodide is strongly adsorbed, which is then eluted from the column using a higher concentration of nitrate (2–2.5 mol/l). Organic iodine may not be absorbed on the column. After iodate is reduced to iodide, the water is passed through an anion exchange column; the inorganic iodine is absorbed onto the column, whereas the organic iodine remains in the effluent. The separated iodine is then detected using NAA or ICP-MS.

Speciation analysis of volatile organic iodine

Volatile organic iodine compounds have been determined by gas chromatography (GC) with MS (Schall *et al.*, 1997). Figure 15.2 shows the schematic of the analytical procedure. Seawater (50–100 ml) is injected into the purging unit of sample treatment with a syringe, where it is degassed; the degassed substances are then transferred with helium into a cold trap, which is cooled with liquid nitrogen. After degassing, the trapped substances are transferred to the separation column by removing liquid nitrogen and heating the trap. The organic iodine compound is separated

using a capillary column in GC and heating the trap at different temperatures, ranging from 40 to 240°C. The detection is performed with an electron capture detector (ECD) and/or ICP-MS; the ICP-MS is more sensitive for iodine than the ECD. The absolute detection limit for ICP-MS is 0.5 pg for iodinated volatile organic compounds. Figure 15.3 shows a chromatographic spectrum of iodinated and brominated volatile organic compounds measured by GC-ICPMS/ECD (Schwarz and Heumann, 2002).

Speciation analysis of iodine in freshwater

The concentration of iodine in freshwater is normally much lower than that in seawater; however, in some particular regions, the groundwater may contain higher levels of iodine. The iodine in precipitation and river water is normally 0.5–5 ng/l, which is more than 10 times lower than that in seawater. The more sensitive ICP-MS and NAA are normally used for the speciation of iodine in freshwater (Reifenhauser and Heumann, 1990; Wuilloud *et al.*, 2003; Hou, 2004). Reifenhauser and Heumann (1990) developed a method by combining isotope dilution mass spectrometry

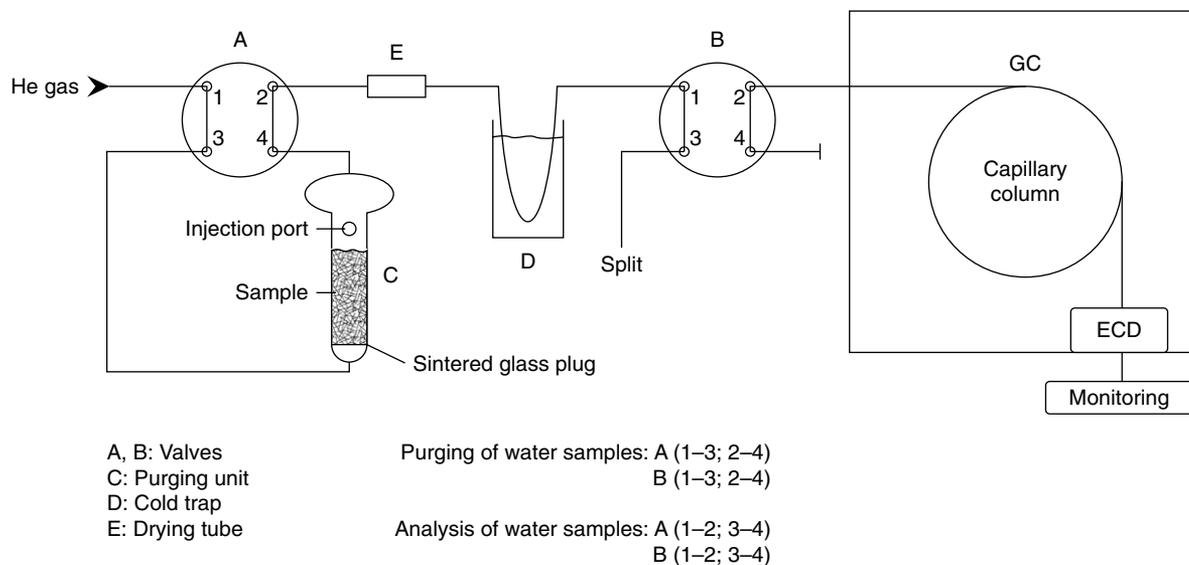


Figure 15.2 A schematic of the analytical purge and trap – GC system for the determination of volatile organic iodine in seawater. Reproduced from Schall *et al.* (1997). The water sample is injected into column C with a syringe and then transferred with helium from valve A through column C and dry tube E and collected into a cold trap, D, which is cooled with liquid nitrogen. After degassing, the trapped substances are transferred to the separation column in a gas chromatography system by removing liquid nitrogen and heating the trap. The organic iodine compound is separated using a capillary column in a gas chromatograph and heating the trap at different temperatures from 40 to 240°C, and detected with an electron capture detector (ECD) and/or ICP-MS.

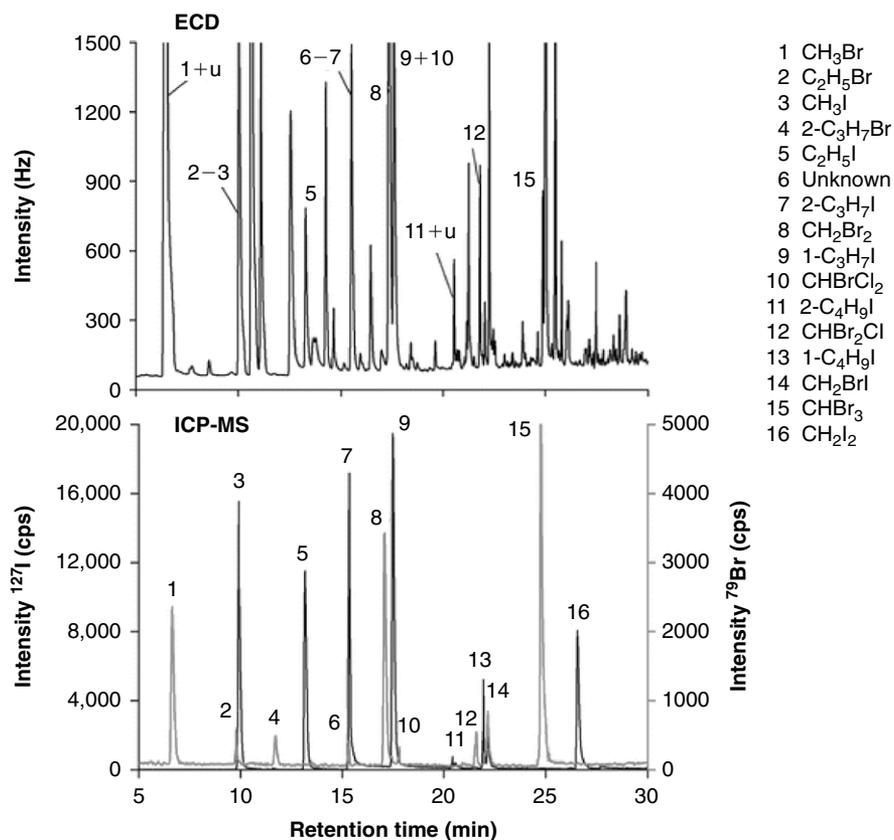


Figure 15.3 Simultaneous ECD and ICP-MS chromatograms of a seawater sample for the determination of brominated and iodinated volatile organic compounds. Reproduced from Schwarz and Heumann (2002). The top figure shows the chromatograms of brominated and iodinated volatile organic compounds in seawater measured by ECD, the bottom figure shows the chromatogram measured by inductively coupled plasma mass spectrometry (ICP-MS).

(IDMS) with anion exchange separation to investigate the species of iodine in freshwater. Besides iodide and iodate, anionic organic iodine and nonelutable organic iodine were also determined. They observed that most iodine existed as organic iodine in lake and river water. Wuilloud *et al.* (2003) and Kannamkumarath *et al.* (2004) developed an analytical methodology using GC or capillary electrophoresis (CE) coupled to ICP-MS to analyze iodophenols, such as 2-iodophenol, 4-iodophenol, and 2,4,6-triiodophenol. Solid-phase microextraction (SPME) was used for preconcentration of the iodophenols from water samples; the concentrated substances were then desorbed from the GC injector at 290°C, or injected into a CE system for separation of different species of iodophenols. The separated species was then introduced to ICP-MS for quantitative detection of iodine. The method was applied for speciation analysis of iodophenols in river, tap and bottled water samples. Radlinger and Heumann (1997, 2000) developed a method for the separation of different fractions of humic substances (HS) by their molecular weight using size exclusion chromatography (SEC). The separated iodine in different species of HS was detected by online coupling of SEC with ICP-MS. The natural water and wastewater were analyzed. In the case of natural samples, iodide was exclusively fixed by HS fractions where natural HS/iodine species have also been observed in the original sample. In wastewater samples collected from sewage disposal plants, organoiodine compounds were also identified which were not affected by the transfer of iodide.

Chemical Speciation of Iodine in Air

Iodine species

The concentration of iodine in the atmosphere ranges from 0.2 to 10 ng/m³; a high iodine concentration is observed in urban areas due to combustion of oil and coal. In the atmosphere, iodine exists as particle-bound iodine (particulate iodine), inorganic gaseous iodine (I₂, HI, HOI) and organic gaseous iodine (CHI₃, CH₂I₂, CH₃CH₂CH₂I); their concentrations vary with various parameters, such as location, season and climate (Noguchi and Murata, 1988; Gabler and Heumann, 1993; Yoshida and Muramatsa, 1995; Rahn *et al.*, 1976).

Speciation analysis of iodine

A series filter was used to separate atmospheric particulate iodine, HI and I₂, HOI and organic iodine. The particulate iodine is usually separated and collected using micropore filters or glass microfibrer. The distribution of iodine in the different sizes of particulates is collected by a multi-stage cascade impact collector. Wimschneider and Heumann (1995) used a six-stage slot cascade impactor to collect particulates

with an aerodynamic diameter of 7.2, 3.0, 1.5, 0.95, 0.45, and <0.45 μm. Iodine concentration in the collected particulates was measured by isotope dilution ICP-MS. The soluble species of iodine in the air particulates was investigated by leaching the particulates with water, and determination of iodide and iodate in the leachate. It was reported that iodide dominates the soluble iodine in the air particles (Baker, 2004; Baker *et al.*, 2001). I₂ and HI can be separated by adsorption using a LiOH (NaOH)-impregnated filter (Gabler and Heumann, 1993; Yoshida and Muramatsa, 1995) or silver screens (Noguchi and Murata, 1988). HOI is normally collected by tetrabutylammonium hydroxide (TBAH)-impregnated filter or charcoal filter, and organic iodine by a charcoal bed or triethylene diamine (TEDA)-impregnated charcoal bed (Noguchi and Murata, 1988; Gabler and Heumann, 1993; Yoshida and Muramatsa, 1995; Wershofen and Aumann, 1989). Figure 15.4 shows a diagram of a multi-stage sampler for iodine in air. The separated species of iodine were determined by NAA or ICP-MS.

Speciation analysis of volatile iodine

GC combined with ICP-MS or ECD is quite often used for the determination of volatile organic iodine. A system similar to that used for seawater was used for the analysis of air samples (Schwarz and Heumann, 2002). Wevill and Carpenter (2004) reported a similar system for the speciation

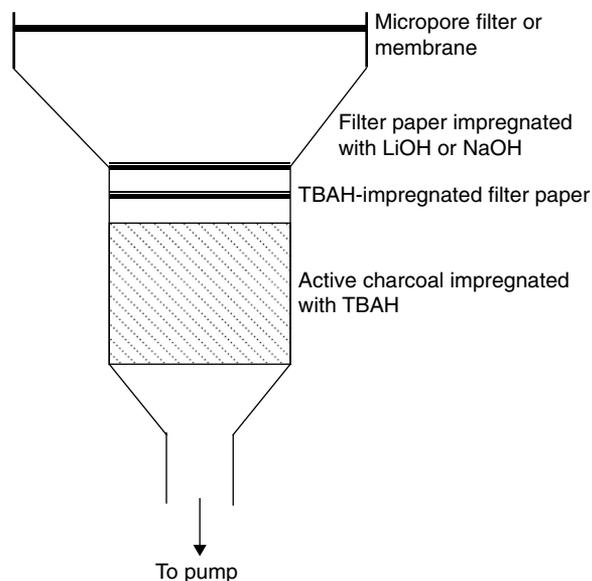


Figure 15.4 A diagram of an air sampler for the collection of different species of iodine from air. The air is sucked by a pump through the system. The particle-associated iodine is collected in the first micropore filter; the gaseous elemental iodine I₂ and HI are absorbed in the second filter, made of cellulose and impregnated with LiOH or NaOH solution; the gaseous HOI is absorbed on the third filter, made of cellulose and impregnated with TBAH solution; and various volatile iodated organic compounds, such as CH₃I and C₂H₅I, in the remaining gas are absorbed in an active charcoal column impregnated with TBAH.

analysis of volatile organic halocarbons, such as CH_3I , CHCl_3 , $\text{C}_2\text{H}_5\text{I}$, $2\text{-C}_3\text{H}_7\text{I}$, CH_2Br_2 , CH_2ClI , CHBr_2Cl , $1\text{-C}_3\text{H}_7\text{I}$, CH_2BrI , CHBr_3 and CH_2I_2 . Detection limits were between 0.02 and 0.12 pptv (parts per trillion by volume).

The photolysis of volatile gaseous iodine could generate I, which would interact with atmospheric species such as O_3 , H_xO_y , and NO_x to produce IO, HOI, ION_2 and I_2 . Production and cycling back to I could cause catalytic removal of tropospheric O_3 . A mixing ratio up to 6.6 ppt

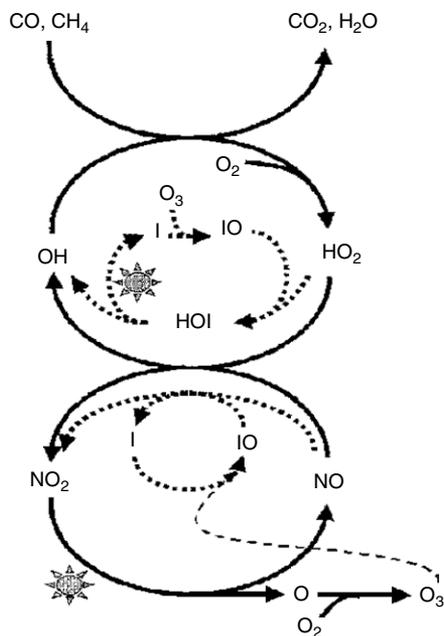


Figure 15.5 A schematic of the photochemical reaction process of iodine species in the production and removal of ozone. The figure shows the reaction process of iodine species in the removal of O_3 : $\text{O}_3 + \text{I} = \text{IO} + \text{O}_2$; $\text{HO}_2 + \text{HO}_2 + \text{IO} = \text{HOI} + \text{O}_2$; $\text{HOI} + h\nu = \text{OH} + \text{I}$; $\text{IO} + \text{NO} = \text{I} + \text{NO}_2$.

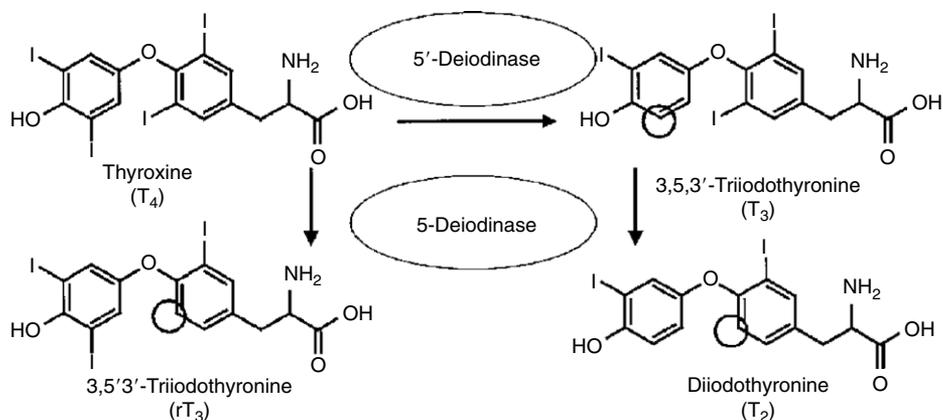


Figure 15.6 Structure of iodinated hormones and their metabolism process. Thyroxine (T_4) synthesized in the thyroid was deiodinated in the blood and tissues by 5-deiodinase to 3,5,3'-triiodothyronine (rT_3) and by 5'-deiodinase to 3,5,3'-triiodothyronine (T_3), which is then further deiodinated by 5-deiodinase to diiodothyronine (T_2).

has been measured at Mace Head, Ireland. **Figure 15.5** shows a schematic of photochemical reaction of iodine species in the removal of ozone (Stutz *et al.*, 1999).

Chemical Speciation of Iodine in Tissues and Biological Samples

Species of iodine

Iodine is known to be utilized by the thyroid gland for biosynthesis of the thyroid hormones T_4 and T_3 . These hormones have an important influence on a wide of biochemical reactions. Besides T_3 and T_4 , iodine also exists as MIT, DIT, T_3 and rT_3 , which are mainly bound with proteins in thyroid, but also function as free T_3 and T_4 . **Figure 15.6** shows the structure of iodinated hormones and their metabolism. Beside thyroid, iodine is also distributed in many other tissues (Hou *et al.*, 1997a).

Speciation analysis of iodine in tissues

Radioimmunoassay methods are widely used for the determination of T_3 , T_4 and rT_3 in blood for diagnosis of thyroid diseases. Hou *et al.* (1999a) investigated the distribution of iodine in various subcellular fractions of human liver using gradient centrifugation coupled with NAA, and observed that iodine content is in the order of nuclei > cytosol > mitochondria > lysosome > microsome (Table 15.2). The proteins in the liver cytosol were fractionated by 2.0, 3.0 and 4.0 mol/l $(\text{NH}_4)_2\text{SO}_4$ precipitation successively into four parts, including three precipitates and a supernatant. Each was dialyzed (MWCO = 8000–10000) against distilled deionized water to remove the salt. They reported that most of the iodine bound to proteins mainly in the 4.0 mol/l $(\text{NH}_4)_2\text{SO}_4$ precipitate; only a small part existed as inorganic ions or small molecular bound states. They further

Table 15.2 Distribution of iodine in subcellular fractions of human liver

Fraction	Weight percentage in total liver (%)	Iodine concentration ($\mu\text{g/g}$ dry mass)		Percentage of iodine in total liver (%)
		Fraction	Protein	
Liver homogenization	–	0.321	4.472	–
Nuclei	26.0	0.932	6.413	48.0
Mitochondria	8.4	0.825	7.015	15.7
Lysosome	4.0	0.681	9.283	10.6
Microsome	3.3	0.171	1.471	1.0
Cytosol	58.3	0.135	1.120	17.7

Notes: The subcellular fractions of human liver were separated by ultracentrifuge. The second column shows the weight percentage of individual fractions in the entire liver sample. The third column shows the iodine concentration ($\mu\text{g/g}$ dry mass) in different fractions as well as in the original liver homogenization. The fourth column shows iodine concentration in the proteins, which is calculated from the protein and iodine concentrations in the different fractions. The fifth column shows the percentage of iodine of the various fractions in the human liver sample.

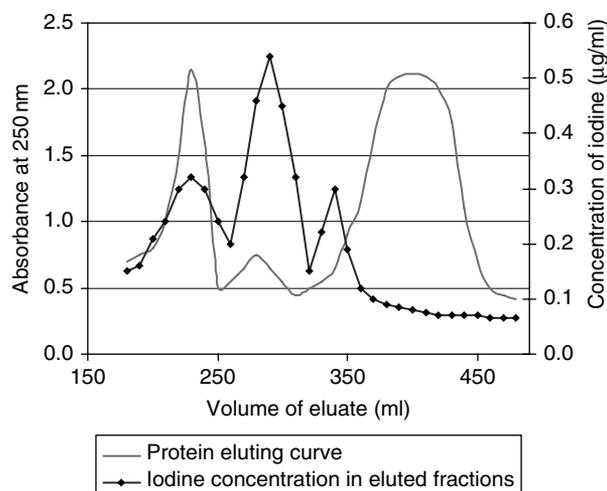


Figure 15.7 Distribution of iodine-bound proteins in the cytosol of human liver. The protein in the cytosol of human liver was separated by gel chromatography (Sephadex G-2000); the protein was directly monitored by UV detector at 280 nm. The chromatogram of protein is shown in the protein elution curve; iodine-associated proteins are shown in the curve of the iodine in the eluted fraction.

investigated the iodine-bound proteins in the cytosol of human liver using gel chromatography and observed three iodine proteins, in which iodine is mainly bound with mid- and high-molecular-weight proteins (Figure 15.7).

Speciation analysis of iodine in hydrolyzed solution of tissues, urine and serum

Because most iodine is bound with macromolecular protein, the sample has to be enzymolyzed; then iodine ion and

various iodo-amino acids can be separated and measured for analysis of the concentrations of iodo-amino acids. High-performance liquid chromatography (HPLC) was successfully used to determine various chemical species of iodine, such as I^- , MIT, DIT, T_3 , T_4 , rT_3 and iodo-polypeptide in hydrolyzed solution of thyroid, tissues, serum and urine (Meurizis *et al.*, 1982; Michalke *et al.*, 2000; Leiterer *et al.*, 2001; Takatera and Watanabe, 1993). Takatera and Watanabe (1993) used two similar RP-HPLC-ICP-MS methods to measure iodine species in thyroid gland protease digestion – C_{18} 3.5×0.4 cm CAPCELL column and a low MeOH concentration was used for the speciation analysis of small iodine species such as iodide, MIT and DIT, whereas a high MeOH concentration was used for speciating rT_3 , T_3 and T_4 . Michalke *et al.* (2000) modified this method to analyze six iodine species in a single run. The method was applied to urine and “normal” or pathological serum. In urine, predominantly iodide was seen. In human serum the six iodine species were seen in a range: $10 \mu\text{g/l}$ for iodide; $\sim 1.5 \mu\text{g/l}$, MIT and DIT; $3 \mu\text{g/l}$, rT_3 ; $5 \mu\text{g/l}$, T_3 ; and $45 \mu\text{g/l}$, T_4 . However, the pathological serum was different. T_4 was drastically reduced and T_3 was almost totally lacking, which was considered a severe health risk. In contrast, rT_3 was found in great excess.

Michalke and Schramel (1999) reported a method of CE coupled to ICP-MS for the speciation analysis of iodine. A buffer comprising phosphate (pH 2.3), NaOH, sodium dodecyl sulfate (SDS) and borate (pH 8.3) for stacking was employed for the separation of iodide, iodate, T_4 and T_3 . The separated four iodine species were subsequently detected during a pressure-driven detection step (baseline-separated) at 19.5, 29.1, 36.6 and 42.2 s. The detection limits were determined at $0.08 \mu\text{g I/l}$ (iodide), $0.3 \mu\text{g I/l}$ (iodate), $3.5 \mu\text{g I/l}$ (T_4) and $2.5 \mu\text{g I/l}$ (T_3). This method has been applied for iodine speciation in human serum and urine. The serum from a healthy person contained iodide ($13 \mu\text{g I/l}$), T_4 ($61 \mu\text{g I/l}$) and T_3 ($7.5 \mu\text{g I/l}$), whereas the serum from a thyroid-operated person lacked T_3 .

Speciation analysis of iodine in human urine samples has also been carried out by ion chromatography (IC) combined with ICP-MS (Stark *et al.*, 1997). Because of the possible interconversion of the iodine species, depending on the pH value, different eluent–column combinations were used for acidic or alkaline sample solutions. Iodide, iodate and several unidentified, presumably organo-iodine species could be separated and detected.

Chemical Speciation of Iodine in Foodstuffs and Environmental Samples

The source of iodine for humans is mainly foodstuffs, as well as drinking water. The investigation on speciation of iodine in foodstuffs mainly focused on milk and

seafood (seaweed and fish), because of the importance of milk iodine to humans, especially to infants, and the high concentration of iodine in these types of foodstuffs. In addition, lack of iodine supplementation to newborns can result in slow brain development, leading to severe damage to the central nervous system (Brätter *et al.*, 2000). It is therefore supposed that an iodine transporter and a peroxidase enzyme are involved in iodine accumulation in mammary glands. These facts make iodine speciation an important factor in human milk. Further, the bioavailability of various species of iodine may be quite different, especially for iodine-bound macromolecules. Investigation on speciation in different types of milk and formula, and other kinds of food, becomes important to accurately estimate the status of iodine nutrition.

Speciation analysis of iodine in milk

Brätter *et al.* (1998) developed an online method for the investigation of iodine species in human milk – SEC separation coupled with ICP-MS detection. They reported that 80% of iodine in human milk was present as iodide; besides iodide, another six high-molecular-weight iodine-containing molecules (5–300 kDa) were also observed. The total iodine in European breast milk samples was determined to be $95 \pm 60 \mu\text{g/l}$.

Michalke (2006) also investigated iodine species in breast milk. He reported that total iodine varied according to lactation state, beginning at 60 mg/l on the 2nd day (postpartum) reaching 100 $\mu\text{g/l}$ on the 3rd day, and decreasing to 80 mg/l (6th day) or 60 mg/l constantly from 9th to the 60th day. A prefractionation by centrifugation showed that iodine is associated with fat at approximately 30%, and 70% of the low-molecular-weight fraction. Speciation analysis of iodine in milk whey (pooled human milk) was carried out by SEC-ICP-MS and IC using a strong anion exchange column combined with ICP-MS. The SEC showed predominantly iodide with about 37 $\mu\text{g/l}$, as well as two more iodine species with 1.5 and 1.0 $\mu\text{g/l}$ having retention times pointing to T_4 and T_3 . IC-ICP-MS results indicated iodide to be the major iodine species in human milk. Leiterer *et al.* (2001) also used an IC coupled with ICP-MS for the speciation of iodine in human milk. They also observed that iodide is the main iodine species in milk, but in a few samples it also has traces of iodate and several unidentified, presumably organoiodine, compounds.

Sanandez and Szpunar (1999) determined iodine species in milk and infant formulas using SEC-ICP-MS. Iodine species were quantitatively eluted with 30 mM Tris buffer within 40 minutes and detected by ICP MS with a detection limit of 1 $\mu\text{g/l}$ (as I). A systematic study of iodine speciation in milk samples of different animals (cow, goat), humans of different geographic origin (several European

countries) and in infant formulas from different manufacturers was carried out. Whey obtained after centrifugation of fresh milk or reconstituted milk powders contained more than 95% of the iodine initially present in milk in all the samples investigated, with the exception of the infant formulas in which only 15–50% of the total iodine was found in the milk whey. An addition of sodium dodecyl sulfonate (SDS) considerably improved the recovery of iodine from these samples into the milk whey. Iodine was found to be present principally as iodide in all the samples except infant formulas. In the latter, more than half of the iodine was bound to a high molecular weight (>1000 kDa) organic molecules.

Speciation analysis of iodine in fish

Simon *et al.* (2002), using LC-ICP-MS, investigated iodine speciation in whole-body homogenates of adult male and female zebrafish (*Danio rerio*) and tadpoles of the African clawed frog (*Xenopus laevis*) at two different developmental stages (NF58 and 61) according to Nieuwkoop and Faber. A Capcell-C18 column and a mobile phase comprising Tris-HCl and methanol were used for chromatographic separation. Iodide, MIT, DIT, T_4 , T_3 and rT_3 were observed in these samples. In addition, another five species of iodine were also identified in the samples.

Speciation analysis of iodine in seaweeds

Due to the high concentration of iodine in marine vegetation, the chemical species of iodine in plants is mainly focused on seaweed. Hou *et al.* (1997b, 2000) developed a method for the determination of various chemical species of iodine in seaweed, such as water-soluble iodine, soluble organic iodine, iodide, iodate and protein-, pigment-polyphenol-, or polysaccharide-bound iodine. First the soluble iodine was separated from the seaweed by water leaching. Then, iodide in the leachate was separated by BiI_3 precipitation, while iodate in the filtrate was reduced to iodide and precipitated. Organic iodine remained in solution. The separated fractions were then analyzed by NAA for iodine concentration. They reported that 9–99% of iodine in seaweed is water soluble. In addition, the percentage of water-soluble iodine is the highest in brown algae and lowest in green algae. In the water leachate of seaweed, iodine exists mainly as iodide, the percentage of organic iodine ranges from 5 to 40%, and the iodate is lower than 5% in all 30 species investigated. In biological macromolecules, iodine is mainly bound with proteins, polyphenol and pigments, but few is bound with polysaccharide. Tables 15.3 and 15.4 show the iodine speciation in *Sargassum kjellmanianum* (a brown seaweed).

Shah *et al.* (2005) also investigated iodine species in commercially available commonly consumed seaweed samples

Table 15.3 Leaching rate of iodine with various solvents from *Sargassum kjellianum* and spinach

Solvent	<i>S. kjellianum</i>		Spinach	
	Concentration ($\mu\text{g/g}$)	Leaching rate (%)	Concentration ($\mu\text{g/g}$)	Leaching rate (%)
Whole sample	140.6 \pm 7.6	–	0.196 \pm 0.043	–
Water (20°C)	149.7 \pm 8.2	39.7	0.310 \pm 0.032	29.4
Water (99°C)	153.2 \pm 5.8	40.0	–	–
Ethanol	121.6 \pm 6.4	27.8	0.231 \pm 0.028	43.9
Ether	129.5 \pm 5.2	8.9	–	–
0.1 mol/l HCl	151.9 \pm 9.2	39.2	0.334 \pm 0.029	28.4
0.1 mol/l KOH	66.0 \pm 3.2	93.3	0.177 \pm 0.017	70.3

Notes: The seaweed *S. kjellianum* and spinach were leached with various solvents. After leaching, the remaining samples were dried and the iodine concentrations in the leached and the original samples were measured by neutron activation analysis. The values are shown in the second and fourth columns. Due to the loss of salt and some components of seaweed after leaching, the weight of leached seaweed is less than that of the original. The percentage (or ratio) of total iodine that remained in the leached seaweed to that in the original seaweed was calculated; the values are listed in the third and fifth columns.

Table 15.4 Concentration of iodine in biological macromolecules in *S. kjellianum*

Sample	Fractions		Iodine	
	Weight (g), dry	Percentage (%)	Concentration ($\mu\text{g/g}$)	Percentage (%)
Whole algae	20.0	100	153.5 \pm 2.4	100
Algin	3.05	14.3	1.86 \pm 0.30	0.185
Fucoidan	0.25	1.17	6.10 \pm 0.25	0.05
Protein	1.30	6.5	403.2 \pm 7.4	65.5
Polyphenol	0.53	2.50	178.7 \pm 3.6	3.09
Pigment	0.35	1.64	138.7 \pm 1.7	1.57

Notes: The different components of seaweed *S. kjellianum* were separated using different processes. The second column shows the weight of different components, and the third column shows the weight percentage of different components in the entire sample. The fourth column shows the iodine concentration in the different components, and the fifth column shows the percentage of iodine component in the entire sample. It shows that the iodine is mainly associated with proteins, pigments and polyphenol.

using a multidimensional chromatographic approach coupled with ICP-MS. A similar profile of iodine species such as those observed by Hou *et al.* (1997b, 2000) was found in the analysis of the alkaline extract (0.1 mol/l NaOH) by SEC-ICP-MS, where iodine associated with both high-, as well as low-molecular-weight fractions in Wakame, while in case of Kombu, only low-molecular-weight iodine species were found. A likely association of iodine with protein, as well as polyphenolic species, was indicated in the case

of Wakame. Anion-exchange chromatography coupled to ICP-MS confirmed that the most predominant inorganic iodine species present in both types of seaweeds is iodide. Protein-bound iodinated species were hydrolyzed by enzymatic digestion using proteinase K. Analysis of the hydrolyzate using reversed-phase HPLC-ICP-MS revealed the presence of MIT and DIT in Wakame.

Bioavailability and Toxicity of Iodine Species

It is known that iodide or iodate have a high bioavailability (> 95%) in humans and animals. However, iodine in the diet may combine with different components and exist as organic iodine, which may have a low uptake in the digestive tract. During the last decade the iodine supply in many countries has increased significantly, but there is still a high frequency of goiter. This may be related to the iodine bioavailability in foodstuffs (Hurrell, 1997).

Bioavailability of iodine species

It was reported that the bioavailability of pure mineral iodine, such as potassium iodide, was 96.4% in normal humans, while that of pure organic iodine, such as mono-iodotyrosine, was 80%. A higher bioavailability of iodine in the seaweeds *Gracilaria verrucosa* and *Laminaria hyperborea* (80–99%) was also observed (Aquaron *et al.*, 2002). A similar high bioavailability of iodine in the diet was also reported by Jahreis *et al.* (2001). They investigated the uptake of iodine in 12 women and found that 89% of the iodine was excreted in the urine and 11% in the feces. However, Wahl *et al.* (1995) reported a very low uptake of iodine from a normal diet; they observed that only 16–18% of the alimentary iodine was excreted with the urine. This may indicate that the type of diet and the species of iodine in the foodstuff are very important with respect to the nutritional status of iodine. A relatively lower water (or acid) leaching rate of iodine (28–40%) from vegetables (spinach and green seaweed) was reported by Hou *et al.* (1997b) (Table 15.2).

Toxicity of excessive intake of iodine

It has been known that excessive iodine intake results in goiter, hypothyroidism, or hyperthyroidism in humans (Institute of Medicine, 2001). The biological basis for iodine-induced hyperthyroidism (IIH) appears most often to be mutational events in thyroid cells that lead to autonomy of function. When the mass of cells with such an event becomes sufficient, and the iodine supply is increased, the subject may become thyrotoxic. These changes may occur in localized foci within the gland or

during the process of nodule formation. IIH may also occur with an increase in iodine intake in those whose hyperthyroidism (Graves' disease) is not expressed because of iodine deficiency. The risks of IIH are principally for the elderly who may have heart disease and those who live in regions where there is limited access to medical care (Stanbury *et al.*, 1998).

Acute toxicity of iodine to animals has resulted in death at levels of 200–500 mg/kg-day, while levels of iodine greater than 10 mg/day, due to the intake of iodine-containing drugs or as a result of accidental poisoning, were toxic to some humans (Backer and Hollowell 2000). Forty-eight individuals were reported to have adverse effects, including goiter, hypothyroidism and sensitivity reactions, from iodine levels less than or equal to 10 mg/day (IPCS, 1988). One study reported that long-term intakes greater than 18 mg/day increased the risk of goiter (Wolff, 1969), while others demonstrated that high iodine intake is associated with an increased risk of thyroid papillary cancer in humans (Franceschi, 1988; Lind *et al.*, 1998). The WHO has set a PMTDI for iodine of 1 mg/day, which was based on the observation that an iodine intake of 1 mg/day or less is probably safe for the majority of the population, but may cause adverse effects in some individuals, e.g., people with thyroid disorders or those that are particularly sensitive to iodine (IPCS, 1988).

Toxicity of iodine species

The observation of toxicity of iodine mainly focused on the iodide or iodate, which is normally present in iodized salt, milk, water and leachate of foodstuffs. However, the toxicity of some other species of iodine may be much higher than that of iodide and iodate. For the prevention of iodine deficiency disorders, iodized oil was used as an injection or administered orally in many countries: iodized oil is normally produced by binding iodine atoms to the polyunsaturated fatty acid in the oil (Zimmermann *et al.*, 2000). After administration, it was supposed that iodine is released gradually as iodide to maintain a constant supply of iodine to the body. Experience in the past decades shows that the utilization of iodized oil is safe. However, acute poisoning of iodized oil to children who are orally administered was reported in China in 1998; this may be related to the species of iodine, which may be more toxic than iodide or iodate. Iodine has been used as an effective, simple, and cost-efficient means of water disinfection (Backer and Hollowell, 2000), in which the active disinfectant species are elemental iodine and hypiodous acid. Doses of iodine below 1 mg/l kill bacteria within minutes. Elemental iodine and hypiodous acid remain in the disinfected water, which may be toxic to humans.

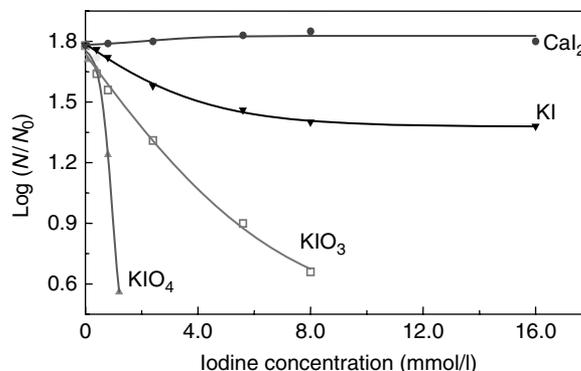


Figure 15.8 The effect of iodine-containing compounds on the growth of *Tetrahymena pyriformis*. *T. pyriformis* is cultured in a solution of various iodine-containing compounds for 34 h. The growth of the cells in various solutions was shown as $\log(N/N_0)$, where N is the number of cells after 34 h culture and N_0 is the number of cells at the beginning of the experiment. A high N/N_0 indicates a lower toxicity of compound to the cells.

Laverock *et al.* (1995) have investigated the acute toxicity (96-h LC_{50}) of aqueous stable iodine species (iodide, iodate and elemental iodine) to rainbow trout and *Daphnia magna*. They reported that rainbow trout was most sensitive to I_2 (LC_{50} greater than or equal to 0.53 mg/l), and much less sensitive to IO_3^- (LC_{50} greater than or equal to 220 mg/l) or I^- (LC_{50} greater than or equal to 860 mg/l). *D. magna* was equally sensitive to I_2 (LC_{50} greater than or equal to 0.16 mg/l) and I^- (LC_{50} greater than or equal to 0.17 mg/l), but less sensitive to IO_3^- (LC_{50} greater than or equal to 10.3 mg/l).

The author has investigated the biological toxicity of iodide, iodate, elemental iodine and periodate by exposing a single celled organism, *Tetrahymena pyriformis*, in various culture solutions with different concentrations and chemical species of iodine. He found that CaI_2 slightly improves the growth of *T. pyriformis*, while other iodine-containing compounds inhibit its growth compared with the control group. KIO_4 and I_2 at high concentration seriously inhibit the growth of *T. pyriformis* in the initial stage; this inhibition was reduced in the later period because of the decomposition of KIO_4 and I_2 . Figure 15.8 compares the effect of iodine-containing compounds on the growth of *T. pyriformis*. It shows that the toxicity of five iodine compounds increases as follows: $CaI_2 < KI < KIO_3 < I_2 < KIO_4$.

Summary Points

- In seawater, most iodine occurs as iodide and iodate, with minor amounts of organic iodine. In open and deep seawater (oxic) iodine mainly exists as iodate, while the iodide concentration is relatively higher in surface and coastal water.
- Titrimetry, colorimetry and differential pulse polarography methods are normally used for the determination of

iodate in seawater while cathodic stripping voltammetry is normally for iodide; the two species can also be separated by IC and determined by NAA or ICP-MS.

- In the air, iodine exists as particles associated, inorganic gaseous iodine (I_2 , HIO) and organic iodine (CH_3I , CH_2I_2 , etc.). The different species of iodine in the air can be separated and collected in a series filter and then measured.
- The volatile iodine species in water and air are normally determined by GC combined with an ECD or ICP-MS.
- In the human and mammal body, iodine exists as thyroid hormones T_4 and T_3 , as well as MIT, DIT and rT_3 , which are mainly bound with proteins in thyroid and other tissues.
- In milk and urine, most iodine occurs as iodide, but are some species of organic iodine also found. The iodine species in fish are similar to that in the human body.
- In seaweed, iodine species vary widely with the species of seaweed. In brown seaweed, most iodine exists as iodide; while in green seaweed, iodine is mainly bound to organic molecules, such as proteins and polyphenol.
- The most commonly used methods for speciation analysis of iodine in tissues and food are chromatographic techniques, such as anion exchange, size exclusion and reverse-phase chromatography, coupled with ICP-MS detection.
- Iodide and iodate have a low toxicity and high bioavailability, whereas the toxicity of elemental iodine and periodate is high. The bioavailability of organic iodine, especially iodine associated with macromolecules, is low.

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Iodine in Farm Animals

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Abstract

Iodine deficiency depletes thyroid iodine stores, inducing hypothyroidism and goiter formation. Development is retarded, particularly of the fetus, resulting in a high rate of stillbirths. In later life, hypothyroidism results in general depression of metabolism and growth. The thyroid gland weight is found to be increased after slaughter or at autopsy. The majority of farm animal feed is plant matter which usually contains little iodine (<20–50 µg/kg dry matter) and the iodine content of water is generally low (2–7 µg/l). Animal feeds vary greatly in composition and water content. For comparison, feeds are either standardized to dry matter or to grain dry matter equivalents, i.e., approximately 880 g grain dry matter equivalent/kg feed. Animal nutrition societies recommend iodine supplements of 120–250 µg/kg dry matter for growing pigs and beef cattle. This prevents iodine deficiency, facilitates high performance, maintains adequate iodine stores and sustains thyroid function. The recommendations for lactating animals are higher (500–600 µg/kg dry matter) resulting in iodine concentrations above 100 µg/l milk. Piglets, calves and lambs require iodine to maintain their rapid rates of growth. German feed manufacturers usually add three to seven times the recommended amounts to pig and cattle diets. Thus, the iodine status of farm animals in Germany is generally good. Deficiency has been virtually eliminated and toxicity is rare. Hypothyroidism is generally due to a combination of insufficient dietary iodine and consumption of iodine antagonists. Iodine antagonists include nitrates, nitrites, thiocyanates and the products of cyanogenic glycoside and glucosinolate degradation. Protein feeds based on rapeseed contain glucosinolates. Iodine supplements are best administered orally. Premixed vitamin and mineral supplements are added to feeds in intensive animal husbandry. Mineral blocks or iodized salt licks are used on pastureland with large numbers of roaming livestock. Administration of large doses of iodine (>4–10 mg·iodine/kg feed) to dairy

cows and laying hens greatly increases the iodine concentrations of milk and eggs. However, in growing pigs the effects on the iodine content of meat are small. Excessive iodine intake in humans can induce hyperthyroidism, and so the iodine content of milk and eggs must be controlled. In 2005 European Union legislation limited the maximum iodine content of cow and hen diets to 5 mg/kg grain dry matter equivalent (previously 10 mg/kg). This should protect consumers and presumably farm animals, but could perhaps be reduced further for pigs. This chapter reviews the effect of iodine intake on the health of animals and the food obtained from animals (e.g., milk, eggs and meat).

Abbreviations

A(F)CR	Agricultural (Food) Research Council
BW	Body weight
EDDI	Ethylenediamine dihydroiodide
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GfE	Gesellschaft für Ernährungsphysiologie Society of Nutritional Physiology
ICP-MS	Inductively coupled plasma-mass spectrometry
Ig	Immunoglobulin
NOEL	Non-observed effect level
NRC	National Research Council (NRC) of the United States
RDA	Recommended daily allowance
RDI	Recommended daily intake
SCN	Thiocyanate
SCP	Secondary plant compounds
TG	Thyroglobulin

TPO	Thyroid peroxidase
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
UL	Upper limit

Introduction

A significant component of the diet of humans consists of animal products (e.g., milk, eggs, meat). Thus, the iodine content of farm animals' feed directly affects iodine nutrition in man. Sadly, goiters remain a prevalent sign of the lack of iodine in human food and animal feed. This global problem reflects the wide distribution of subsistence agriculture with huge numbers of livestock reared on large farms, often without supplemental iodine in feeds.

In 1985, to combat iodine deficiency in the former state of East Germany, salt for human consumption was iodized and iodine was added to animal feed (Anke *et al.*, 1993). Eliminating iodine deficiency in farm animals improved the iodine content of milk and eggs. This further increased the dietary iodine intake of East Germans (see section "The Effect of Iodine Intake on the Iodine Content of Eggs, Meat and Milk"). The program successfully reduced iodine deficiency in both humans and farm animals, and could be used as a model for iodine supplementation in other countries.

This chapter discusses the importance of iodine supplementation of animal feed. The iodine content of animal feed and the impact on the health of animals and the food obtained from animals (e.g., milk, eggs and meat) are considered.

The majority of animal feeds consist of plants and plant matter which rarely contain enough iodine to meet animals' needs. There are approximately 1.6 billion cattle and buffaloes, 1.0 billion pigs, 1.9 billion sheep and goats (FAO, 2006) in the world. In addition, poultry, camels, llamas and other livestock are farmed on a smaller scale. The amount of feed required by all the livestock in the world is several times more than the amount of food consumed by humans.

Legislation on feeds

Despite the common European Union (EU) legislation covering both human food and animal feed (regulation EC No. 178/2002, EU 2002) their composition and assessment are fundamentally different. Most human food is refined. In contrast, animal feed mainly consists of partly processed or unprocessed plant matter (including residues from the food industry). As a rule feed consumed by animals contains more fiber, fertilizer residues (nitrate) and secondary plant compounds (SCP) than the foods produced by the animals that consume the feed. The SCP contain cyanogenic glycosides and glucosinolates which act as iodine antagonists (see section "Response Criteria and Dose-Response Studies

of Iodine Requirement in Farm Animals"). The impact of the glucosinolates on hypothyroidism and animal iodine requirements is discussed below, in the light of the increasing use of rapeseed as oilseed/protein feed in Europe, China, Canada and Australia.

Beside the safety of human food and animal feed as an integral part of legislation (EU, 2002) there are three basic requirements for food-producing animals which are reflected by demands on feed quality (Table 16.1). The profitability of farm animals depends on their health and yield, as well as the quality of the produce. A high-quality feed meets the animals' nutritional needs "at point," reducing feed costs in terms of the amount of feed consumed per unit of produce obtained. The quality of a feed can be expressed as a feed:yield ratio; yield may be considered in terms of quantity of milk produced (kg) or gain in body weight (BW) per animal per day (g). Animal feeds vary greatly in composition and water content, therefore animal nutrition societies prefer to use feed dry matter weights for comparison. However, the feed industry and legislation standardize feed contents to grain dry matter equivalents, i.e., approximately 880 g grain dry matter equivalent/kg feed.

Table 16.1 Desirable qualities of animal feed and farm animals producing human food

Animal ^a	Feed ^a
<i>Health</i> No illness Normal blood, urine, milk and organ parameters	Reduce content of undesirable and toxic constituents (e.g., fiber or glucosinolates and other iodine antagonists) to below dietary threshold levels Meets animals' requirements for nutrients including micronutrients and energy Adequate content Adequate availability High acceptance/feed intake
<i>High performance^b</i> 30–40kg daily milk yield per cow 750–800 daily weight gain per pig Low feed:gain ratio for good profitability and minimizing the nitrogen and phosphorus output per unit milk and meat produced	
<i>Product quality</i> Adequate color, consistency and flavor (good sensory properties) Essential nutrients in milk, meat and eggs and their concentration	Optimization, mainly dietary energy, protein, fat and fortification of micronutrients (e.g., iodine) resulting in an improved supply for humans

^aThe legal upper limits for the amounts of residues and microbial hazards in feeds and food were not included in this table. However, safety of food and feed for consumption is a fundamental requirement.

^bData for poultry and horses were not included. Data for fertility (e.g., duration and number of matings required for successful fertilization, birth weight of the newborns, number of piglets per litter born alive) were not included.

Roughage, concentrates and compound feeds

Animal feeds are classified as either roughage or concentrates. Roughage consists of grass (from pastures, meadows, or from cropping) or other fodder crops, (e.g., maize, legumes, cruciferous plants). Roughage is predominantly given to ruminants who digest fiber with the assistance of microbes in their forestomach (rumen), and may be supplied fresh (green) or preserved (ensiled, sun dried, or artificially dried). The concentrates mainly consist of cereal and legume grain (generally below the quality required for human consumption) and residues of flour, starch and alcohol produced from cereal grains, i.e., bran-, corn-, or wheat-gluten feed, or of oil extraction, i.e., solvent extracted meals from soyabean, rapeseed, sunflower, cottonseed, linseed, peanut, safflower and other oilseeds. The diets of pigs and poultry consist entirely of concentrates, and 1/3–1/2 of the dry feeds offered to high-yielding ruminants are concentrates.

In developed countries food produced by farm animals, i.e., milk, eggs and meat, represents 1/4–1/3 of the estimated mean human consumption of dry food matter per capita per day (400g). Farm animals, therefore, provide a significant proportion of human nutrition. The large demand for milk, meat and eggs can only be met by using high-yielding animals of specific breeds. These animals are partially or entirely fed compound feeds, which are manufactured from a mixture of feed materials and fortified with coated vitamins and minerals including iodine.

Compound feeds are designed to meet the nutritional needs of specific animals and encourage a predetermined level of production. Regarding the optimal nutrient content for required animal responses, the grain-dominated and therefore “one-sided” diets must contain adequate energy and protein, as well as sufficient vitamins and minerals (major elements, trace elements) in feed stable preparations.

Recommendations for iodine supplementation

Early animal studies focused on preventing marked symptoms of iodine deficiency diseases (e.g., goiter) and reduction of yield, mainly in growing animals. More recent dose–response studies in animals have investigated levels of iodine in the thyroid gland, serum concentrations of iodine and thyroid hormones, the milk iodine concentration of dairy cows, lactating ewes, goats, or sows, and the iodine content of excrements.

The recommended dietary iodine intakes represent amounts of iodine sufficient to prevent symptoms of deficiency and impairment of health or performance while maintaining the body’s store of iodine (see sections “Requirements/Recommendations for Iodine Supplementation” and “Response Criteria and Dose–Response Studies of Iodine Requirement in Farm Animals”). Recommended daily

intakes (RDI) are more than the requirements, because RDI include safety margins which take into account periods when the availability of nutrients may be reduced, e.g., due to the presence of antagonists in feed or losses through feed storage.

Animal nutrition as an applied science has to balance the economic constraints of the farming industry with animal health and performance. Animal nutritionists have responded to the challenge of increasing the amounts of trace elements in milk, eggs and meat by recommending the use of iodine fortified feed (Swanson *et al.*, 1990; Kaufmann and Rambeck, 1998). The development of our understanding of iodine physiology and maintenance of iodine nutrition in animals requires collaboration between farmers, feed compounders, food chemists, veterinarians, clinical chemists and officers in the agricultural food industry.

Studies of the effects of feed iodine and iodine antagonists on iodine status in animals could help to advance understanding of human iodine nutrition and physiology (Laurberg *et al.*, 2002). This chapter focuses on iodine nutrition and physiology in pigs. Pigs are among the most sensitive farm animals to feed quality and iodine deficiency. Of the mammals used in the farm industry, the digestive system and metabolism of pigs bears most similarity to that in man. Iodine nutrition in ruminants (mainly lactating cows) and poultry are also discussed.

Iodine Deficiency due to Low Iodine Intake and Iodine Antagonists

Signs of iodine deficiency

In both humans and animals iodine deficiency reduces the level of thyroid hormones resulting in hypothyroidism. This can be induced in animal experiments by thyroidectomy or administration of large dosages of antithyroid and thyroid blocking agents. Enlargement of the thyroid gland with loss of functional tissue is a sign of iodine deficiency and hypothyroidism in several species. However, unlike calves (Figure 16.1), lambs and kids, in pigs and poultry goitrous swelling of the neck is not apparent. The effects of iodine deficiency on metabolic rate and growth also differ between farm animals, depending on species, gender, age and level of performance (Underwood, 1977).

Iodine deficiency in the fetus

The effects of deficiency are most pronounced pre- and perinatally due to the rapid rate of growth and the nutritional demands of the embryo and fetus. In the first half of pregnancy thyroid hormones are supplied to the fetus by the dam. Later in the pregnancy the fetal thyroid is able to produce thyroid hormones (Nathanielsz, 1976), but is

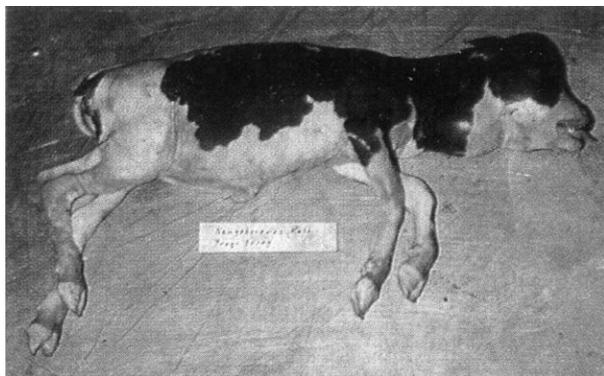


Figure 16.1 Photograph of a stillborn calf with a goiter. The calf was stillborn because of iodine deficiency and hypothyroidism *in utero*. The hypothyroid mother did not exhibit signs of iodine deficiency.

dependent on transfer of iodine across the placenta and therefore dependent on maternal iodine intake. Iodine deficiency in female farm animals increases intrauterine mortality. This has been observed and documented in cows, sows, ewes and goats, for nearly 100 years (Smith, 1915; Welch, 1928; Devilat and Skoknic, 1971; Gürtler *et al.*, 1982; Groppel *et al.*, 1983; Körber *et al.*, 1985; Schöne *et al.*, 1986; Kursa *et al.*, 1998). Rates of embryo resorption are increased in the first trimester, while rates of stillbirths and placental retention increase later in pregnancy. Stillbirths result from prolonged gestation, possibly because the production of glucocorticoids by hypothyroid offspring is decreased. This weakens the signal sent by the mature fetus to the mother to start labor (Slebozinski, 1979).

Iodine-deficient piglets and calves are partially or totally hairless (Hart and Steenbock, 1918; Andrews *et al.*, 1948; Kursa *et al.*, 1998). The newborn babies of hypothyroid sheep were immature and had reduced vitality. This manifested as retarded growth of the heart, brain and skeleton of the fetuses (Hetzel *et al.*, 1990). There was also evidence of histopathological changes, mainly in the nervous system (Potter *et al.*, 1982).

Iodine deficiency in pregnant animals

In contrast with the severely affected fetus, the hypothyroid dam, despite lacking T_4 in the serum, appears clinically indistinguishable from normal females. The maternal thyroid gland becomes extremely efficient at removing iodine from plasma and recovering iodine from the degradation of thyroid hormone and thyroglobulin (TG). It has been reported that other criteria used to assess reproductive health and performance (e.g., the estrus frequency and expression, the libido and frequency of mating) are either less affected or not affected by iodine deficiency (EFSA,

2005). However, in contrast with these studies, in cow herds in goitrous areas in Finland, iodine supplementation was found to reduce the irregularity of estrus cycles and improve first-service conception rate (Hetzel and Maberly, 1986).

Groppel *et al.* (1986) found that the mating frequency of goats and sheep fed casein, starch and cellulose diets without additional iodine was similar to that of animals given supplemental iodine (0.4 mg-iodine/kg-diet). However, the dietary iodine deficit increased the abortion rate. As with hypothyroid sows, the gestation period was prolonged and the live-born goitrous kids were less viable than controls.

Iodine deficiency in growing animals

The neurological disorders of the hypothyroid newborn were similar to the psychomotor retardation of growing pigs with experimentally induced iodine deficiency. In these studies iodine deficiency was induced by feeding pigs grain soyabean diets with added potassium thiocyanate, or rapeseed feed diets containing the iodine antagonists glucosinolates without supplemental iodine (Sihombing *et al.*, 1974; Lüdke and Schöne, 1988; Schöne *et al.*, 1988). The hypothyroid animals were lethargic and showed delayed flight behavior. Their voice changed from a light squeal to a pitiful growl. Acute constipation was, at least in part, due to decreased feed intake. Depression of the rate of growth of animals resulted in stunting, to the point of dwarfism in some cases. Hypothyroid pigs had shortened trunks and legs and myxedema (infiltrations visible as skinfold thickening mainly in the abdominal region). Dry and brittle hair, bristles, or wool are common symptoms of iodine deficiency in farm animals (Underwood, 1977).

The lower rectal temperature of hypothyroid pigs compared to healthy pair fed littermates (who were given iodine supplements) represents a decreased metabolic rate (Schöne *et al.*, 1988). Newborn piglets with mild hypothyroidism induced by feeding rapeseed meal to sows (with sufficient dietary iodine) responded to cold stress by further reduction of the rectal temperature (Berthon *et al.*, 1993). The thermogenic response to cold exposure was reduced.

Hypothyroidism disrupts many organ systems and results in, e.g., impaired digestion (Levin, 1969) and disturbed protein and energy metabolism. Pigs made hypothyroid by diets with rapeseed feed without added iodine, i.e., <100 µg-iodine/g thyroid, undetectable or traces of T_4 in serum (<10 nmol/l), decreased, increased, or normal T_3 serum concentration, showed:

- reduced erythropoiesis resulting in a lower hemoglobin content of red blood cells (Schöne *et al.*, 1986, 1990c)
- reduced osteocyte activity, hypophosphatemia and reduced alkaline phosphatase activity (Schöne *et al.*, 1987; Njandak *et al.*, 1990)

- impaired immune response, resulting in reduced antibody titers against specific administered antigens (Schöne *et al.*, 1987)
- disturbed zinc, copper and vitamin A status (Schöne *et al.*, 1990c, 1989). Liver and bone zinc were decreased. Liver copper increased while serum copper decreased. The liver accumulation of copper may result from cholestasis. Serum ceruloplasmin decreased. Liver vitamin A decreased while serum vitamin A increased. The increased serum vitamin A reflects increases in retinol-binding protein which is normally excreted via the kidneys but accumulated in serum as a result of impaired renal function in our pig model of hypothyroidism.

The iodine content of feeds

Despite the low iodine content of plants, the development or severity of iodine deficiency is ameliorated by the trace amounts of iodine in some feeds, drinking water and even air. As a result of the iodine content of seawater and its vaporization and precipitation; air, water, soil and plants in coastal regions contain more iodine (McDowell, 2003). Livestock kept on coastal farms are therefore less at risk of iodine deficiency than those reared on mountains. In addition, the types and amounts of iodine antagonists, the iodine reserves and salvaging mechanisms vary between farm animal species, categories, herds and individuals.

Plants, unlike animals, do not require iodine. Despite the absence of functional targets in plants, the use of iodized fertilizers resulted in a greater concentration of iodine in leaves (spinach) than roots (carrots; Dai *et al.*, 2004). Standardized to dry matter, the vegetative parts (green) of plants contain more iodine than the generative parts (seeds).

The iodine content of plants reflects passive uptake from contamination or diffusion of iodine in the air, water and soil. The iodine concentration in German and Austrian soils is around 1–3 mg/kg (Anke *et al.*, 1993; Jopke *et al.*, 1997; Gerzabek *et al.*, 1999). Whitehead (1979) reported higher concentrations in soil samples from the UK. There is a correlation between the clay and organic carbon content of a given soil and the iodine content.

There are several reports of feed iodine content however, only a few are reliable. Data from older German feed tables (DLG, 1973) suggested that some feeds contained several 100 µg·iodine/kg dry matter. This is significantly more than the dietary recommendations for farm animals. Iodine deficiency would be highly unlikely if that much iodine was ingested. This suggests overestimation of the iodine content of feeds in these tables. The methods used to measure iodine concentration in feeds (e.g., ashing) may have produced falsely high values in low iodine feeds. This overestimation of iodine content has been recently demonstrated for several foods (Haldimann *et al.*, 2000; Remer and Fonteyn, 2004).

In investigations using inductively coupled plasma-mass spectrometry (ICP-MS) (Fecher *et al.*, 1998; Leiterer *et al.*, 2001) the iodine concentrations of barley and wheat were undetectable (<20 µg/kg; Schöne *et al.*, 2001a). Low concentrations of iodine (6 µg/kg) were detected in durum wheat (*Triticum durum*) flour by ICP-MS (Haldimann *et al.*, 2005) and neutron activation analysis (3 and 9 µg/kg; Dermelj *et al.*, 1991). Iodine concentrations in the range 2–30 µg/kg (mean 6.1, median 4.6) were detected in cereal grains from 38 different sites in Austria using neutron activation (Shinonaga *et al.*, 2001). Using ICP-MS we found that the iodine concentration of soyabean meal was 51 µg/kg (Schöne *et al.*, 2001a) while Dermelj *et al.*, 1991, found concentrations of 15 and 19 µg/kg in two samples of this feed using neutron activation analysis.

This concentration range was similar to the relatively low iodine content of three maize silage samples from Thuringian farms which contained 34, 12 and 11 µg/kg dry matter (Leiterer and Kirmse, unpublished data, 2006). However, a grass–maize–silage feed used in a study on cows contained 120 µg·iodine/kg dry matter (Schöne *et al.*, 2006a). The iodine content of the green fodder from the partly chalky, partly loamy Thuringian soils is in the range 10–50 µg/kg dry matter, depending on the degree of soil contamination during the harvest and preservation of the feed. Soiling of maize and grass when harvesting and ensiling or drying of feed on the field can increase the ash content from <50 to >100 g/kg dry matter. This could increase feed iodine content by at least 100 µg/kg dry matter relative to clean feed.

In many laboratories the methods used to determine feed iodine were and probably still are unreliable. For example, pigs fed a grain soyabean diet reported to contain 690 µg·iodine/kg (approximately 4 times the required amount) developed large goiters (500 mg thyroid weight/kg BW; Petersen *et al.*, 1979). Control pigs which received a diet with only 30–90 µg added iodine/kg in mineral form had normal thyroid weights (80–90 mg/kg BW). As the data regarding iodine content were thought to be erroneous, the subcommittee on Swine Nutrition of the National Research Council (NRC) of the United States did not include data on iodine concentrations in the table in their report entitled *The mineral composition of some feed ingredients commonly used for swine* (NRC, 1998).

Although trace amounts of supplemental iodine (50–100 µg/kg feed dry matter) effectively prevent goiter, they are currently difficult to detect and measure accurately. More sensitive methods to determine the iodine content of feed, including matrix disintegration, are required. Methods with the potential to reliably detect <20 µg·iodine/kg should be critically evaluated. The reliability of the reported feed iodine content should be questioned in animal studies if goiter or other symptoms of iodine deficiency are reported, despite the use of diets thought to contain more than 100 µg detected iodine/kg

dry matter. In Germany the currently recommended method to determine iodine concentrations in mineral vitamin premixtures and compound feeds is ICP-MS. The officially recommended method of ICP-MS detects iodine concentrations in the range 0.4–101 mg/kg (VDLUFA, 2006), which is sufficient for the needs of the feed industry (Grünewald and Steuer, 2006). The method should be improved and standardized for feed with < 0.4 mg (added) iodine/kg to enable assessment of the very low native iodine content of unsupplemented feed.

Requirements/recommendations for iodine supplementation

To prevent and cure deficiency iodine must be administered, as a rule orally. Premixed vitamin and mineral feed supplements are used in more intensive husbandry. Mineral blocks and iodized salt licks are used on pastureland (Cheeke, 1991). As iodine is water-soluble and may precipitate, the feed should be sheltered or protected by covered troughs.

Alternatively, if huge numbers of livestock are kept on a large area of pasture, parenteral supplementation may be preferable. For example, iodine supplementation provided by an intramuscular injection of a slow release preparation, such as iodized oil, can be sufficient for several months (Chambon and Chastin, 1993). Parenteral administration of iodine is beyond the scope of this chapter and only dietary supplementation of iodine is discussed here.

Iodine feed supplements must be stable to meet the needs of several groups of farm animals. Iodates [$\text{Ca}(\text{IO}_3)_2 \times 6\text{H}_2\text{O}$; $\text{Ca}(\text{IO}_3)_2$] and iodides [NaI ; KI] are both approved feed supplements in the EU (2003, 2005). Iodates are more stable than iodides as feed additives and so are used preferentially. Cheeke (1991) suggests that ethylenediamine dihydroiodide (EDDI) and pentacalcium orthoperiodate may be suitable feed iodine additives, but does not include information on their stability. In our studies we found that a casein-bound iodine supplement produced by milling casein with KI was highly stable (Schöne *et al.*, 1997c).

Animal nutrition societies generally recommend iodine intakes in the range of 120–250 $\mu\text{g}/\text{kg}$ feed dry matter for growing pigs and beef cattle (excluding the recommendations of the NRC; 500 $\mu\text{g}/\text{kg}$ dry matter for beef cattle; NRC, 1996). These intakes prevent iodine deficiency, facilitate a high performance (e.g., weight gain and low feed:gain ratio), maintain adequate iodine stores (> 500 $\mu\text{g}/\text{g}$ thyroid), and sustain thyroid function. The recommendations for lactating animals (cows and sows) suggested in the German tables (GfE, 2001, 2006) and in the older and unrevised A(F)RC tables (AFRC, 1981) are higher (500–800 $\mu\text{g}/\text{kg}$ feed dry matter; Table 16.2; excluding the recommendations of the NRC; 160 $\mu\text{g}/\text{kg}$ dry matter for sows; NRC, 1996). Lactating swines, ewes, and cows ingesting the recommended amounts of iodine produce

Table 16.2 Required and recommended iodine supplementation of feed of cattle, pigs, and poultry in the US, UK, and Germany ($\mu\text{g}/\text{kg}$ feed dry matter)

	US NRC (1994, 1996, 1998, 2001)	UK Agricultural (Food) Research Council (1981)	Germany GfE (1995, 1999, 2001, 2006)
Dairy cows	500	800	500
Calves, growing bulls	500	120	250
Sows	160	500	600
Growing pigs	160	No data	150
Laying hens	320–490		500
Broiler chickens	350		500

Note: Data for sheep with 100–800 $\mu\text{g}/\text{kg}$ dry matter (NRC, 1985) and for goat with 300–800 $\mu\text{g}/\text{kg}$ feed dry matter (GfE, 2003) were not included above.

milk containing more than 100 μg iodine/l milk. This ensures an adequate supply of iodine for their piglets, calves and lambs who require very high serum concentrations of thyroid hormones to maintain a rapid rate of growth.

Recent iodine recommendations from the British Society of Animal Science do not differentiate between the various categories of farm animal. For example, addition of 0.2 mg iodine/kg diet is recommended for all categories of pigs (Whittemore *et al.*, 2003). Most of the recommendations are of the same order of magnitude, suggesting that the British guidelines were established empirically. We suggest that this approach is not appropriate and that guidelines should be based on targeted dose–response studies in animals using growth and performance data, as well as laboratory diagnostic criteria.

Response criteria and dose–response studies of iodine requirement in farm animals

The growth intensity is classically measured to determine the response to iodine supplementation in farm animals, as well as in laboratory animals. Iodine supplementation is not always required to maintain growth. Growth retardation may not occur in animals fed grain or refined diets free of antagonists. The growth and feed intake of sheep, cows and pigs receiving typical diets without supplemental iodine were not depressed (Aumont *et al.*, 1989a, b; Groppe *et al.*, 1983; Schöne *et al.*, 1990a). The feed:gain ratio and feed costs were not increased. A markedly increased thyroid weight and decreased T_4 serum concentration suggest that only small amounts of iodine are required to sustain growth. Administering diets of casein, starch, cellulose and an iodine-free premixed vitamin and mineral supplement to weaned female goats led to growth depression only after 20 weeks of the experiment

(Groppel *et al.*, 1989). In pigs fed typical grain diets growth depression and visible iodine deficiency only occurred if insufficient iodine supplementation occurred in the presence of iodine antagonists, such as rapeseed glucosinolates.

Although farm animals can adapt to diets poor in iodine, the prevention of deficiency requires the use of simple and valid laboratory criteria to diagnose iodine deficiency. As stated above, the thyroid iodine content of hypothyroid animals with goiter is low. As a result levels of T_4 in the serum are undetectable, but reduction of T_3 , the more active thyroid hormone, only occurs if iodine deficiency is severe. Elevation of T_3 may occur in early hypothyroidism; however, this is not consistent and can not therefore be used to diagnose iodine deficiency (Schöne *et al.*, 1991).

Dose–response studies have determined the amount of thyroid iodine produced at given levels of dietary iodine intake. These studies have investigated the effect of dietary iodine on the thyroid iodine concentrations (indicating the extent of TG iodination), thyrocyte structure (epithelial cell height and thyroid weight), and function, and the synthesis of T_4 . However, antagonists, such as the rapeseed glucosinolates may reduce the effective dose of dietary iodine. To achieve a given response in the presence of an iodine antagonist, an increase in the dose of supplemental iodine may be required.

Our studies in pigs investigated the effect of varying amounts of supplemental iodine and iodine antagonists (glucosinolates) on serum T_3 and T_4 concentrations, as well as the thyroid weight and iodine content. The aim was to determine the sensitivity of the diagnostic criteria for the

detection of iodine deficiency and the extent to which increasing the dose of iodine can compensate for the presence of antagonists.

We conducted seven experiments with a total of 319 growing pigs and investigated high rapeseed feed diets with up to 160 g/kg solvent extracted meal and press cake from rapeseed of older (high glucosinolate) or newer (low glucosinolate) varieties. The diets contained 0.5–19 mmol·glucosinolates/kg·diet. Control groups were fed diets without rapeseed (no glucosinolates). The effect of supplemental iodine; 0 (control), 62.5, 125, 250, 500, and 1000 $\mu\text{g}/\text{kg}\cdot\text{diet}$ were also investigated (Schöne *et al.*, 1990b, 1991, 1997a, c, 2001b). The Sandell–Kolthoff reaction was used to detect dietary and thyroid iodine in the first four studies (Schöne *et al.*, 1990b, 1991, 1997a, c). The data in Schöne *et al.* (2001b) includes serum iodine concentrations measured using ICP-MS. The serum concentrations of T_3 and T_4 were determined by radioimmunoassay. The aim was to define a non-observed effect level (NOEL) for dietary glucosinolate and determine whether iodine supplementation can be used as an antidote.

Growth response of pigs fed iodine antagonists (glucosinolates)

Visible iodine deficiency (clinical hypothyroidism) and depression of feed intake and growth rates occurred in pigs fed rapeseed meal diets without supplemental iodine. These effects occurred significantly earlier in the group fed the rapeseed meal diet with the higher dose of glucosinolate (Figure 16.2, Schöne *et al.*, 1990b). Thyroid iodine

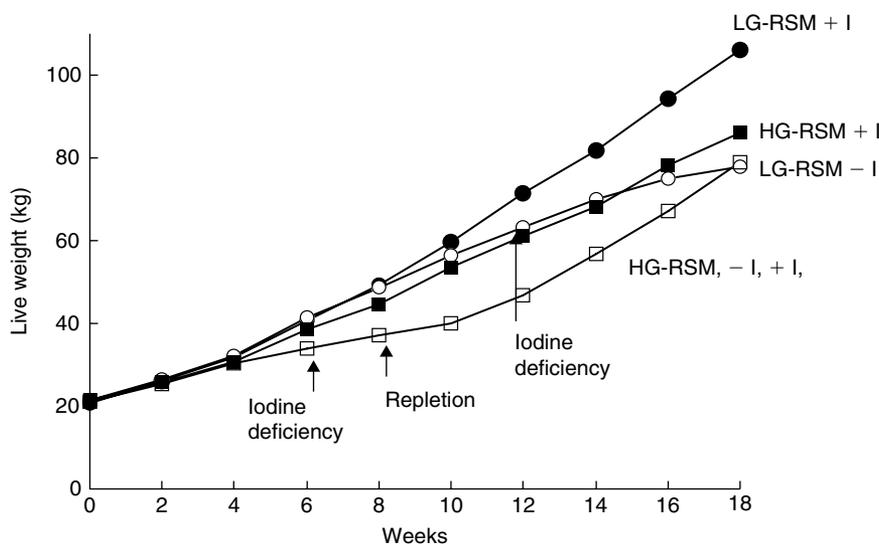


Figure 16.2 Growth of pigs fed rapeseed meal (RSM) without or with added iodine. Pigs were divided into 3 groups and fed diets containing different amounts of glucosinolate (as rapeseed meal; RSM). 1. No glucosinolates, i.e. without RSM (control). 2. low glucosinolate content (LG) - 6 mmol/kg diet as 160 g RSM/kg diet. 3. High glucosinolate content (HG) 19 mmol/kg diet as 160 g RSM/kg diet. Each of these groups was divided into 4 subgroups of 4 animals each and given different amounts of iodine supplementation (0 (no iodine supplementation) to 1000 μg iodine/kg diet). The growth of pigs fed diets free of RSM (not shown) was rapid and similar to the group with LG -RSM + I.

stores were depleted by the lack of dietary iodine resulting in reduction of serum T_4 (Schöne *et al.*, 1991). The rate of depletion was faster in the group fed the higher dose of dietary glucosinolates. Animals fed the control diet without rapeseed glucosinolates or supplemental iodine developed goiter, but growth was not affected and symptoms of deficiency did not occur.

Supplemental iodine prevented iodine deficiency in the group fed the older rapeseed meal diet which had a higher glucosinolate content (19 mmol/kg). The growth responses of the pigs fed this diet with and without supplemental iodine were identical until shortly before symptoms of hypothyroidism developed in the group without supplemental iodine. Thus, utilization of nutrients and a high rate of growth can be sustained while iodine stores and thyroid hormones are depleted.

Despite iodine supplementation the growth rates and feed intake were more depressed in the group fed the higher dose of glucosinolate (McKinnon and Bowland, 1977; Schöne *et al.*, 1990b) than in the group with the lower dose of glucosinolate. Increasing the dose of iodine may not be able to completely reverse the effects of antagonists.

The effect of iodine antagonists (glucosinolates) on thyroid iodine status

The serum concentration of T_4 was less sensitive to changes in dietary iodine and glucosinolates than thyroid weight (standardized to body weight) and thyroid iodine concentration (Figures 16.3–16.5). The reduction of thyroid iodine concentration was mainly due to the “dilution effect” of the enlarged gland. The reduction of total thyroid iodine content due to glucosinolates was also significant (Schöne *et al.*, 1990b, 1991, 1997a, c, 2001b) in most cases. In contrast, the serum T_4 concentration remained relatively constant over a wide range of supplemental iodine (125–1000 $\mu\text{g}/\text{kg}$ -diet) and doses of iodine antagonists (2–10 mmol/kg-diet). This suggests that the synthesis and/or degradation of T_4 is tightly controlled and well-regulated.

The store of precursor hormone in the thyroid gland was studied by administration of thyroid-stimulating hormone (TSH) or thyrotropin-releasing hormone (TRH) (Christison and Laarveld, 1981) to pigs fed rapeseed meal with either high, low, or no (control) glucosinolate content. The serum T_4 of the animals in the control group

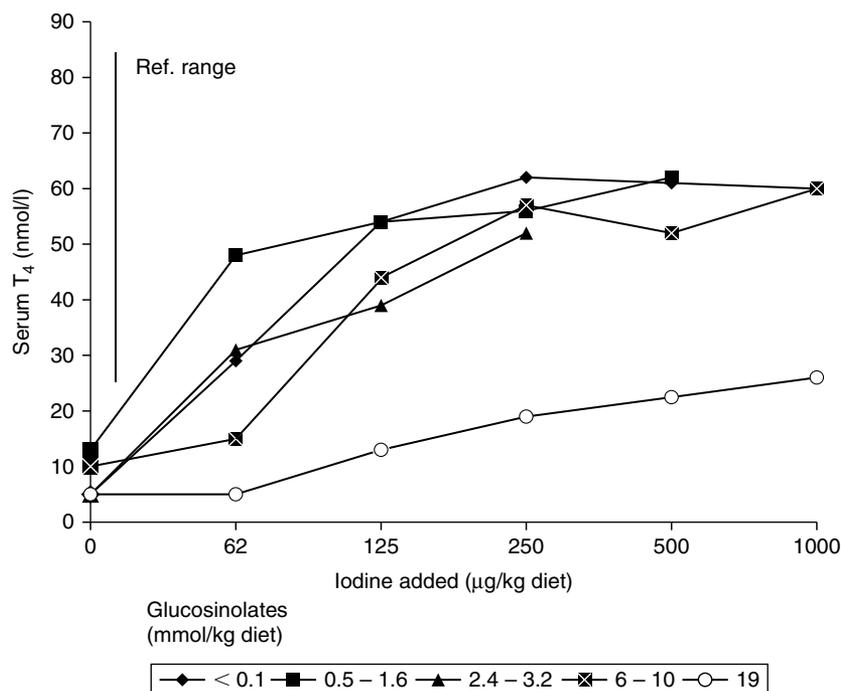


Figure 16.3 The effect of dietary glucosinolate and iodine supplementation on serum T_4 concentration. The data in this figure are derived from a meta-analysis of several studies of the effect of dietary glucosinolate (as rapeseed meal) and iodine supplementation on serum T_4 concentration in pigs (Schöne *et al.*, 1990b; 1991; 1997a,c; 2001b). The serum T_4 concentration of the control group (without glucosinolates) and the group fed the lowest glucosinolate dose (0.5–1.6 mmol/kg diet) increased steeply with supplemental iodine (62–1000 $\mu\text{g}/\text{kg}$). More iodine was required to produce 50 nmol T_4/L serum in the group fed diets with the higher glucosinolate doses (2.4–3.2 and 6–10 mmol/kg diet). The serum T_4 concentration in the animals fed diet containing the highest glucosinolate dose (19 mmol/kg diet) was impaired despite supplementation with 1000 μg iodine /kg diet, i.e. significantly more than the recommended amounts. Pigs fed diets containing 62 μg added iodine/kg diet without iodine antagonists had serum concentration of T_4 above the lower limit of the reference range.

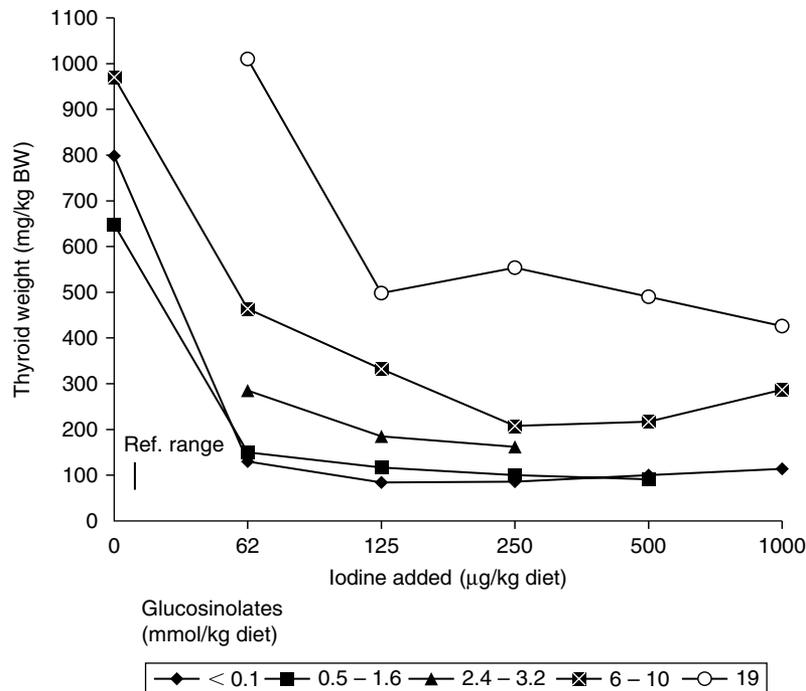


Figure 16.4 The effect of dietary glucosinolate and iodine supplementation on thyroid weight. The data in this figure are derived from a meta-analysis of several studies of the effect of dietary glucosinolate (as rapeseed meal) and iodine supplementation on thyroid weight in pigs (Schöne *et al.*, 1990b; 1991; 1997a,c; 2001b). Without iodine supplementation all diets, including the control diet without rapeseed feed, induced marked goitre. However, in the control group (without glucosinolates) fed 62 µg iodine/kg diet the thyroid weight was in the reference range (60–120 mg/kg BW shown on the left). In the group fed the lowest glucosinolate dose (0.5–1.6 mmol/kg diet) 125 µg iodine/kg diet restored thyroid weight. In the groups fed the higher doses of glucosinolate, the goitre persisted despite large doses of supplemental iodine. In the groups fed 2.4–3.2, 6–10 and 19 mmol glucosinolates/kg diet the thyroid weights were still increased by factors of 2, 2–3 and 4–5 respectively despite iodine supplementation.

immediately increased. In the group fed the low glucosinolate diet (meal from 00 rapeseed) the serum concentration of T_4 did not increase, but instead remained at its initial level, similar to the initial level of the control group. The serum T_4 concentration of the group fed the high glucosinolate diet did not increase, but persisted at its initial level, one-third lower than that of the control group.

Hepatic 5-monodeiodase catalyzes the conversion of T_4 to T_3 . This enzyme was decreased in rapeseed-fed pigs (Spiegel *et al.*, 1993b) suggesting that the serum T_3 status is impaired. Other studies found that the T_3 serum concentration was inconsistent in glucosinolate stressed hypothyroid pigs (Schöne *et al.*, 1990a, 1991).

The effects of dietary iodine and antagonists on the regulation of thyroid hormone supply and consumption by TSH and thyroid peroxidase (TPO) could not be investigated until recently. The effect of glucosinolates on the TRH and TSH axes of pigs was indicated by enlargement of the thyroid. Although the thyroid microarchitecture of pigs given rapeseed feed with iodine supplementation was normal, the greater TSH release was demonstrated by the significantly increased epithelial cell height of the thyrocytes (Schöne *et al.*, 1991). In pigs fed rapeseed press cake

with supplemental iodine Spiegel *et al.* (1993a) also found parenchymatous goiters with increased thyroid weight and epithelial cell height. The follicle and colloid area increased with moderate glucosinolate intake (1–2.5 mmol/kg·diet), but decreased with higher glucosinolate loads (5–7 mmol/kg·diet). This is consistent with the finding that in rats exposed to nitrate there is a tendency toward a shift from the colloid to the epithelial cells and interstitium (Jahreis *et al.*, 1989).

The large goiters that developed in the animals fed without supplemental iodine were manifestations of the irregular distribution of epithelial cells and sparsely filled colloid (Schöne *et al.*, 1991). This is classified as diffuse goiter (Seffner and Heller, 1979). Diets containing 19 mmol glucosinolates/kg reduced thyroid iodine concentration and production of T_4 in pigs, despite large doses of supplemental iodine (1 mg/kg·diet). At this level of exposure to antithyroid agents it is not possible to overcome the toxic effects with supplemental iodine.

Glucosinolates are mainly degraded to nitriles and thioureyne compounds such as oxazolidinethiones (Figure 16.6). The nitriles are detoxified by sulfur transferases (Lang, 1933) to thiocyanate. Increased serum thiocyanate (SCN)

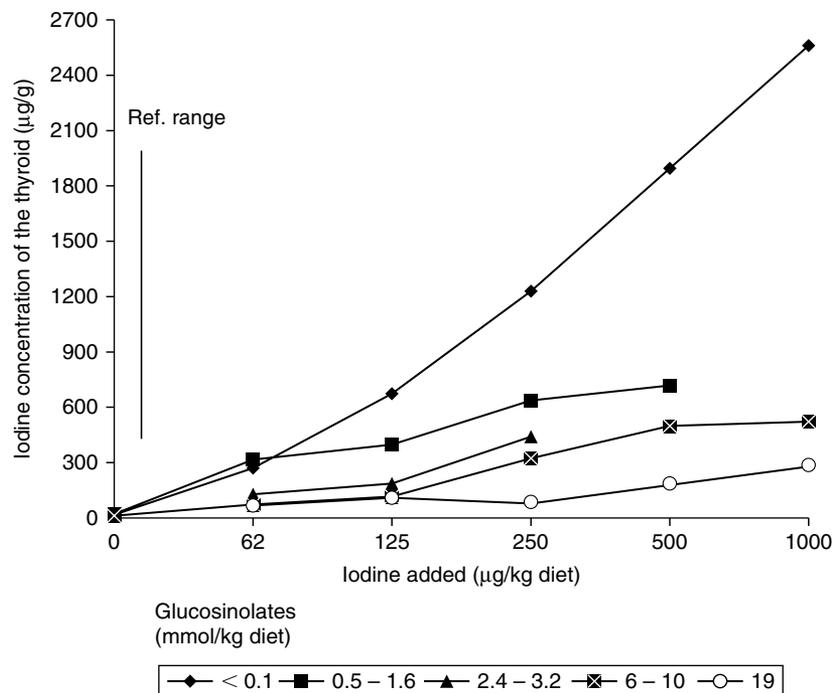


Figure 16.5 The effect of dietary glucosinolate and iodine supplementation on thyroid iodine concentration. The data in this figure are derived from a meta-analysis of several studies of the effect of dietary glucosinolate (as rapeseed meal) and iodine supplementation on thyroid iodine concentration in pigs (Schöne *et al.*, 1990b; 1991; 1997a,c; 2001b). The animals' main iodine store (12-18 µg iodine/g thyroid) was depleted in those groups fed diets without supplemental iodine. The thyroid iodine content was related to dietary iodine by a quadratic function. Iodine supplementation at the lower iodine dosages (62, 125 and 250 µg/kg diet) greatly increased thyroid iodine concentration. However there was a flattening of the effect of increasing supplementation at the higher dosages (500 and 1000 µg/kg diet). The steepest increase curve was seen in the control group (without glucosinolates). The presence of glucosinolates in the diet inhibited the storage of iodine within the thyroid and reduced thyroid iodine concentration. The effect of the lower doses (0.5-1.6, 2.4-3.2 mmol/kg diet) was less than that of the higher doses (6-10 mmol/kg diet and 19 mmol/kg diet). Pigs fed diets containing 125 µg added iodine/kg diet without iodine antagonists had thyroid iodine concentrations above the lower limit of the reference range.

can be used as marker of consumption of glucosinolates. Antithyroid effects of large doses of SCN (administered as KSCN) are reversed by even moderate amounts of supplemental iodine (Schöne *et al.*, 1997a). This indicates that SCN contributes very little to the overall antithyroid effect of glucosinolates. Cyanogenic glycosides from linseed release nitriles which are detoxified to SCN. Even a high serum SCN concentration had no effects on the thyroid hormone status of piglets (Schöne *et al.*, 1997b) and poultry (Richter *et al.*, 1997, 1998).

However, as explained below, the oxazolidinethiones must be eliminated by oxidation and impair iodine and thyroid hormone status. Large doses of methimazole or methylthiouracil (1000 mg/kg·diet; approximately 35 mg/kg BW/day) also impair thyroid hormone production and do not respond to dietary iodine supplementation. After exposure to methimazole or methylthiouracil for several weeks, the pigs developed severe hypothyroidism. This did not respond to iodine supplementation, even at doses significantly above the recommended amounts (Hennig *et al.*, 1969; Schöne *et al.*, 1997a).

Iodide (I^-) is taken up via the iodide sodium symporter on the basal membrane of the thyroid epithelial cell (Spitzweg *et al.*, 1998). I^- is oxidized to elemental iodine in a reaction catalyzed by TPO in the apical membrane of the epithelial cells. Iodine is then added to the tyrosyl residues of TG and stored in the thyroid as iodized TG. TPO is also involved in the oxidation of oxazolidinethiones, products of glucosinolates degradation (Kohler *et al.*, 1988). The presence of oxazolidinethiones reduces the availability of TPO for oxidation of I^- , resulting in a relative enzyme deficiency. The excess I^- in animals exposed to glucosinolate increases the proportion of total serum iodine which is nonhormone iodine (Schöne *et al.*, 2001b). Higher urine and fecal iodine concentrations also occur in mother swine fed rapeseed meals (Schöne *et al.*, 2001a), and higher urinary iodine occurs in dairy cows (Šustala *et al.*, 2003) fed rapeseed meals. In contrast, the milk iodine concentration was reduced by rapeseed glucosinolates (Papas *et al.*, 1979b; Schöne *et al.*, 2001a; Šustala *et al.*, 2003). The mammary-gland-epithelial cells contain peroxidase enzymes and the mechanisms

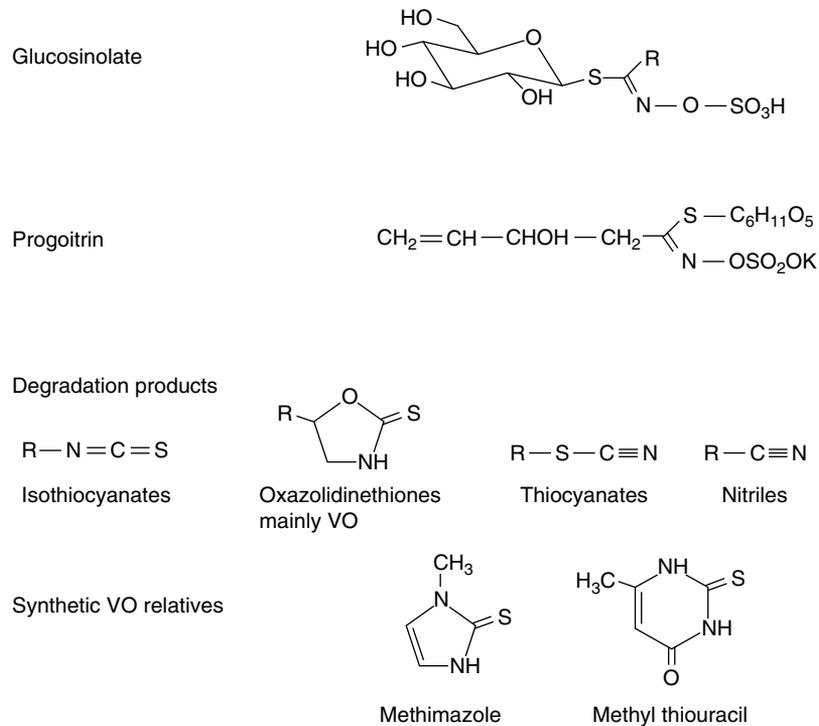


Figure 16.6 Chemical formulae of glucosinolates and glucosinolate degradation products. This figure illustrates the chemical formulae of glucosinolates, the main rapeseed glucosinolate progoitrin and the glucosinolate degradation products. Methimazole and methylthiouracil are related to L-5-vinylloxazolidinethione (VO) the main example of an oxazolidinethione. The thiocyanate ion, SCN^- was not depicted. R radical.

underlying the reduction of both milk iodine and thyroid iodine concentrations by glucosinolates are similar. The iodine content of eggs laid by hens fed rapeseed meal is also reduced (Papas *et al.*, 1979a). However, the transfer of iodine to eggs and the effect of iodine antagonists on the development of eggs in the ovaries were not investigated until recently.

In vitro models of TPO activity and which use thioureyne compounds, as well as iodine (Taurog, 1996) and H_2O_2 , and similar models of lactoperoxidase activity (Edelhoch *et al.*, 1979) demonstrate that other substrates are often oxidized in preference to iodide. The peroxidase catalyzes the formation of disulfide compounds. These are subsequently oxidized and excreted, but only in the presence of iodine. Absolute iodine deficiency or relative deficiency in the presence of high concentrations of thioureyne compounds results in recovery of the thioureyne from the disulfide and irreversible inhibition of peroxidase (Taurog, 1996). In the sow and cow experiments inactivation of thioureylenes by TPO at the expense of iodide oxidation, and therefore storage of elemental-iodine in the thyroid, resulted in increased iodine (and presumably iodide) concentrations in urine and feces, respectively.

Animal nutrition societies recommend adding 120–250 $\mu\text{g}\cdot\text{iodine}/\text{kg}\cdot\text{diet dry matter}$ to the feed supplied to growing pigs, sheep, goats and cattle.

Lactating animals (sows, ewes and cows) should have 500–600 $\mu\text{g}\cdot\text{iodine}/\text{kg}\cdot\text{diet dry matter}$ added to their feed (see section “Requirements/Recommendations for Iodine Supplementation”). These recommendations are for animals fed diets which do not contain any iodine antagonists. Dose–response studies on rapeseed feeds have only been performed in pigs. The rumen microorganisms of cows, sheep and goats can detoxify glucosinolate, so these animals usually consume more rapeseed feed than pigs. Furthermore, the studies of rapeseed feed in pigs generally present the “worst case scenario.” For pigs fed iodine antagonists, German guidelines recommend supplementation with twice the RDI of iodine (Table 16.2; GfE, 2006). This recommendation should probably be applied to most farm animal categories, despite their differing tolerance of diets with rapeseed feeds.

The Effect of Iodine Intake on the Iodine Content of Eggs, Meat and Milk

Egg iodine

Iodine in eggs is concentrated in the yolk. In hens it was found that 90–96% of the total egg iodine was contained in the yolk (Richter, 1995). Increasing dietary iodine increases the iodine concentration of eggs, egg yolks

and egg whites (Richter, 1995; Kaufmann *et al.*, 1998; Kroupová *et al.*, 1999; Yalcin *et al.*, 2004). However, in these studies the yolk iodine concentration varied significantly for any given level of iodine supplementation. For example, supplementation with 5 mg·iodine/kg feed for at least 4 weeks resulted in concentrations of 1500 (Richter, 1995), 1000 (Kaufmann *et al.*, 1998), and 1400 µg/kg total egg content (Yalcin *et al.*, 2004), while Kroupová *et al.* (1999) achieved levels of 3900 µg/kg total egg content with less supplemental iodine (3.5 mg·iodine/kg feed). Recent studies found that eggs contain 1070 µg·iodine/kg total egg content if hens are supplied 3.4 mg·iodine/kg feed. The more common dose of 0.5–1 mg·iodine/kg hen feed results in concentrations of 400–700 µg·iodine/kg (Schöne *et al.*, 2006b).

Meat iodine

Using ICP-MS we found that the iodine concentration of pork, beef and mutton was in the range <1.6–16 µg/kg (Schöne *et al.*, 2002). The lower limit (<1.6 µg/kg) represents the threshold for quantification of iodine as a result of freeze-drying weighed fresh meat, analysis of the ground lyophilisate, and recalculation of the results for the fresh meat. A threshold for quantification of 6.63 µg/kg lyophilisate corresponds to 1.6 µg·iodine/kg fresh meat (Schöne *et al.*, 2006c).

The muscle iodine concentration in the range 4–10 µg/kg was consistent with the data from three muscle samples assessed by neutron activation analysis (Dermelj *et al.*, 1996). However, these observations are only one-twentieth to one-tenth of the results of other studies (Swanson *et al.*, 1990; Rambeck *et al.*, 1997; Herzig *et al.*, 2005) and the data presented in the German nutrient tables (Scherz and Senser, 2000). The high muscle iodine concentrations reported in the literature probably result from the use of methods of iodine detection which were not specific for low iodine matrices.

Recent dose–response experiments in growing pigs (0.1–4 mg·iodine/kg dry matter) and growing bulls (0.5–10 mg/kg dry matter) resulted in a maximum concentration of 17 µg·iodine/kg in pork and 80 µg·iodine/kg in beef (samples from the lumbar portion of *M. longissimus*; Schöne *et al.*, 2006c; Franke *et al.*, 2008; Meyer *et al.*, 2008).

We recently determined the iodine distribution in a slaughtered pig (100–120 kg BW) using ICP-MS. Only 2–5% of body iodine was found in the muscles and adipose tissue; 2% was in the skeleton; and 9% in the blood and viscera (Franke, Schöne, and Flachowsky, 2007, unpublished). Over 85% of body iodine was stored in the thyroid gland.

Milk iodine

In East Germany, in the early 1980s, the milk iodine concentration was below 20 µg/kg. This is characteristic of cow feed without supplemental iodine (Anke *et al.*, 1993).

This increased to 100 µg/kg when feed was supplemented with iodine (Jahreis *et al.*, 1999). Mean concentrations in the range 100–300 µg·iodine/kg milk were found in more recent studies in Germany (Bader *et al.*, 2005; Preiss *et al.*, 1997; Schöne *et al.*, 2003; Launer and Richter, 2005) and other European countries, e.g., Denmark (Rasmussen *et al.*, 2000) and Norway (Dahl *et al.*, 2003). The German Society of Nutrition's recommended daily allowance (RDA) of iodine for young people and adults is 200 µg iodine (D-A-CH, 2000). Half a liter of milk with 100 µg·iodine/kg would provide a quarter of the RDA, but half a liter of milk with 200 µg·iodine/kg would provide half.

In the summer, when cows are allowed to roam on pastureland, the milk iodine concentration tends to be lower than in the winter, when they are kept in barns and given feeds fortified with iodine. It is not uncommon to keep herds of high-yielding cows indoors all year round. The milk from these conventional intensive dairy farms has higher concentrations of iodine than milk from organic farms, where legislation governs the grazing of cows and farmers have reservations about using feed additives including iodine. The studies cited above which measured the iodine concentration of milk did not consider the cows' iodine intake. We therefore investigated the effect of varying the dietary intake of dairy cows on the milk produced (Schöne *et al.*, 2006a).

In the dose–response experiment, five Holstein cows in the last lactation third (7–9 months after the calving) were fed increasing amounts of iodine as calcium iodate-hexahydrate (as a mineral mixture, 150 g/day) over four study periods of 14 days, each. In addition to the unsupplemented feed (control) the cows were given feed supplemented with 65 and 132 mg·iodine/cow/day. These doses provided 3 (control), 17, 68 and 134 mg·iodine/day, i.e., 0.2 (unsupplemented diet), 1.3, 5.1 and 10.1 mg·iodine/kg dry matter. Three samples of milk were taken from each cow during the last 5 days of each study period. The daily yield of milk was 20 kg.

The milk iodine concentration did not differ between sampling days ($P = 0.89$; two-way ANOVA). However, increasing dietary iodine intake significantly increased the iodine content of milk. A fourfold increase in iodine supplementation from 15 mg·iodine/day in period 2 to 65 mg·iodine/day in period 3 resulted in a 3.5-fold increase in milk iodine concentration (Figure 16.7). Doubling the daily iodine dose from 65 mg·iodine/day (period 3) to 132 mg·iodine/day (period 4) resulted in a further 2.3-fold increase in milk iodine concentration. Thirty to forty percent of the ingested iodine was transferred into the milk in the three groups given supplemental iodine. In the period without dietary iodine supplementation (only basal content), it appeared that three quarters of the ingested iodine was transferred to the milk. This was probably overestimated, because iodide released by breakdown of thyroid hormones is recycled and could contribute to the iodine content of milk if there is a dietary iodine deficit.

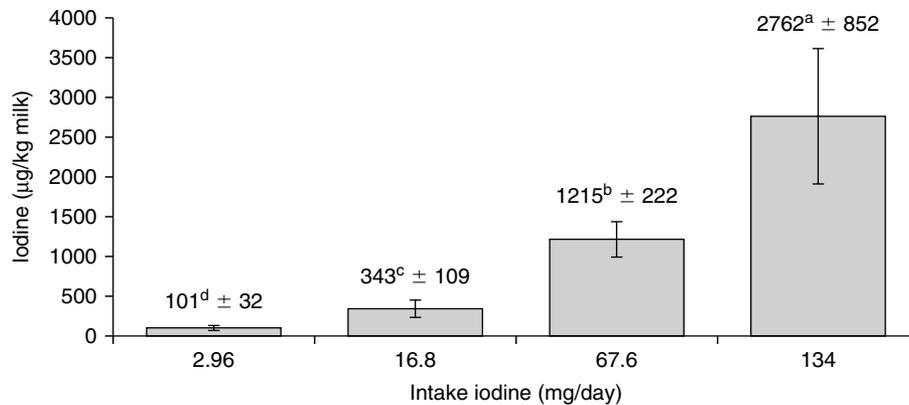


Figure 16.7 The effect of iodine supplements on milk iodine concentration. This figure from a dose-response study conducted in dairy cows demonstrates the effect of increasing doses of supplemental iodine on milk iodine concentration (shown as mean \pm standard deviation of 3 samples taken from the 5 cows in each group, ^{abcd}different indices characterize significant differences, $p < 0.05$, found using the Dunnett test). The supplementation was given exclusively as mineral feed (150 g/day). 6 mg iodine/kg was detected in the mineral feed without supplementation, the other components (maize and grass silage) contributed 2 mg iodine per day (Schöne *et al.* 2006a).

The upper limit (UL) for human daily iodine intake recommended by the German, Austrian and Swiss Societies of Nutrition (D-A-CH, 2000) is 500 $\mu\text{g}/\text{day}$. Consumption of only 250 ml of the milk produced during period 4 of the study would result in ingestion of more than 500 μg iodine. As a result of this experiment, the European Food Safety Authority reduced the maximum allowed iodine intake of cows and hens in the EU from 10 mg/kg feed to 5 mg/kg grain dry matter equivalent (EU, 2003, 2005; EFSA, 2005).

Despite controversy over the relatively low German UL, iodine supplementation, like selenium, has a high application risk (BfR, 2006). This is demonstrated by the slight difference between the RDA of iodine (200 $\mu\text{g} \cdot \text{iodine}/\text{adult}/\text{day}$) and the UL or upper tolerable level (500 $\mu\text{g} \cdot \text{iodine}/\text{adult}/\text{day}$; D-A-CH, 2000). The UL differs from the RDA by a factor of 2.5. Thus supplementation with more than the 2.5 times the RDA to prevent or treat deficiency and hypothyroidism increases the risk of producing the adverse effects of excess iodine (hyperthyroidism).

For human consumption, a cows' milk iodine concentration of 200–300 $\mu\text{g}/\text{L}$ milk is desirable. This requires iodine supplementation of cows feed with 1 mg iodine/kg feed DM (Figure 16.7). This exceeds the GfE (2001) recommendation of 0.5 mg iodine/kg DM but is below the cited approved maximum amount (5 mg iodine/kg grain equivalent feed: EU, 2005). Feed compounders and dairy farmers generally aim for a total feed iodine content of 1 to 1.5 mg/kg DM using premixed vitamin and mineral supplements and compound feed (Grünewald and Steuer, 2006). In view of the recommendations of animal nutrition societies and cow feeding in practice it is a challenge for EU legislation to further decrease the maximum feed iodine content.

Food produced by farm animals can provide a significant proportion of the iodine required by humans. Supplementation of iodine in the feed of hens, dairy cows, ewes (Aumont *et al.*, 1989a; Hampel *et al.*, 2004), or goats (Groppe and Anke, 1983) increases the concentration of iodine in eggs and milk, respectively. In most industrialized countries the mean daily intake of liquid milk products per adult is 300–500 ml. Consumption of 300–500 ml of milk with an iodine concentration in the range 150–300 $\mu\text{g}/\text{kg}$ could provide up to half the daily RDA for adults (200 $\mu\text{g} \cdot \text{iodine}$). Production of cows' milk with an iodine concentration within the desired range requires an iodine intake of 10–20 mg $\cdot \text{iodine}/\text{cow}/\text{day}$ (0.5–1 mg/kg dry matter, i.e., 1–2 mg/kg compound feed).

Dipping teats in iodine-containing milk sanitizers has a small effect on milk iodine concentration. In recent investigations the increase in milk iodine concentration from the pretreatment period (without the iodophor) to the treatment period with the iodophor was of the order of one-tenth to one-half of the initial concentration (Galton, 2004; Flachowsky *et al.*, 2007). Cheese is not a good source of iodine for humans. When cheese is made iodine does not remain in the curd, but distributes between the curd and the whey (Zimmermann *et al.*, 2005).

Effects of Excessively High Dietary Iodine Intake on Farm Animals and Poultry

Toxicity: excess in farm animals including poultry

Misuse of mineral premixtures and mineral blocks containing concentrated iodine could cause excessive iodine intake. The use of supplements without defined amounts

of iodine must be discouraged. Excessive iodine intake affects the health of the animals, and may affect consumers as iodine is concentrated in milk and eggs (see section "The Effect of Iodine Intake on the Iodine Content of Eggs, Meat and Milk").

Symptoms of excessive dietary iodine intake in calves (Newton and Clawson, 1974a) and dairy cows (Olson *et al.*, 1984) include coughing, profuse nasal discharge, and skin and hair changes, e.g., alopecia (Mangkoewidjojo *et al.*, 1980). In three 16–20-week experiments in calves (80–120 kg BW), feed intake and growth intensity were reduced when iodine was administered at doses of 100 or 200 mg/kg·diet (ground grain and hay, 880 g dry matter/kg feed), i.e., 4–7 mg·iodine/kg BW/day. No effects on thyroid weight were observed however, the mean (130–170 mg thyroid/kg BW) appeared relatively high. In studies conducted on herds of cows Olson *et al.* (1984) reported that dairy cows fed over 68–600 mg EDDI daily (55–482 mg·iodine; i.e., 0.08 – approximately 0.75 mg·iodine/kg BW/day for a cow weighing 600–700 kg), for between 1 month and 7 years, developed the symptoms of excessive dietary iodine intake described above. The milk yield of cows fed ≥ 0.55 mg·iodine/kg BW/day for 1 month was reduced by 15%. The symptoms improved over 4 weeks when the dietary iodine supplementation was reduced to a level close to the RDI (12 mg/day = 0.02 mg·iodine/kg BW/day).

Furthermore, in several cattle herds, the use of large doses of iodine to treat respiratory and other diseases was found to increase the symptoms described above (McCauley and Johnson, 1972). Indeed, iodine excess is associated with suppression of cellular and humoral immunity, as well as increased incidence of diseases of the respiratory system and skin. Administration of an iodine load to young cattle resulted in a reduction of the blastogenic response of lymphocytes to B and T cell mitogens, depression of phagocytic capacity, and shortening of the persistence of the antibody response to bacterial mitogens (Haggard *et al.*, 1980).

The risk of excessive iodine intake in sheep is high if mineral blocks are used for supplementation. When the effects of the major and trace elements in the amounts administered via the mineral blocks were assessed separately, only iodine reduced plasma immunoglobulin (Ig)G concentration (Boland *et al.*, 2004). Rose *et al.* (2007) fed lactating ewes 5.5, 9.9, 14.8 and 21 mg·iodine/kg·diet dry matter. The lambs of the mothers given the two highest iodine doses had only half the IgG in the plasma of those born to mothers fed the lowest dose (Rose *et al.*, 2007). Absorption of sufficient Ig from the mother is required to maintain the health of the mammalian neonate. A deficiency of Ig in the neonate increases the incidence of pneumonia, septicemia and navel infections, increasing neonatal mortality.

Excess iodine in poultry delays sexual maturation in males and females. In laying hens excess iodine intake progressively reduces egg yield. At 2500 mg iodine/kg·diet (approximately 100 mg iodine/kg BW/day) ovulation is

inhibited and laying stops (Lewis, 2004). Hatchability is more sensitive to iodine intake. In turkeys 35 mg·iodine/kg in the diet did not affect number or quality of eggs, but did reduce the number of fertile eggs (as a percentage of total number of eggs laid, Christensen and Ort, 1991).

In pigs given 10–1600 mg·iodine/kg·diet (0.42–68 mg·iodine/kg BW/day) for 12 weeks, growth, feed intake, and feed:gain ratio were not affected up to 400 mg·iodine/kg·diet (19 mg·iodine/kg BW/day; Newton and Clawson, 1974b). This NOEL is high and suggests that pigs are more tolerant of iodine excess than cattle. In pigs, serum iodine concentrations of 93 (control), 392, 1065, 1871 and 2935 μ g·iodine/l were detected when doses of <0.05 (control = recommendation), 0.42, 0.86, 1.8 and 3.5 mg·iodine/kg BW/day were administered respectively. In a 2nd 16-week experiment 171 (control), 1365, 2325, 3336, 5888, 11200, 16825, 24312 μ g/l were detected when doses of <0.05 (control = recommendation), 1.2, 2.3, 4.5, 9.4, 19, 34 and 68 mg·iodine/kg BW/day were administered respectively. The thyroid was weighed at slaughtering, and it was found that weight increased with the dose of iodine supplementation. The increase in thyroid weight achieved significance with the addition of 80 mg·iodine/kg·diet in the first experiment, and with the addition of 1600 mg·iodine/kg·diet in the second.

Effects of large doses of iodine on the growth and thyroid status of pigs

We compared the effects of large doses of iodine (10 mg/kg·diet) with iodine supplements within the recommended range (100 and 1000 μ g/kg·diet) on a total of 120 pigs (3 groups of 40 animals; Schöne, 1999). Iodine supplementation did not affect feed consumption (Table 16.3) or growth (daily weight gain). The serum iodine concentration was significantly increased in the pigs receiving the highest dose of iodine. However, the serum T₄ concentration did not differ significantly between the three groups. The percentage of total serum iodine which was present in T₄ fell from about half in the groups given the low and moderate doses of iodine, to about one-third in the group given the highest dose.

However, in this study the serum iodine concentration of pigs receiving 1 and 10 mg·iodine/kg·diet was relatively low because blood was sampled when the animals were slaughtered and feed had been withdrawn 24 h prior to this. The serum iodine concentration peaks a few hours after ingestion of iodine and declines rapidly with fasting, because any surplus iodide is rapidly excreted by the kidneys. After administration of doses of iodine which are several times more than the recommendation/requirement (Table 16.2), the serum concentration may vary from 50 to 100 μ g/l iodine up to several 100 μ g/l serum, depending on the duration of feed withdrawal (Schöne *et al.*, 2006c).

Table 16.3 Effect of low, moderate and high dietary iodine intakes on the performance, iodine and thyroid hormone status of pigs

	Added iodine ($\mu\text{g}/\text{kg} \cdot \text{diet}$)		
	100	1000	10,000
Performance – 40 pigs/group			
Feed intake (kg/day) NS	2.32 \pm 0.08	2.36 \pm 0.06	2.37 \pm 0.08
Weight gain (g/day) NS	757 \pm 92	751 \pm 96	742 \pm 121
Feed:gain ratio (kg feed/kg gain) NS	3.06 \pm 0.14	3.14 \pm 0.12	3.19 \pm 0.14
Serum parameters – 10 pigs/group			
Iodine ($\mu\text{g}/\text{l}$)	56 ^b \pm 10	54 ^b \pm 20	81 ^a \pm 17
T ₄ (nmol/l) NS	51 \pm 5	48 \pm 15	47 \pm 8
T ₃ (nmol/l)	1.2 ^a \pm 0.2	0.9 ^{ab} \pm 0.3	0.8 ^b \pm 0.2
rT ₃ (nmol/l) NS	1.0 \pm 0.3	1.0 \pm 0.3	1.2 \pm 0.3
Thyroid parameters – 10 pigs/group			
Weight (g)	9.9 ^b \pm 1.9	9.7 ^b \pm 1.8	17.8 ^a \pm 4.3
Weight/body weight (mg/kg)	88 ^b \pm 18	88 ^b \pm 11	163 ^a \pm 37
Concentration iodine ($\mu\text{g}/\text{g}$)	705 ^c \pm 75	2737 ^b \pm 353	3320 ^a \pm 378
Total iodine (mg)	7.1 ^c \pm 2.0	26.8 ^b \pm 7.4	59.9 ^a \pm 20.5
Stored iodine % of intake (%), for calculation see the notes	20 ^a \pm 8	9 ^b \pm 3	2 ^c \pm 1

Notes: This table demonstrates the effect of low, moderate and high dietary iodine intakes on the performance, iodine and thyroid hormone status of pigs. 40 and 10 animals per group, respectively. Initial weight 28.1 \pm 0.8 kg (mean \pm standard deviation; Schöne, 1999).

The low dosage tested represents the magnitude of the requirement/recommendation of the German animal nutrition society (GfE, 2006), the medium dosage corresponds with the supplementation applied to the compound feed and the high dosage represents the upper limit of dietary supplement allowed for pigs.

Calculation storage (%) = $\frac{\text{total thyroid iodine at slaughtering, mg} - \text{estimated total iodine at experiment's start}}{\text{total iodine feed supplement's intake, mg}} \times 100$;

At experiment's start thyroid iodine was estimated at 2 mg (resulting from 75 mg thyroid/kg BW = 2g total thyroid weight and a concentration of 1000 μg iodine/g organ).

^{abc}Different indices in the same line characterize significant differences ($P < 0.05$) found using the Student–Newman–Keuls test. NS=not significant.

When serum iodine is used as a diagnostic test for iodine deficiency blood should be sampled within 12 hours of a meal at most.

The dosage of 10 mg·iodine/kg·diet decreased serum T₃ concentration significantly and increased (as a tendency) the serum concentration of rT₃. In turkeys, higher doses of iodine, i.e., 20–100 times the recommendation/requirement (Table 16.2) reduced the concentration of T₃ (Christensen and Ort, 1991) which indicates an unfavorable effect on thyroid hormone metabolism.

The weight of the thyroid gland of the pigs given 10 mg added iodine/kg·diet was double that of the pigs given one-tenth or one-hundredth this dose. In more recent studies the addition of 5 mg added iodine/kg·diet (approximately 0.15 mg/kg BW/day) also resulted in significant enlargement of the thyroid glands: 107 mg thyroid weight/kg BW versus 68 mg thyroid weight/kg BW in controls (Berk *et al.*, 2004; Franke *et al.*, 2008).

These findings confirm previous observations. In pigs treated with potassium iodide (5 mg iodine/day = approximately 2 mg·iodine/kg·diet) the thyroid weight doubled, the height of epithelial cells was reduced, and the follicle diameter increased 1.5–3 times in comparison to untreated controls (Seffner and Heller, 1979). The greater

sensitivity to iodine in the older studies may be because the animals used were initially hypothyroid and iodine deficient. If so, the moderate iodine dosage used (2 mg/kg·diet) may have induced colloid goiter and hyperthyroidism. As supplementation is now widespread, animals used in more recent studies are less likely to be iodine deficient, and therefore > 2 mg/kg·diet are required to provoke colloid goiter.

An increase of supplemental iodine from 0.1 to 1 mg/kg feed resulted in a fourfold increase in thyroid iodine concentration (Table 16.3; Schöne, 1999). However, the further increase from 1 to 10 mg·iodine/kg feed increased thyroid iodine concentration by only 1/5. The thyroid iodine concentration eventually reaches a plateau, and does not increase despite further increase in dietary iodine intake. This suggests that the ability of the thyroid gland to store iodine is limited. In the pigs given 0.1 mg added iodine/kg·diet, thyroid iodine was 20% of the consumed iodine, but only 2% of the consumed iodine at the dose of 10 mg added iodine/kg·diet.

In studies in pigs, where large doses of iodine were administered to try to improve meat iodine content, the UL of thyroid iodine concentration was found to be in the range 2000–3000 $\mu\text{g}/\text{g}$ (Schöne *et al.*, 2006). However, Franke *et al.* (2008) reported that the maximum thyroid

iodine concentration was about 1600 µg/g in both the highest doses tested.

In both these pig experiments and in a later experiment with growing bulls (Meyer *et al.*, 2008) the highest tested iodine dose significantly increased the thyroid weight by one-half to about double that of controls. Undoubtedly, iodine excess induces colloid goiter and inhibits iodine uptake and hormone synthesis by the thyroid in laboratory animals (Wolff, 1969), and in human populations consuming seaweed, i.e., food with an extremely high iodine content (Nagataki, 1993).

The effect of dietary iodine on thyroid iodine concentration in various species

There are relatively few investigations of thyroid iodine concentrations in farm animals and poultry fed a broad range of doses of iodine. However, the response to iodine supplementation seems to vary with age and between species.

In bulls (100–300 kg BW) supplementation of feed with 0.4–0.7 mg added iodine/kg feed dry matter resulted in thyroid iodine concentrations of 800 µg/g thyroid (Groppel *et al.*, 1989). Meyer *et al.* (2008) found thyroid iodine concentrations of 800 µg/g thyroid in bulls (240–550 kg BW) fed the relatively high dose of 10 mg added iodine/kg feed dry matter. Interestingly, a thyroid iodine concentration of 370 µg·iodine/g thyroid was achieved with only 0.5 mg added iodine/kg·diet dry matter.

Goats and sheep fed only 600 µg added iodine/kg feed (i.e., grain dry matter equivalent) had thyroid iodine concentrations in the range 900–1300 µg/g gland, with no differences between the dams and their kids and lambs, respectively (Groppel *et al.*, 1989).

Birds may store large amounts of iodine. In experiments with Japanese quails, the female adults had mean thyroid iodine concentrations of 500–4000 µg·iodine/g thyroid when diets without supplementation and up to a maximum of 1200 µg added iodine/kg·diet were administered. Remarkably, the thyroid iodine concentration was 3000 µg·iodine/g with only 150 µg added iodine/kg·diet and remained at this level with 300–600 µg added iodine/kg·diet (McNabb *et al.*, 1985). The maximum thyroid iodine concentration found in the 1-day-old chicks of these quail hens was 900 µg·iodine/g, i.e., only 1/4 of the maternal thyroid iodine concentrations (Stallard and McNabb, 1990).

Laying hens also had high thyroid iodine concentrations of 4400–6300 µg/g when only 0.34 mg·iodine/kg·diet was added (Richter *et al.*, 1998). The diets contained linseed or linseed press cake whose cyanogenic glycosides increased serum SCN concentration (compared to a control diet without linseed feeds). However, there were no effects on thyroid weight, thyroid iodine content, or concentrations of T₄ and T₃ in the serum.

Upper limits of added iodine in animal feed

The ULs for iodine supplementation of the feeds of cattle and pigs – 50 mg/kg cattle diets (NRC, 1980) and 400–800 mg/kg pig diets (NRC, 1980; Kirchgeßner, 1992) were mainly derived from studies of chronic toxicity. However, the doses, duration and response criteria of these experiments vary considerably and extrapolation of the findings should be done with caution.

An increase in thyroid weight and incidence of colloid goiter are useful in the diagnosis of iodine excess. The finding of goitrous foals from mares consuming a highly iodized feed (Baker and Lindsey, 1968) is justifiably a frequently cited example of iodine excess in domestic animals.

In the 1990s iodine supplementation in animal nutrition was driven by the desire to maximize the concentration of this trace element in milk, meat and eggs. As a result, even as late as 1994 German feed legislation allowed a maximum of 40 mg added iodine/kg feed (i.e., grain dry matter equivalent) of cattle, pigs and poultry. However, horses were limited to a maximum of 4 mg·iodine/kg feed (Weinreich *et al.*, 1994). Three years later, the maximum was reduced to 10 mg/kg feed grain dry matter equivalent in cattle, pigs and poultry. The UL in horse feed remained at 4 mg added iodine/kg feed (Weinreich *et al.*, 1997).

There is now a real risk of iodine toxicity in humans from consumption of milk or eggs produced by animals given too much iodine. The EU has therefore set an UL of 5 mg·iodine/kg feed (i.e., grain dry matter equivalent) for the dairy cows and laying hens (EU, 2005). However, recent experiments on growing pigs and bulls have demonstrated that this may still be too high, as supplements at this dose increase thyroid weight and inhibit storage of iodine within the gland. In view of the risks to thyroid status and the lack of effect of higher doses on meat iodine content, reduction of the UL in pig feed to below 3 mg·iodine/kg·diet should be considered. This UL for addition of iodine to feeds is still many times the recommendations/requirements advised by the animal nutrition societies (Table 16.2).

Summary Points

- Iodine deficiency results from consumption of feeds which contain little (or no) iodine and iodine antagonists.
- The requirements/recommendations for iodine supplementation vary between animal categories. To ensure an adequate iodine content in milk, lactating animals require more iodine than growing animals.
- This chapter describes some dose–response studies of the iodine requirement of farm animals fed typical grain diets without or with glucosinolates as iodine antagonists.
- The iodine content of eggs, milk, and to some extent meat, produced by farm animals reflect the feed iodine supplementation. The effects of increasing iodine intake

on the concentration of iodine in animal products are discussed above.

- Excessive intake of iodine is toxic to animals and may affect humans if the produce of these animals is consumed.

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Abstract

Radioiodine plays an important role in the diagnosis and treatment of various thyroid disorders. Production methods for various iodine isotopes, namely, ^{131}I , ^{120}I , ^{123}I , ^{124}I and ^{125}I are briefly described in this paper. The chemistry of iodine and radiation effects in aqueous solutions and isotopic exchange reactions are also reviewed. An understanding of the chemistry of iodine is essential in isotope production, and for developing the procedure to prepare the radioactive iodine labeled pharmaceuticals. In radiochemical analysis of iodine, most environmental and biological samples can be accurately analyzed by neutron activation at trace levels. The use of potassium iodide (KI) has become an important remedy to prevent the harmful effects of radioiodine exposure under nuclear accident conditions. The inhibitory effect of KI administration on thyroid radioactive iodine uptake is discussed.

Abbreviations

ENAA	Epithelial neutron activation analysis
HPGe	High-purity germanium
KI	Potassium iodide
MDA	Minimum detectable amount
NAA	Neutron activation analysis
NIS	Sodium iodide symporter
RAIU	Radioactive iodine uptake
T_3	Triiodothyronine
T_4	Thyroxine

Introduction

Iodine-rich thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) are necessary for growth and development. They stimulate all aspects of cell metabolism, including protein synthesis and oxygen consumption. It has been recognized for more than 50 years that iodine

is an essential component of the thyroid hormones T_4 and T_3 , and that severe iodine deficiency (less than $50\mu\text{g}$ iodine intake daily) is the major cause of mental retardation and endemic goiter and cretinism worldwide.

Radioiodine plays an important role in the diagnosis and treatment of various thyroid disorders. Treatment of thyroid carcinoma and hyperthyroidism with ^{131}I -pharmaceuticals has been practiced for years, but other isotopes, i.e., ^{120}I , ^{123}I , ^{124}I and ^{125}I are also produced and used in various medical applications. Radioiodine concentrated by the thyroid in large amounts can cause cell death, primarily because of ^{131}I 's beta radiation. Large doses of ^{131}I are, therefore, given to treat patients with hyperthyroidism. In contrast, low-dose exposure damage does not kill thyroid cells, but can induce radiation damage and mutations, which can result in thyroid cancer.

Radioiodine is also identified as one of the most important fission products that can be released from nuclear facilities, particularly under accident conditions, in terms of its radiological effects on the environment, especially human exposure. A large amount of ^{131}I delivered to the thyroid almost always leads to hypothyroidism, because of permanent radiation-induced destruction of thyroid cells. The use of potassium iodide (KI) helps in preventing such harmful effects.

The analysis of trace iodine in biological, as well as environmental, samples is of immense interest in this review. Special attention is given to the radiochemical analysis with neutron activation of ^{127}I and ^{129}I .

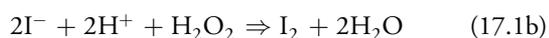
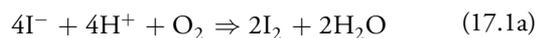
The behavior of iodine at trace levels is often anomalous compared with that at macro concentrations. Because of these anomalies, in radiochemical investigations of iodine, either trace analysis or synthesis of labeled compounds, care must be taken to develop proper procedures to deal with potential problems. At the beginning of this review, it is necessary to briefly discuss the major chemical reactions of iodine, which are of importance to radiochemical studies and may be involved in chemical analysis and isotope production. Also included in this discussion are

isotopic exchange reaction and radiation effects on iodine in aqueous solutions. Radiochemical technology plays an important role in isotope production and pharmaceutical preparation.

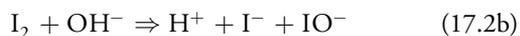
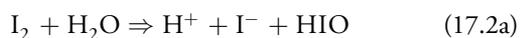
Chemistry of Iodine: Relevance to Radiochemical Studies and Nuclear Properties of Iodine Isotopes

Major iodine reactions in aqueous solutions

The chemistry of iodine in aqueous solutions is very complex and extremely sensitive to oxidizing and reducing impurities and radiation effects, particularly at very low concentrations dealing with radioiodine. Iodide ions may be oxidized in acidic solutions by oxygen and/or hydrogen peroxide, as shown in the following reactions:



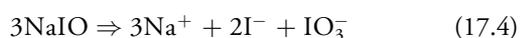
Molecular iodine, I_2 , is easily hydrolyzed in neutral and basic solutions:



HIO may dissociate into H^+ and IO^- :



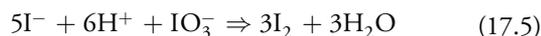
and IO^- may disproportionate to form IO_3^- in basic solution:



The kinetics of these reactions has been reviewed by several investigators (Beahm *et al.*, 1986, 1992; Bell *et al.*, 1982a, b; Weber *et al.*, 1992).

Apparently, the rate of Reaction (17.1a, b) will be dependent on the concentrations of all three reactants involved. At high pH and low O_2 or H_2O_2 , the I_2 formation should be very minimal. If I_2 is formed in acidic solution, it will be hydrolyzed (Reaction 17.2a, b) rapidly to form HIO, which may partially dissociate into H^+ and IO^- . Although it seems thermodynamically possible for HIO to disproportionate to form IO_3^- and I^- ions, it is known to be a second or higher order of reaction (Haimvich and Treinin, 1965), which is almost impossible to proceed, to any extent, at very low iodine concentrations. In fact, no credible experimental data has been reported

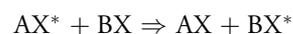
for the formation of IO_3^- at very low concentrations (Bell *et al.*, 1982b), and no iodate was found in a laboratory study with I_2 as a starting material dissolved in water (Lin, 1981). On the other hand, at higher iodide concentrations, iodide ions can be effectively oxidized by IO_3^- in acidic solution according to the classical Dushman reaction (Reaction 17.5) (Fureichi, 1972), which is frequently used in radiochemical analysis,



Although it is well-known that iodine may exist in a number of oxidation states, the behavior of trace-level iodine often differs from the behavior of iodine at macro concentrations (Eiland and Kahn, 1961; Kahn and Wahl, 1953; Studier *et al.*, 1962; Wille and Good, 1957; Wolfenden, 1957). Care should be exercised when dealing with radioactive iodine in solution at very low concentrations.

Isotopic exchange reactions

Isotopic reaction is a powerful tool in the studies of chemical reaction mechanisms and kinetics, as well as analytical chemistry. It is also frequently used in the preparation of labeled compounds. The exchange reaction can be represented by a simple reaction:



where X^* denotes the radioactive species. The isotopic reaction was first described in detail by Wahl and Bonner (1951). In a general case with reactant concentrations $[\text{A}]$ for $(\text{AX} + \text{AX}^*)$ and $[\text{B}]$ for $(\text{BX} + \text{BX}^*)$, the first-order reaction rate is R with a rate constant k .

$$R = k[\text{A}][\text{B}]$$

where X^* denotes the radioactive atom.

The extent of reaction at time t can be estimated by the following equation:

$$Rt = -\ln(1 - F) \frac{[\text{A}][\text{B}]}{([\text{A}] + [\text{B}])}$$

where F is the fraction of exchange at time t . This equation can be rewritten as:

$$\ln(1 - F) = -kt([\text{A}] + [\text{B}])$$

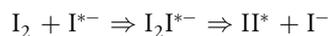
and the half time of the reaction can be easily estimated by

$$t_{(1/2)} = \frac{0.693}{([\text{A}] + [\text{B}])}$$

Experimentally, the exchange rate constant is first measured in a reaction at various time intervals, and the

exchange rate is calculated from the known reactant concentrations. In most cases, when the concentration of initially radioactive species is very small (i.e., $[A] \ll [B]$), the reaction half time may be inversely proportional to $[B]$.

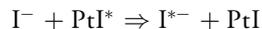
As an example, the well-known exchange between elemental iodine and iodide ions in solution is completed within a few seconds (Dodson and Fowler, 1939):



The exchange reaction of iodine atoms between CH_3I and I_2^* or I^{*-} has been studied in either organic solvents or alcohol-water mixtures (Lin, 1968; Noyes and Zimmerman, 1953):



The exchange rate varies depending on not only the concentration of reactants, but also the characteristics of the solvent. Heterogeneous exchange reactions between iodide, elemental iodine, and other iodine species with radioactive iodine atoms adsorbed on platinum surfaces have also been reported (Toth, 1972). This is of interest, and probably very useful in preparing labeled compounds by this heterogeneous exchange reaction:



Exchange reactions among the iodine species have been studied to a certain extent and the results are qualitatively summarized in Table 17.1. Application of isotopic exchange reactions to prepare the labeled compounds will be discussed in "Production of Iodine Isotopes".

Radiation chemistry of iodine in aqueous solutions

Radiation chemistry of iodine at very low concentrations in aqueous solutions was first investigated by Lin (1980b) and later by others (Ishigure *et al.*, 1988). Iodide ions can easily be oxidized to I_2 at higher concentrations in acidic

Table 17.1 Major exchange reactions among iodine species in aqueous solutions

State	Pt-I	I_2	I^-	IO^-	IO_3^-	CH_3I
Pt-I	-	Rapid	Rapid	Rapid	NE	Moderate ^b
I_2	-	-	Rapid	Rapid	Slow	Moderate ^b
I^-	-	-	-	Reacts in acid	Slow, reacts in acid	Moderate ^b
IO^-	-	-	-	-	NE ^a	NE ^a
IO_3^-	-	-	-	-	-	NE ^a
CH_3I	-	-	-	-	-	-

^aNE, not examined.

^bStudied in organic solvents or alcohol-water solutions only.

solutions (Figure 17.1), the yield of I_2 formation as a function of radiation dose is shown in Figure 17.2. At lower concentrations, iodide (I^-) is oxidized to iodate (IO_3^-) through HIO as an intermediate. Radiation-induced iodate formation increases with increasing dose rate, and the chemical yield is enhanced by lower concentrations (Lin, 1980b; Ishigure *et al.*, 1988).

The reaction mechanism of iodine radiolysis can be described by the following reactions, and the mechanism of formation of IO_3^- at very low concentrations is believed to be the result of successive radiolytic oxidation of I^- and/or HIO (Lin, 1980b).

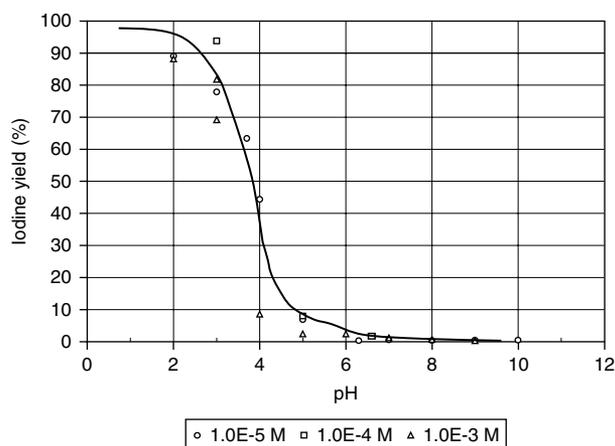
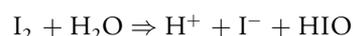
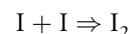
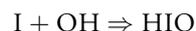
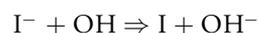


Figure 17.1 Effect of water pH on iodine (I_2) formation.

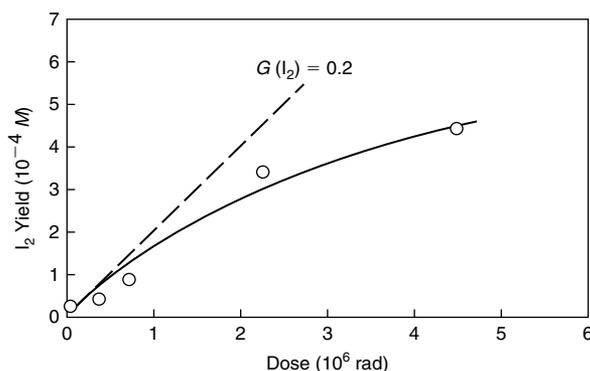


Figure 17.2 Yield of I_2 as a function of gamma-ray radiation dose. Lin, 1980b.

Nuclear properties of iodine isotopes

Major iodine isotopes of interest in this review are summarized in Table 17.2. Among all these isotopes, with the exception of ^{131}I , all fission products are beta emitters with various half lives. They cannot easily be isolated and purified for medical applications. Thus, they are exclusively used in monitoring fuel integrity in nuclear power plants. Formerly reactor-made ^{131}I was used almost exclusively in nuclear medicine. Now it is going to be replaced by cyclotron-made isotopes, particularly ^{123}I . This is because these isotopes generally have shorter half lives and give a lower internal radiation dose to the patient, and their gamma ray energy is ideally suited to many gamma cameras.

In neutron activation analysis (NAA) of trace iodine, the reactions $^{127}\text{I}(n,\gamma)^{128}\text{I}$ and $^{129}\text{I}(n,\gamma)^{130}\text{I}$ are used. ^{128}I and ^{130}I have proper gamma rays for gamma spectrometric analysis.

Production of Iodine Isotopes

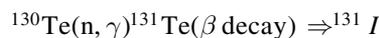
Production of carrier-free iodine-131

The term “carrier-free” stands for “isotopically pure.” However, the carrier-free ^{131}I product, obtained from either fission or tellurium target, generally contained small amounts of inactive ^{127}I and long lived ^{129}I . The contamination of ^{127}I and ^{129}I , however, can hardly act as a carrier for the ^{131}I as long as no carrier is intentionally added.

Table 17.2 Nuclear properties of major iodine isotopes

Isotope	Half life	Decay mode	Major gamma rays (keV)	Major production mode
^{120}gI	1.35	h	EC, β^-	$^{120}\text{Te}(p,n)^{120}\text{gI}$
^{123}I	13.2	h	EC	$^{123}\text{Te}(p,n)^{123}\text{I}$ $^{124}\text{Te}(p,2n)^{123}\text{I}$
^{124}I	4.18	d	EC, β^+	$^{124}\text{Te}(p,n)^{124}\text{I}$
^{125}I	60.1	d	EC	$^{124}\text{Xe}(n,\gamma)^{125}\text{Xe}$ $^{125}\text{Xe}(\beta^+ \text{ decay}) \Rightarrow ^{125}\text{I}$
^{126}I	13	d	EC, β^-, β^+	$^{127}\text{I}(n,2n)^{126}\text{I}$
^{128}I	25.0	m	β^-, β^+ , EC	$^{127}\text{I}(n,\gamma)^{128}\text{I}$
^{129}I	1.57×10^7 y		β^-	Fission
^{130}I	12.4	h	β^-	Fission, $^{129}\text{I}(n,\gamma)^{130}\text{I}$
^{131}I	8.02	d	β^-	$^{130}\text{Te}(n,\gamma)^{131}\text{Te}$ $^{131}\text{Te}(\beta \text{ decay}) \Rightarrow ^{131}\text{I}$ Fission
^{132}I	2.30	h	β^-	Fission
^{133}I	20.8	h	β^-	Fission
^{134}I	52.6	m	β^-	Fission
^{135}I	6.61	h	β^-	Fission

The development of ^{131}I preparation techniques has been reviewed from time to time by Kahn (1962) and by IAEA (1966) and Constant (1970). The production of ^{131}I from nuclear fission of uranium, which was employed earlier at some nuclear facilities, requires elaborate equipment, purification procedures and waste handling facilities. The method of ^{131}I production employed in most production facilities nowadays is by irradiation of Te metal or TeO_2 based on the following reactions:



Both wet and dry distillation methods are employed to recover ^{131}I produced from the tellurium targets.

Distillation Methods Distillation methods have been widely used in iodine isotope production. Since iodine may be converted to a volatile form (I_2), either wet distillation or dry distillation has been employed. A general distillation procedure for carrier-free ^{131}I purification has been reported earlier by Kahn and Freedman (1954). In a wet distillation method (IAEA, 1966), irradiated Te metal is dissolved in a chromic acid– H_2SO_4 mixture. After complete dissolution, the iodate (IO_3^-) formed is reduced to elemental iodine (I_2) with oxalic acid and then distilled off from the solution. The distillate is trapped in alkaline sulfite solution. This solution is then purified by an oxidation–reduction cycle and finally redistilled into dilute alkaline solution. In another wet distillation method, irradiated TeO_2 is dissolved in NaOH and the sodium tellurite is oxidized with H_2O_2 in the presence of a catalyst, sodium molybdate. The mixture is then acidified with H_2SO_4 and the iodine is distilled off and trapped in ice-cold water.

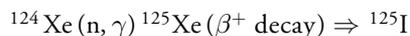
In a typical dry distillation method, the irradiated telluric target, Te or TeO_2 , is heated to 700–800°C in a stream of air and the iodine produced is distilled off and trapped in diluted NaOH or NaHCO_3 solution (Evans and Stevenson, 1956).

Adsorption Method Iodine separation by the platinum adsorption technique was first reported by Toth (1961) and Lin *et al.* (1963). Oak Ridge National Laboratory has adopted an adsorption procedure (Case and Acree, 1966) for the purification of ^{131}I produced from fission by a distillation process. In 1966, in comparison with other methods of ^{131}I , Baker (1966) has concluded that the adsorption technique is the most efficient and economical method. In this method, a metallic Pt plate or felt is used to adsorb carrier-free iodine from an acidic solution containing target materials. The Pt plate or felt with iodine activity is then removed from the solution and thoroughly washed with water. The activity is then desorbed in a slightly basic solution. Application of electrical current could enhance the desorption process. Nearly quantitatively the adsorbed iodine activity can be desorbed from the Pt surfaces.

Iodine adsorbed onto the Pt surface is believed to be in the atomic state, and can be easily exchanged with non-radioactive iodine, I_2 or I^- , in solution. This isotopic exchange procedure is very convenient for preparing labeled compounds, as discussed in "Radiation chemistry of iodine in aqueous solutions".

Production of iodine-125

A traditional method of producing ^{125}I is by neutron irradiation of ^{124}Xe in a reactor through the following reactions:



In the irradiated Xe gas container, ^{125}I is washed out with dilute NaOH solution (Baker and Gerard, 1972). The solution may be further purified through distillation. In recent years, ^{125}I has also been produced from tellurium targets by activation with cyclotron-produced particles.

Production of iodine-120, 123, 124

Formerly reactor-made ^{131}I was used almost excessively. However, now most ^{131}I will be replaced by cyclotron-made isotopes, especially ^{123}I . ^{123}I is primarily a gamma emitter with a short physical half life of 13 h, and ^{131}I is a beta and gamma emitter with a longer physical half life of 8 days. Since greater cellular damage or cell death is produced by the higher energy beta emission of ^{131}I than by the gamma emission of either isotope, ^{123}I is now the preferred choice for diagnostic studies of the thyroid.

These three radionuclides can be all produced by the (p, n) reaction using isotopically enriched solid TeO_2 targets (Scholten *et al.*, 1995; Qaim *et al.*, 2003; Comor *et al.*, 2004). The radioiodine produced can be separated rapidly from the target material by dry distillation of the melted target after irradiation, as described earlier ("Distillation Methods").

Preparation of radioiodine in chemical forms other than iodide

Practically, all radionuclides of iodine produced from target materials are purified and dissolved in dilute alkaline solution, or solution containing reducing agent to ensure that iodine stays in the reduced form of iodide (I^-). However, in many cases where iodine activity occurs at very high concentrations in solution, a small fraction of iodate (I^-) is formed, most likely due to radiation-induced oxidation (discussed in "Radiation chemistry of iodine in aqueous solutions"). This may cause some inconvenience when iodide is used. However, it is of interest to know that when iodine activity is administered for goiter prevention, radioiodine in the form of iodate is taken up by the thyroid rapidly as effectively as iodide (Cronquist *et al.*, 1971).

In some laboratory applications, iodine activity may be needed in other chemical forms. Elemental iodine labeled with ^{131}I can be prepared easily by several techniques. Oxidation of iodide in acidic solution with KIO_3 (Myer and Kennedy, 1950) (see Equation 17.5) is a popular method, and isotopic exchange reaction (see later) between I_2 in organic solvent and carrier-free $^{131}I^-$ in aqueous solution is another (Kahn and Freedman, 1954). Preparation of labeled organic iodides, such as CH_3I , can be achieved by isotopic exchange reaction in alcohol-water solutions (Atkins and Arkell, 1965). Carrier-free CH_3I (-131) has been prepared by the reaction of dimethyl sulfate $(CH_3)_2SO_4$ and carrier-free $^{131}I^-$ in the presence of $CaCO_3$ (Nebeker *et al.*, 1971). The preparation of carrier-free iodine activity in iodate (I^-) by irradiation of iodide in a neutral solution has been proposed by Lin (1980a). With high-intensity gamma ray (at $>10^6 R \cdot h^{-1}$) irradiation for a few minutes, more than 98% conversion can be obtained.

Radiochemical Analysis of Iodine

Radiochemical procedure for iodine

The earlier chemical procedures for iodine, which are of interest to radiochemists, have been reviewed by Kleinberg and Cowan (1960). A widely-accepted standard radiochemical procedure for iodine purification was developed by Glendenin and Metcalf (1951). The procedure uses sodium hypochlorite and hydroxylamine hydrochloride in successive steps to ensure radiochemical exchange with the iodine carrier (generally I^-), followed by carbon tetrachloride-aqueous extractions with nitrite and sulfite for the separation of iodine. The purified iodine is finally precipitated as silver iodide for weighing and counting. Since this procedure has been widely employed, a step-by-step procedure is described below.

- Step 1. Add to the sample, in a 40-ml centrifuge tube, about 10 ml of 2 M Na_2CO_3 (note 1) and 20 mg of I^- carrier. Add 2 ml of 5% NaClO, mix well, and heat to boil for 5 min.
- Step 2. Cool the solution and transfer to a 60-ml separator funnel. Acidify the solution by slowly adding 3 ml of conc. HNO_3 . Add 3 ml of 1 M $NH_2OH \cdot HCl$ and extract the I_2 produced into 10 ml of CCl_4 (note 2).
- Step 3. Shake the CCl_4 layer with 10 ml of water containing a few drops of 1 M $NaHSO_3$ until both phases are colorless, and discard the CCl_4 .
- Step 4. Add 1 ml of 6M HNO_3 and a few drops of 1M $NaNO_2$ to the aqueous phase. Extract the I_2 into another 10 ml of CCl_4 .
- Step 5. Repeat Step 3.
- Step 6. Transfer the aqueous phase to a 40-ml centrifuge tube. Add 1 ml of 6M HNO_3 and heat nearly to

boiling. Add 2 ml of 0.1 M AgNO_3 drop by drop with stirring, and digest the precipitate of AgI for a few seconds by gentle boiling and brisk stirring.

Step 7. Filter with suction onto a weighed filter paper in a small Hirsch funnel; wash three times with 5 ml of H_2O and three times with 5 ml of ethanol. Dry at 110°C for 10 min, weigh and mount (note 3).

Notes:

1. The sample should not contain more than 5 g of uranyl nitrate. Since the carbonate is used to form a soluble complex with uranium, NaOH can be used instead in other samples.
2. Recently, since CCl_4 has been identified as a carcinogen and hence prohibited in most laboratories, cyclohexane is recommended as a substitute in this procedure.
3. If the recovery of iodine from AgI is required, the AgI sample can be dissolved in conc. NH_4OH in the presence of metallic zinc (Wahl, 1955).

Radiochemical separation

On many occasions, such as in the study of short-lived iodine isotopes, a rapid ($<10\text{s}$) radiochemical procedure is needed to separate the target material and impurities (Wunderlich, 1967). A simple separation is required for initial separation, followed by further purification for iodine activity measurement. Ion exchange with AgCl (Denschlag, 1969) for separation from fission products is one example, wet or dry distillation (Greendale *et al.*, 1966) developed to separate iodine from the target materials is another. In the determination of iodine-131 activity contamination in milk, a general procedure is to separate iodine from milk by anion exchange resin; approximately 95% of the iodine-131 content of milk is present as iodide and readily removed by ion exchange resin.

Separation of iodine oxidation states

Fractional precipitation (Boyd and Larson, 1969) and ion exchange chromatography (Kleeman and Hermann, 1960) have been reported. The paper chromatographic method for the separation of radioiodine with I^- , IO_3^- and IO_4^- carriers has been reported by a number of investigators (Naumann, 1965; Cvoric, 1969). Generally, the alcohol- H_2O - NH_3 solvent systems have frequently been used. An example of paper chromatographic analysis is shown in Figure 17.3.

Lin (1980a) has developed a solvent extraction procedure to separate iodine species in reactor water samples. This procedure consists of the following steps (Figure 17.4): (1) Extract 100 ml of water sample containing radioiodine with 100 ml of CCl_4 in a 250 ml separator funnel. Organic iodine and I_2 are extracted into the CCl_4 phase.

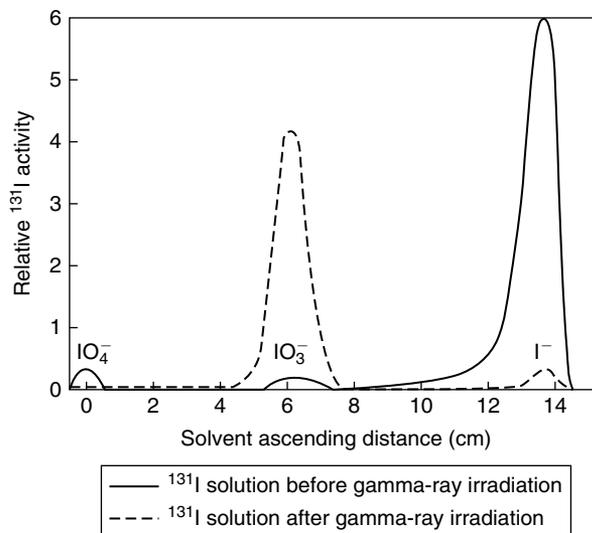


Figure 17.3 Paper chromatographic analysis of iodine sample before and after gamma-ray irradiation. Lin, (1980b).

(2) Extract the CCl_4 phase with 100 ml of freshly prepared 0.5 M KI solution. I_2 is extracted into the aqueous phase via exchange between I_2 and I^- and organic iodine stays in the CCl_4 phase. (3) Extract the aqueous phase from step (1) with 100 ml of CCl_4 containing 0.3 g of I_2 . Iodide and HIO are extracted into the CCl_4 phase via exchange (in case of HIO , the fast hydrolysis equilibrium $\text{I}_2 + \text{H}_2\text{O} \leftrightarrow \text{HIO} + \text{I}^- + \text{H}^+$ is involved) leaving IO_3^- and IO_4^- in the aqueous phase. In each step in this procedure one extraction should be able to separate one state from another at $>98\%$.

By using this procedure, Lin (1980a, 1992) has successfully analyzed the chemical forms of iodine activities in BWR coolant. Under normal operational conditions, the chemical forms of ^{131}I have been measured to be $\sim 65\%$ I^- and $\sim 35\%$ IO_3^- , while more than 90% of the iodine activity is oxidized to IO_3^- during reactor shutdown.

Gamma ray spectrometric analysis of radioiodine

Radiochemical analysis of five major iodine activities (^{131}I , ^{132}I , ^{133}I , ^{134}I and ^{135}I) to monitor fuel integrity and activity transport is an important task in nuclear power plant operation (Lin, 1996). The following procedure is commonly employed in the measurement of iodine activity in the reactor coolant. One hundred milliliter of reactor water sample is obtained from the sample line, and the activity content may be measured with a calibrated gamma-ray spectrometric system. If the sample contains relatively little corrosion product activity, the sample may be counted directly with a high-purity germanium (HPGe) detector with associated gamma ray spectrometric

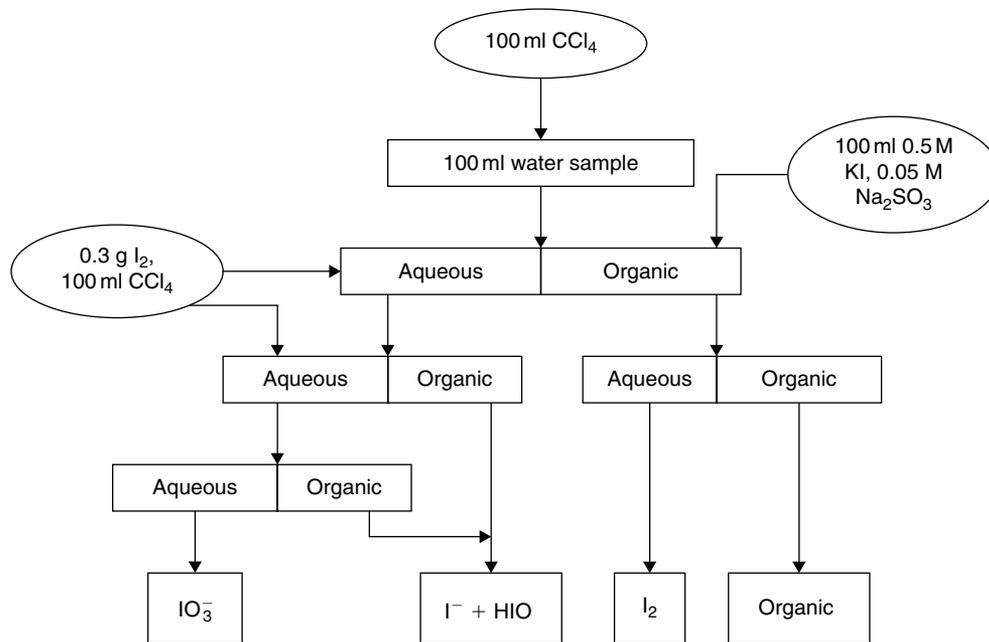


Figure 17.4 Solvent extraction procedure for iodine species analysis. Lin, (1980a).

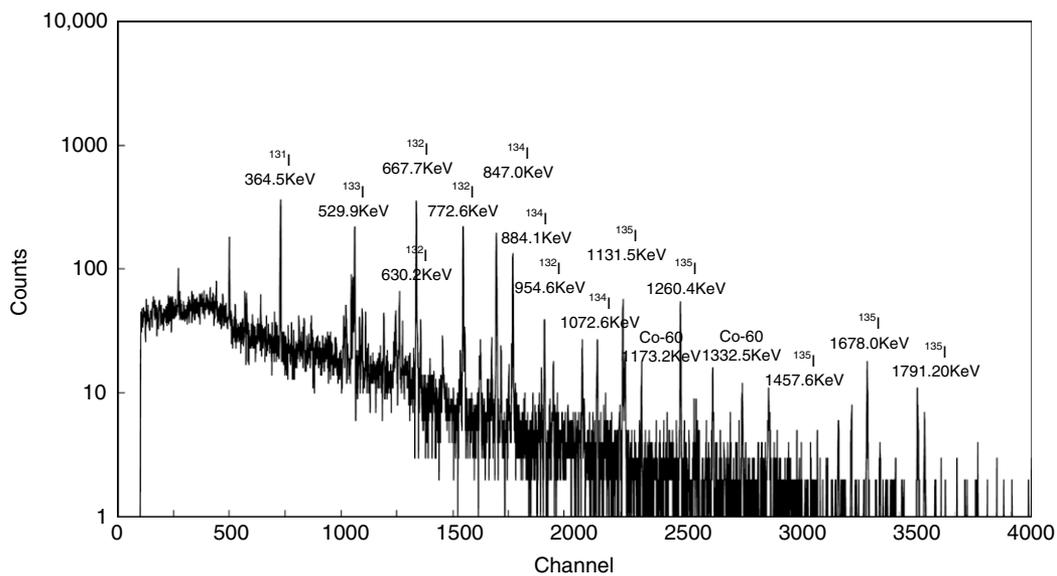


Figure 17.5 Gamma ray spectrum of iodine activities in a typical reactor water sample.

equipment. To minimize the inference in spectrometric analysis by other nuclides, the sample may be purified through a procedure described previously (“Radiochemical procedure for iodine”), or simply separated from other activity by filtering through an anionic filter membrane. The sample is normally counted repeatedly 2–3 times over a 24-h period to ensure accurate determination of ¹³¹I activity in the sample. A typical example of a gamma ray spectrum of reactor coolant sample is shown in [Figure 17.5](#).

Neutron Activation Analysis

Chemical analysis of trace iodine, in either biological or environmental samples, always encounters problems of interference from impurities and uncertainty in chemical yield of analysis. As discussed previously in “Chemistry of Iodine Relevance to Radiochemical Studies” and “Nuclear Properties of Iodine Isotopes”, the chemistry of iodine is very complex and isolation or purification of iodine from the sample is a major obstacle in a traditional chemical analysis.

NAA, however, is not only able to avoid such problems, but also is more sensitive than traditional chemical analysis. In addition, neutron analysis has the advantage of being a nondestructive process over chemical analysis. NAA has been well-developed and widely accepted for trace iodine analyses of naturally existing stable ^{127}I (Akhter *et al.*, 2004; Fardy and McOrist, 1984; Landsberger *et al.*, 2005; Sato and Kato, 1982) and radioactive ^{129}I in various samples.

Neutron activation analysis of iodine (^{127}I)

Thermal neutrons are readily captured through the $^{127}\text{I}(n,\gamma)^{128}\text{I}$ reaction to produce the radioactive nuclide ^{128}I ($t_{1/2} = 25$ min), which emits a characteristic gamma energy of 443 keV in conjunction with beta decay. In order to determine quantitatively the iodine content in the sample, a known quantity of iodine standard is irradiated in parallel with the sample for direct comparison in gamma ray spectrometric analysis.

Since neutron activation with thermal neutrons may generate many undesirable activated products from the sample, which may interfere with gamma ray spectrometric

analysis, neutron filters (boron carbide and cadmium) are introduced to allow only epithermal neutron to irradiate the samples namely, epithermal neutron activation analysis (ENAA), which can improve the peak-to-background ratio in the gamma ray spectrum and the sensitivity of analysis to a certain extent.

After irradiation, some commonly observed short-lived radionuclides from the sample, such as ^{28}Al ($t_{1/2} = 2.24$ min), will decay away within a few minutes of cooling, while the relatively longer-lived ones, mainly ^{24}Na ($t_{1/2} = 15.02$ h) and ^{38}Cl ($t_{1/2} = 37.2$ min), may exist throughout the life span of ^{128}I . An HPGe detector, in conjunction with a multichannel analyzer, is commonly employed in gamma-ray spectrometric analysis. Typical gamma ray spectra of a kombu sample after neutron activation with and without thermal neutron filter are shown in Figure 17.6.

In a spectrometric analysis, the contribution of Compton scattering due to ^{24}Na ($E_r = 1369$ and 2754 keV) and ^{38}Cl ($E_r = 1642$ and 2168 keV) to the spectral background in the detection of ^{128}I photo-peak can be evaluated for the determination of the minimum detectable amount (MDA) of iodine based on the following equation:

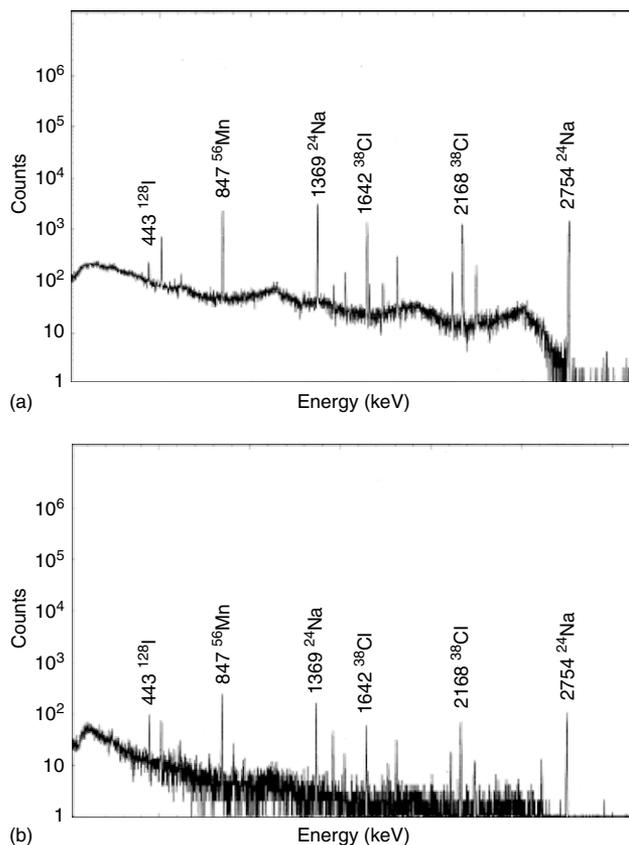


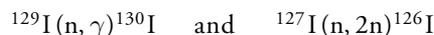
Figure 17.6 Typical gamma ray spectra after neutron activation of a kombu sample: (a) without; and (b) with boron carbide as a thermal neutron filter. Both gamma ray energies (keV) and the respective radionuclides are indicated.

$$\text{MDA} = \frac{2.71 + 4.65\sqrt{B}}{S}$$

where B is the spectral background (counts) and S is the sensitivity ($\text{counts} \cdot \text{g}^{-1}$); both are proportionally increased by prolonging irradiation and measurement time. The MDA of iodine by NAA is very much dependent on the content of interference nuclides (Chao *et al.*, 2002), especially ^{24}Na . The calculated MDA of iodine in a sample, activated with and without neutron filter, is shown in Figure 17.7.

Neutron activation analysis of iodine-129

Iodine-129 is an environmentally important radionuclide with a half life of 1.57×10^7 y. The three main sources of ^{129}I in the environment are nature, nuclear weapons testing and power reactor operation. NAA is usually carried out by measuring the ratio of ^{129}I to ^{127}I after neutron activation by the following nuclear reactions with reactor neutrons:



Chemical purification of a sample is required before and after neutron irradiation. A typical purification procedure is illustrated in Figure 17.8 (Tseng and Chao, 1996). After irradiation, two major radionuclides, ^{130}I and ^{126}I , are analyzed by gamma ray spectrometry. As in the measurement of ^{127}I by thermal neutron activation described previously, a known quantity (^{129}I to ^{127}I ratio) of the standard is also activated in parallel with the test sample. The $^{129}\text{I}/^{127}\text{I}$ ratio is evaluated from the counts under the photo-peaks of 536 keV (or 740 keV) from ^{130}I and 754 keV from ^{126}I .

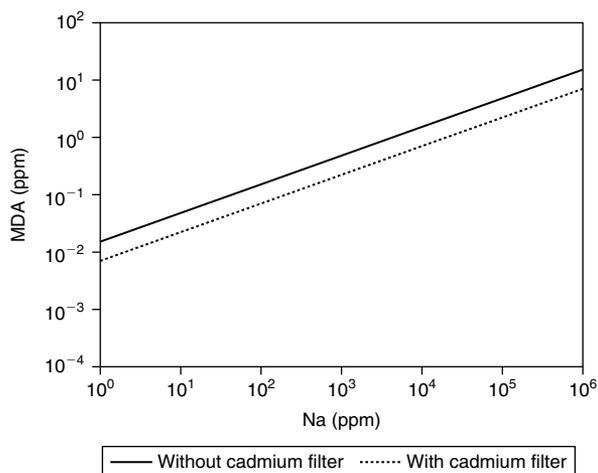


Figure 17.7 Calculated MDA of iodine as a function of sodium concentration in a sample by neutron activation analysis. Measurement condition: neutron flux, $7 \times 10^{12} \text{cm}^{-2} \cdot \text{s}^{-1}$; sample weight, 0.1 g; irradiation time, 30s; counting time, 5min.

By a direct comparison with the measurements from a standard sample, the $^{129}\text{I}/^{127}\text{I}$ ratio in the test sample can be calculated without knowing the recovery yield in the chemical processes. A typical gamma ray spectrum for an irradiated kombu sample is shown in Figure 17.9.

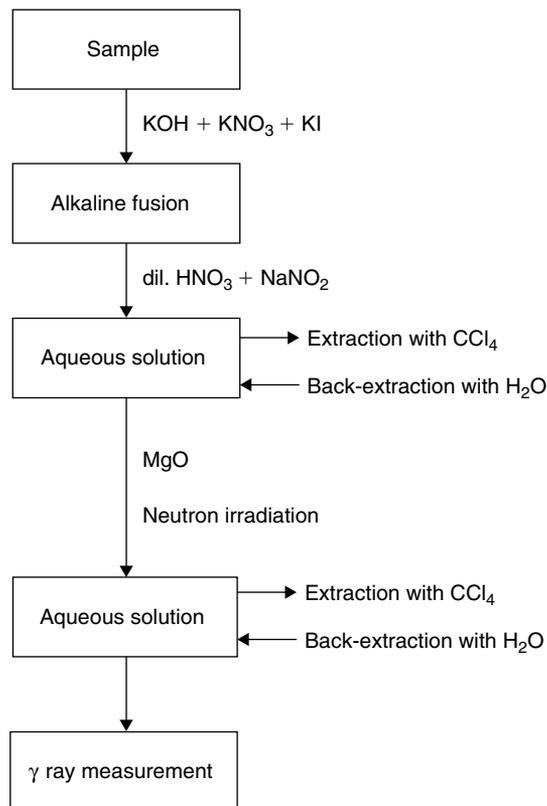


Figure 17.8 Schematic diagram of chemical purification and measurement of ^{129}I in environmental/biological samples.

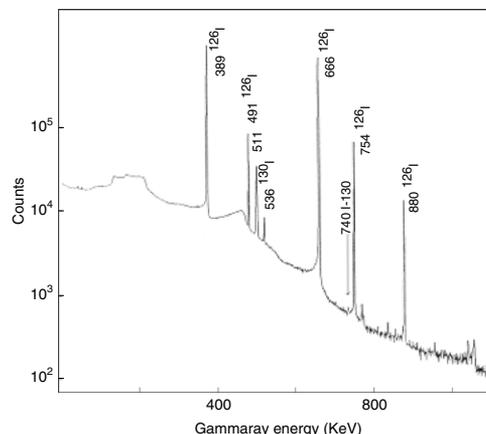


Figure 17.9 Gamma ray spectrum of a kombu sample irradiated by neutrons.

Table 17.3 $^{129}\text{I}/^{127}\text{I}$ ratio in environmental/biological samples worldwide

Area/date	Samples	$^{129}\text{I}/^{127}\text{I}$ ($\times 10^{-10}$)	Remarks	References
Sweden/1993	Deer	1900–4000	Chernobyl accident	Handle <i>et al.</i> , (1993)
Rocky Mt. US/1972–1974	Deer	740–11000	Atmospheric nuclear test	Markham <i>et al.</i> , (1983)
Idaho US/1972–1974	Deer	6500–79000	Nuclear reprocessing	Markham <i>et al.</i> , (1983)
Chile/1993	Bovine	12.9–90		Handle <i>et al.</i> , (1993)
	Human	11–20		
Missouri US/1974/1977	Deer	5.4–460		Oliver <i>et al.</i> , (1982)
	Cow	4–136		
	Human	2.4–133		
Germany/1979–1981	Cattle	49–909		Aumann and Buneitel (1995)
	Hog	4–620		
Taiwan/1995–1996	Hog	0.23–23		Chao and Tseng (1996)
	Bovine	2.5–66		
	Ovine	3.1–82		
	Rain water	5×10^4		
Japan/1979–1983	Pine needle	$<700 - 1.8 \times 10^5$	Nuclear reprocessing	Muramatsu and Ohmomo (1986)
	Algae	$<10-990$		
	Soil	$<10-3000$		
China/1994–1998	Human	4.1–2.0		Hou <i>et al.</i> , (2000)
	Seaweed	1.2–4.1		

As a result of nuclear tests and/or release from nuclear facilities, almost all the $^{129}\text{I}/^{127}\text{I}$ ratios reported during the past decades for environmental and biological samples worldwide (Table 17.3) were higher than the levels of naturally occurring iodine, which has an $^{129}\text{I}/^{127}\text{I}$ ratio of less than 10^{-12} (Edwards, 1962).

Protection from Radioiodine Exposure under Nuclear Accident Conditions*

Humans can be exposed to radioiodine, which is released during nuclear accidents, through many pathways. Radioiodine can exist in many forms: as water-soluble iodide (I^-), as contamination in animal milk, as deposits on vegetation, and more likely as airborne in the environment initially released from an accident. It is of interest to note that regardless of any chemical form of radioiodine (either inhaled or consumed through food chain), they all concentrate effectively in the thyroid.

The use of KI serves as an important remedy to protect from radioiodine exposure under nuclear accident conditions. In principle, under normal circumstances, excess iodine decreases sodium–iodide symporter (NIS) on the thyroid cell surface, thereby inhibiting further access for iodine into the thyroid. Excess iodide administration at the appropriate time decreases thyroid radioactive iodine uptake (RAIU) by increasing the amount of nonradioactive

iodine available for binding to the thyroid cells. In the event of release of radioiodine from nuclear accidents, a marked decrease in thyroid RAIU could be achieved. The inhibitory effect on thyroid RAIU of a single dosage of 130 mg of iodine in KI lasts for about 48 h.

Summary Points

- The relevance of iodine chemistry to radiochemical studies has been briefly reviewed, including major iodine reactions in aqueous solutions, isotopic reactions and radiation effects in aqueous solutions.
- Nuclear properties of major iodine isotopes in medical applications are presented.
- The production of iodine isotopes by nuclear reactions from various target materials for medical applications is described. Separation and purification methods for iodine isotopes, including distillation (wet and dry) and adsorption techniques, are discussed. Also discussed is the preparation of iodine isotopes in chemical forms other than iodide (I^-).
- The analysis of iodine at trace levels is reviewed in detail. Classical radiochemical procedures commonly been used in various samples, but neutron activation is probably more popular in environmental and biological samples. Gamma-spectrometric methods are often used in conjunction with NAA.
- Exposure to radioiodine under nuclear accident conditions has been of concern to the general population residing near nuclear power plants. Protection from exposure by KI administration is suggested, and the inhibitory effect of KI on thyroid RAIU is discussed.

*Note: The information contained in this section is taken partly from the literature “Distribution and Administration of Potassium Iodide in the Event of a nuclear Incident” (National Research Council of National Academies, 2004).

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Section 2.2

Physiology, Metabolism, and Biochemistry

Retention of Iodine in the Body: Biological Half-Life of Iodine in the Human Body

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Abstract

The Human Monitoring Laboratory (HML) has measured the retention of ^{131}I in patients who had received the radioiodine diagnostically. These measurements provided sufficient information so that the biological half-life of iodine could be calculated. The average biological half-life of ^{131}I in 26 euthyroid (normal) subjects was found to be 66.1 ± 6.3 days, which may be statistically significantly lower than the International Commission on Radiological Protection's (ICRP) recommended value of 80 days. Nine hyperthyroid (overactive) patients had a mean biological half-life of 38.2 ± 8.6 days, and in three hypothyroid (underactive) patients the corresponding value was 29.3 ± 8.8 days. Thyroid uptake of iodine was measured as 0.14 ± 0.01 , 0.31 ± 0.03 , and 0.04 ± 0.01 of the administered dose in euthyroid, hyperthyroid, and hypothyroid patients, respectively. The euthyroid range in Ottawa and Canada (0.06–0.22 of uptake) was significantly lower than the ICRP's value of 0.3. The radioiodine retention in athyreotic (no thyroid gland) subjects followed a two-compartment model with biological half-lives of 1.0 ± 0.2 and 18.4 ± 1.1 days.

Abbreviations

BOMAB	<i>Bottle Mannikin Absorber</i>
BRMD	Bureau of Radiation and Medical Devices
HML	Human Monitoring Laboratory
ICRP	International Commission on Radiological Protection
MCS	Multichannel scaling
NaI(Tl)	Sodium iodide doped with thallium

Introduction

Iodine is a trace element essential to the functions of the body and constitutes about 0.00004% of the total human

body weight. As a result, the body has developed mechanisms to retain this element for subsequent use. It is found in highest concentration in the thyroid gland, muscles, and various endocrine tissues. This chapter describes some recent research that was conducted to measure the retention of iodine in persons with underactive (hypothyroid), normal (euthyroid), and overactive (hyperthyroid) thyroid glands, and in persons having no thyroid gland (athyreotic).

In 1996 the Human Monitoring Laboratory (HML) measured the biological retention of iodine in athyreotic patients and those with intact thyroid glands by sequential measurements of the thyroid or whole-body retention of ^{131}I . The advantage of this study was that no person received unnecessary exposure to radioactive materials, as the diagnostic doses of ^{131}I were supplied to all participants of this study. The disadvantage of using volunteers from a population of patients was that the age/gender mix of the subject pool could not be predetermined, and the uncertain extent to which patients may be regarded as "normal" or conform to the desired physiological model. The study commenced in March 1997 and finished in December 1999. The results have been compared to the International Commission on Radiological Protection (ICRP) recommendations and the ICRP metabolic models.

The biological half-life of iodine in the normal human thyroid cannot be assumed to be unvarying over a period of years, even in a given population. In 1959 the ICRP recommended that the biological half-life of iodine should be 138 days (ICRP, 1959). This was revised downwards in 1978 to a value of 120 days (ICRP, 1979), and yet again in 1989 to a value of 80 days (ICRP, 1989). The average fraction of iodine presumed retained by the thyroid after ingestion has not changed from 0.3 over this period.

According to the ICRP (1997), 0.3 of ingested iodine is initially taken up by the thyroid and the remainder is excreted in urine. Most organic iodine that enters the blood from the thyroid is metabolized in tissues and

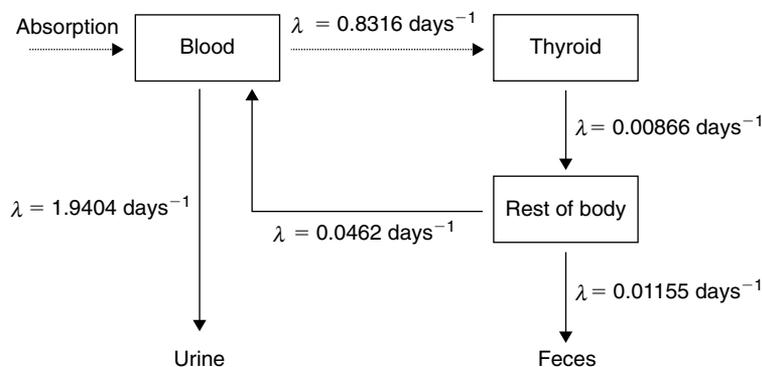


Figure 18.1 Iodine retention model showing the rate constant, λ .

returned to the plasma as inorganic iodine, which is then recycled. About 20% of the iodine released from the thyroid goes to fecal excretion in an organic form, the biological half-lives are: blood, 0.25 days; thyroid, 80 days; rest of the body, 12 days (see [Figure 18.1](#)).

The recycling of iodine can best be described by a two-compartment model but this is not normally seen when using ^{131}I as the 8.04 days radiological half-life precludes the resolution of the two compartments (a single compartment does not necessarily imply that a single physiological mechanism is at work). The ICRP recognizes that the 0.3 figure for initial thyroid uptake is anything but firm, and is much influenced by the level of dietary iodine. [Pittman et al. \(1969\)](#) and more recently [Moorthy et al. \(2001\)](#) have documented the progressive fall in thyroid iodine uptake as a result of increased dietary iodine from iodization of salt, use of certain food additives, and changing dietary habits.

Of the secondary sites for iodine retention, the major salivary glands will make the greatest contribution to the counts measured by a detector placed as for a thyroid uptake measurement. In the normal euthyroid individual, the avidity of the thyroid gland for iodine dominates body retention. However, the ability to concentrate iodine is shared by salivary glands, gastric mucosa, breast tissues, choroid plexus, ovaries, and sweat glands. These secondary sites of iodine concentration and secretion become important in the athyreotic individual after surgery or ablative radioiodine therapy and, presumably, in the much more common situation in which thyroid iodine uptake is suppressed by thyroid hormone replacement therapy. In these circumstances, the majority of ingested radioiodine would be expected to be excreted very rapidly via the urine, with the retention of a small residuum determined by the metabolism of the secondary sites, predominately the salivary glands.

There are two main types of salivary glands: the major salivary glands and the minor salivary glands. The three pairs of major salivary glands are the parotid glands,

submandibular glands, and sublingual glands. The parotid glands are the largest salivary glands and are found on each side of the face, just in front of the ears. They overlie the temporomandibular joint and would not contribute many photons to a collimated detector placed in front of the thyroid gland.

The submandibular glands are the next largest salivary glands and are found on either side of the neck, under the chin and tongue area. The sublingual glands are found deeper in the neck than the submandibular glands, under either side of the tongue. There are about 600–1000 minor salivary glands, which are too small to see without a microscope. These minor salivary glands are located beneath the lining of the lips, tongue, hard and soft palate, inside the cheeks, nose, sinuses, and voice box. These glands are closer to a detector placed in front of the thyroid gland, especially if it has a large diameter, and could contribute photons. The biological half-life of iodine within the salivary gland is reportedly approximately 10 h ([Nishizawa et al., 1985](#)), but iodine recycling may only be evident in the athyreotic subject.

Measuring the Biological Half-Life

HML's low-background counting chamber

The low-background counting chamber that houses HML's whole-body/thyroid counter was constructed in 1959 by the Dominion Bridge Company using material supplied by the Steel Company of Canada. Prior to construction, samples of steel were sent to the Physics Department of the University of Toronto to test for radioactive contamination. Evidence of some contamination (mostly ^{137}Cs and ^{60}Co) was found that was attributed to radioactive fallout from atomic bomb testing in the 1940s and 1950s. The chamber was installed in the Radiation Protection Bureau in 1960, and has since been used in the Health Canada's Human Monitoring Program.

The thickness of the chamber wall, floor, and ceiling is 0.2 m, and the approximate weight of the chamber is 51 metric tons. The wall thickness is sufficient to reduce the γ rays from naturally occurring radioactivity in the surrounding building materials by a factor of about 1000, and the cosmic rays to about 60% of their unshielded intensity. The inner surfaces of the room are covered by 6.3 mm of lead, which reduces the background, below 0.1 MeV, by a factor of two.

The inside dimensions of the chamber are $1.52 \times 2.13 \times 2.13$ m. The chamber is equipped with double doors operated by electric motors controlled from the laboratory. There is a second control in the chamber, which can be used to open the doors from inside in case of emergency. An intercom is also provided for communication between subject and operator, as well as to provide music to relieve the tedium of lengthy counting periods. Subjects may also be viewed through a large water-filled window $0.3 \times 0.46 \times 0.6$ m wide.

Detector systems

The whole-body counter is equipped with six sodium iodide doped with thallium (NaI(Tl)) detectors, combined in two triangular arrays. The upper array consists of three detectors that scan above and the lower array of three detectors that scan below the subject. The upper array is on a moveable arm that can be raised from the bed surface to the roof of the counting chamber. The lower array is in a fixed geometry 12 cm below the bed.

Each detector array is powered by an independent high-voltage supply. The signal from each detector is processed by a preamplifier. The three preamplifiers for the upper array are connected to a dual sum invert amplifier and the three for the lower array to another. These modules sum the incoming signals into single signals. The signals are then processed by two separate amplifiers that are connected to a multichannel buffer. Spectral analysis is performed on a computer using software custom-modified for the HML.

Each detector in the upper and lower arrays is a cylindrical NaI(Tl) crystal that is nominally 12.7 cm in diameter and 10.2 cm in height. The crystal is optically coupled to a low-background photomultiplier tube. The outer casing of the detector is stainless steel 304 (Fe, 70%; Cr, 19%; Ni, 11%; specific gravity, 8.02), which is 0.635 mm thick. The transmission of photons through the outer casing at 100 keV is 83%, rising to 97% at 1000 keV.

Only one detector of the upper array is used for thyroid counting, the other two being removed from the array by disconnecting the signal cables. Normally the detector is placed centrally over the supine subject's thyroid gland at a distance of 14 cm. However, some of the subjects had such high activities that the detector needed to be raised to reduce the dead time to manageable levels.

The detector arrays scan the subject for normal whole-body counting, but the scanning detector geometry can

also identify the location of a radionuclide that is not homogeneously distributed in a person (or phantom), using the multichannel scaling (MCS) mode of the whole-body counter. The latter mode was used in conjunction with the thyroid count mode.

Counting efficiency

The counting efficiency for ^{131}I thyroid counting was determined using the Bureau of Radiation and Medical Devices (BRMD) thyroid phantom (Kramer *et al.*, 1996a, b) placed as the neck section of a Reference Man *Bottle Mannikin Absorber* (BOMAB) phantom (Kramer *et al.*, 1991) using ^{133}Ba as a surrogate for ^{131}I . The thyroid counter was also calibrated using a BOMAB phantom containing ^{133}Ba distributed homogeneously throughout the phantom. The efficiency was determined at the normal counting distance (14 cm) and at larger distances.

The volunteers

A group of athyreotic patients was studied and compared with individuals with intact thyroid glands. The athyreotic subjects had all undergone complete removal of functioning thyroid tissue by a surgical "total thyroidectomy," followed by administration of the sodium salt of ^{131}I and ablation of any residual thyroid tissue at least 3 months prior to entry into the study. This treatment was necessitated by the presence of a carcinomatous tumor within the thyroid gland. The diagnostic dose of radioiodine received by the athyreotic patients was given to test for completeness of thyroid ablation, and for the possible presence of iodine-concentrating metastases. The athyreotic state requires treatment with thyroid hormone replacement. The hormone replacement therapy was withdrawn prior to giving the diagnostic radioiodine, so that each patient was in a hypothyroid state at the time of the radioiodine administration. None of the athyreotic patients was shown to have significant residual thyroid tissue, and none had evidence of iodine-concentrating metastatic disease.

The individuals with intact thyroid glands were selected from among patients referred to for measurement of thyroid uptake and retention of ^{131}I for the confirmation or exclusion of clinically suspected thyroid malfunction. Twenty-six of these patients were shown to have neither under- nor over-activity of the thyroid by evidence not confined to, but including, the radioiodine uptake measurement and not including the biological half-life. None of this euthyroid group with "normal" thyroid function was on treatment with thyroid hormone, antithyroid medication, or other medication known to affect thyroid metabolism. None had recently received significant amounts of extraneous iodine such as radiological contrast.

The subjects participating in this study were solicited at a nearby hospital by the nursing staff following

diagnostic administration of ^{131}I . Informed consent was obtained from each subject prior to any research measurements being made, as directed by the hospital's Research Ethics Board. Patients with intact thyroid glands were being investigated for possible abnormal thyroid function and received oral doses of ^{131}I sodium iodide of approximately 350 kBq. Patients rendered athyreotic by a combination of surgery and ^{131}I therapy in the management of their thyroid carcinoma received a diagnostic dose of ^{131}I of approximately 150,000 kBq prior to whole-body scanning in an attempt to image any metastatic disease. Each volunteer came to the HML for up to 6 counts spanning up to 6 weeks. Volunteers were compensated for traveling expenses for each visit to the HML.

The volunteer group consisted of 34 women and 14 men. The age ranges of the two groups were 18–75 and 25–77, respectively. There were two subsets within the volunteer group: patients receiving a diagnostic dose for measurement of thyroid function (~ 360 kBq) and athyreotic patients undergoing a metastatic survey ($\sim 150,000$ kBq) to ensure that no thyroid function remained.

Nine of the patients were shown to be hyperthyroid clinically, biochemically, and by radioiodine uptake, but not by biological half-life; three patients were found to be hypothyroid by the same criteria. As with the euthyroid patients, none had received thyroid or antithyroid medication or extraneous iodine.

Counting protocol

All subjects were measured in the whole-body counter in a supine position. The first count was a whole-body count with the detectors scanning over the subject. The counts were acquired in MCS mode simultaneously with the normal acquisition in an attempt to determine the location of ^{131}I (other than in the thyroid). The second count was a thyroid count where the subject remained supine, but also extended the neck to raise the thyroid gland above the clavicles. The results from the latter counts were used to determine the retention parameters.

The counting regime for patients with intact thyroid glands was begun within a week of the initial administration of ^{131}I , with repeat counts being performed at approximately 7-day intervals, however, the regime for athyreotic patients had to commence as quickly as possible to measure the rapidly excreted iodine. Repeat counts were daily (where possible) for the first 3–4 days, and the last counts were at a weekly interval.

Half-life determination

The effective decay rate is given by:

$$\lambda_{\text{eff}} = \lambda_{\text{rad}} + \lambda_{\text{biol}} \quad (18.1)$$

where λ_{eff} is the effective decay rate (day^{-1}), λ_{rad} the radioactive decay rate (day^{-1}), and λ_{biol} the biological decay rate (day^{-1}). λ_{eff} is obtained from the linear regression of $\ln(\text{thyroid activity})$ as a function of time. λ_{rad} is 8.04 days (ICRP, 1983), and hence λ_{biol} can be obtained from Equation (18.1). The biological half-life is then obtained from:

$$T_{1/2} = \frac{\ln(2)}{\lambda} \quad (18.2)$$

where $T_{1/2}$ is the half-life (day) and λ is the decay rate (day^{-1}).

Figure 18.2 shows the ^{131}I retention of activity in the thyroid as a function of time for a normal subject, and Figure 18.3 shows a typical plot of ^{131}I retention as a function of time for an athyreotic subject. Figure 18.2 shows that a single compartment is adequate to describe the retention of ^{131}I in the thyroid, which was exemplified by all the subjects with intact thyroid glands measured at the HML. By contrast, Figure 18.3 shows a different pattern of retention for athyreotic subjects. This shows iodine retention described by a two-compartment model that is not evident in subjects with intact thyroid glands.

Table 18.1 gives the biological half-lives and their uncertainties, and ^{131}I thyroid uptake values of the thyroid intact subjects, and Table 18.2 gives the half-lives and apparent thyroid bed uptake data for the athyreotic patients. Table 18.1 shows that the biological half-lives vary from 11.4 to 137 days, with one exception discussed below. Similarly, the uptake values vary from 2 to 57% distributed between three groups: 27 euthyroid patients, of which one was subsequently eliminated, 9 hyperthyroid

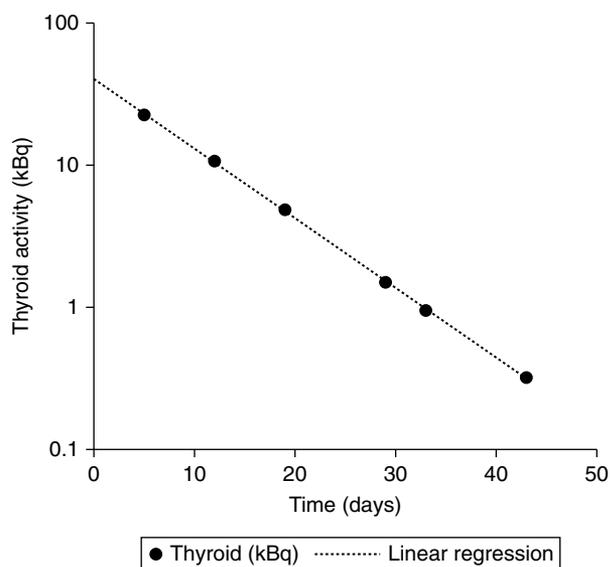


Figure 18.2 Plot of thyroid activity and filled linear regression curve as a function of time for an euthyroid subject.

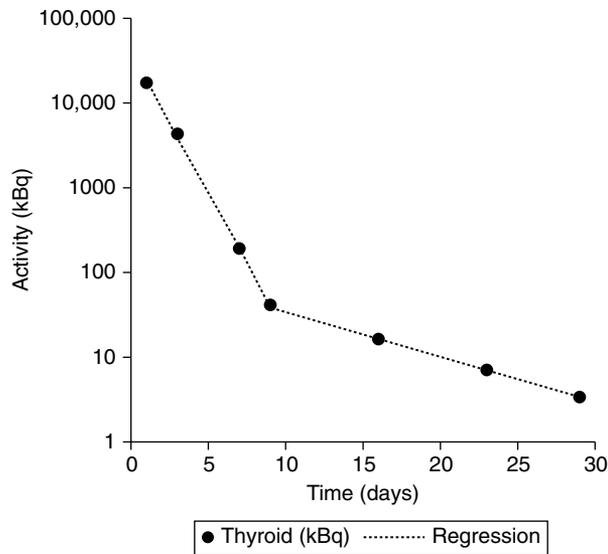


Figure 18.3 Body retention of ^{131}I for a typical athyreotic subject.

patients, and 3 hypothyroid patients (hypothyroid is <6% uptake, euthyroid is in the range of 6–22% uptake, and hyperthyroid is >22% uptake). All 26 of the euthyroid patients were subsequently shown to have neither under- nor over-activity of the thyroid by evidence including, but not confined to, the radioiodine uptake measurement and excluding radioiodine retention measurements. None were on treatment with thyroid hormone, antithyroid medication, or other medication with the potential to affect thyroid metabolism. None had recently received significant amounts of extraneous iodine such as radiological contrast. This does not allow their thyroid function to be strictly defined as “normal,” but neither is their thyroid behavior without relevance to that of a normal healthy North American population.

Table 18.2 shows the results for the patients that received a higher dose of ^{131}I as part of a survey to verify that previous treatments had been successful. There was no evidence of an ^{131}I -concentrating lesion. The short-term retention is predominantly due to salivary gland retention, and the long-term compartment is predominantly “rest of body.”

Table 18.3 summarizes the data from **Tables 18.1** and **18.2**, apart from the exclusion of one euthyroid (by radioiodine uptake) subject. Subject no. 15 (**Table 18.1**), who had an unusually long biological half-life for iodine, has been eliminated from the data set as an extreme outlier and was not considered in the subsequent statistical analysis. It is interesting to note that this patient was found to be on antidepressant medication – Nefazodone Hydrochloride. This drug can infrequently cause goiter and hypothyroidism as side effects in some patients. In this case, it appears to have locked the radioiodine in the thyroid gland for the duration of the monitoring period.

Table 18.1 Biological half-lives of iodine, their uncertainty (σ), the uptake ($t = 24\text{h}$) obtained by the HML, and the uptake obtained by the hospital, H, ($t = 24\text{h}$) obtained from the normal subjects

Subject no.	Biological half-life (days)	σ (days)	Uptake (HML) (%)	Uptake (H) (%)
1	45.2	1.5	12.8	8.0
2	24.2	0.9	15.0	16.6
3	71.0	2.7	14.3	11.0
4	19.3	0.4	6.7	6.4
5	89.3	3.8	12.2	17.0
6	92.1	1.8	20.4	17.9
7	63.5	1.2	14.2	11.8
8	79.5	2.0	11.5	7.5
9	36.1	0.7	31.5	19.7
10	50.9	0.9	22.5	19.0
11	34.2	0.9	16.5	19.8
12	55.9	0.4	17.7	16.6
13	137.3	4.5	11.8	9.0
14	98.8	0.5	23.5	18.0
15	2507.1	62.4	13.3	13.7
16	46.8	1.0	5.9	5.9
17	18.5	1.0	2.3	2.6
18	110.3	3.2	14.0	12.4
19	76.6	1.0	16.0	13.0
20	51.6	0.6	25.4	19.2
21	72.9	2.2	12.4	12.7
22	117.1	1.3	24.5	19.6
23	87.4	1.7	27.4	16.9
24	105.1	0.9	16.5	16.5
25	37.0	0.2	15.3	23.0
26	61.6	0.8	12.2	10.0
27	26.1	0.2	10.8	12.0
28	22.7	0.8	4.7	4.9
29	52.2	2.4	12.1	13.9
30	24.8	0.5	36.0	42.7
31	38.9	0.7	28.1	22.9
32	11.4	0.3	31.2	23.0
33	31.7	0.8	27.9	23.4
34	24.2	0.7	3.5	8.1
35	98.8	1.1	28.5	23.0
36	44.0	0.3	34.4	28.5
37	19.7	0.7	49.3	36.5
38	24.9	0.6	56.8	51.8
39	49.7	1.0	32.6	31.0

Note: Hypothyroid, <6% uptake; euthyroid, 6–22% uptake; and hyperthyroid, >22% uptake.

Table 18.3 shows that the average biological half-life of the subjects with intact thyroid glands is 66.1 ± 6.3 days. This moderate decrease in biological half-life of the North American group that participated in this study from ICRP values is a continuation of the trend identified above.

Direct comparison with the ICRP value of 80 days is difficult, as there are no uncertainties associated with that value. One might assume that the ICRP value has the same relative uncertainty as the value of biological half-life obtained in this work. Performing a one-sided t -test, to test the null hypothesis that there is no difference

Table 18.2 Biological half-lives for the two compartments and ^{131}I “uptake” of the athyreotic subjects

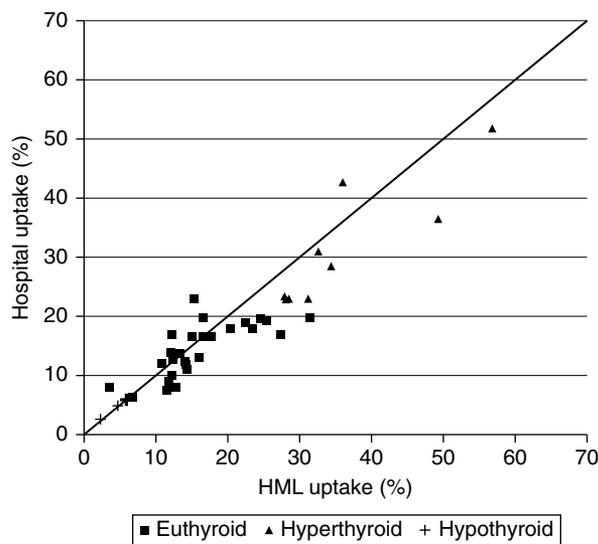
Subject no.	Short-term		Long-term		
	Half-life (days)	σ (days)	Half-life (days)	σ (days)	Uptake ($t = 24\text{h}$)
40	1.69	0.49	19.0	0.5	0.01
41	N/A	N/A	12.2	0.0	N/A
42	0.58	0.01	22.5	0.8	0.04
43	0.96	0.04	19.7	0.2	0.12
44	0.85	0.10	22.3	1.0	0.05
45	0.63	N/A	19.1	0.1	0.05
46	0.77	N/A	15.6	0.3	0.05
47	2.04	0.20	15.3	0.4	0.05
48	0.73	0.12	20.0	0.3	0.04

Note: N/A means that there was insufficient data to determine the value.

between the two values (the alternative hypothesis is that the half-life has decreased), one obtains a t -value of 1.97 ($t_{\text{crit}} = 1.68$, $P = 0.027$), suggesting that the null hypothesis be rejected. In other words, the average biological half-life obtained in this work is statistically significantly lower than the ICRP value of 80 days. It should be pointed out, however, that testing for statistical significance at the $P < 0.05$ level, and accepting any value lower than this, can lead to erroneous conclusions. Sterne and Smith (2001) have shown that P values just below this threshold (0.05) do not provide conclusive evidence against the null hypothesis, but $P < 0.001$ does.

Uptake determination

Once the retention parameters were determined for each individual, the thyroid activity was estimated at time 24 h from the linear regression of the activity as a function of time. The uptake is thyroid burden at time 24 h divided by the decay corrected amount administered by the hospital. The average fractional uptake (0.16 ± 0.01) of the thyroid has declined from the ICRP recommended value of 0.3. A one-sided t -test comparing the average fractional uptake with the ICRP value (assuming that it has a similar uncertainty to the uptake value determined in this work) gives a

**Figure 18.4** Plot of uptake measured versus the uptake calculated the retention parameters.

t -value of 11.36 ($t_{\text{crit}} = 1.68$, $P = 0.000$), strongly suggesting that the null hypothesis be rejected. In other words, the average fractional uptake obtained in this work is statistically significantly lower than the ICRP value of 0.3.

Comparing the uptakes determined at the HML with the uptake determined at the hospital using a two-sided t -test, one finds that the values for the euthyroid patients are not statistically significantly different (t -value = 1.01, $t_{\text{crit}} = 2.00$, $P = 0.27$). Similarly, two-sided t -tests for the hyperthyroid and hypothyroid patients also indicate that there is no difference between the uptake values measured at the HML and at the hospital. The overall relationship of the uptake values is shown in Figure 18.4.

Retention model

Based on the findings of this study the thyroid retention of iodine by normal persons can be written as:

$$R(t) = 0.69e^{(-t \cdot \ln(2)/0.25)} + 0.18e^{(-t \cdot \ln(2)/66.1)} + 0.13e^{(-t \cdot \ln(2)/1.03)} + 0.0008e^{(-t \cdot \ln(2)/18.4)} \quad (18.3)$$

Table 18.3 Statistical summary of the biological half-lives from Tables 18.1 and 18.2

	Normal	Hyperthyroid	Hypothyroid	Athyreotic (short)	Athyreotic (long)	Units
Average	66.1	38.2	29.3	1.03	18.4	Days
Σ	31.9	25.7	15.3	0.53	3.4	Days
$\sigma_{(\text{mean})}$	6.3	8.6	8.8	0.19	1.1	Days
N	26	9	3	8	9	
Median	62.5	31.7	22.7	0.81	19.1	Days

The first exponential in Equation (18.3) describes the retention in the blood stream and was inferred from the ICRP model. The second exponential in Equation (18.3) is the thyroid retention determined here. The last two exponentials in Equation (18.3) are determined from the athyreotic subjects, and are assumed to be present in all subjects.

Summary Points

- The biological half-life of radioiodine in this subset of a North American population is 20% lower than the ICRP recommended 80 days.
- The fractional uptake has decreased from the value recommended by the ICRP and is about 18%.
- Iodine has a half-life of 66.1 ± 6.3 days in normal persons.
- Iodine has a half-life of 38.2 ± 8.6 days in hyperthyroid persons.
- Iodine has a half-life of 29.3 ± 8.8 days in hypothyroid persons.
- Persons with no thyroid gland (athyreotic) do retain iodine, but with much shorter half-lives (13% with 1.03 ± 0.19 days and 0.1% with 18.4 ± 1.1 days).

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19

Mathematical Models of Human Iodine Metabolism, Including Assessment of Human Total Body Iodine Content

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As soon as radioactive tracers of iodine, especially iodide, thyroxine (T₄), and triiodothyronine (T₃), became available after the end of World War II, research began on the kinetics of radioiodine in humans, as well as in experimental animals. As measuring devices improved, radioactivity could be measured not only in the blood plasma, but also in other body fluids, and external measurement of the radioactivity in the thyroid and in other organs of the body soon became feasible. A rich body of experimental data ensued; there was a need to synthesize these data into a unifying theoretical structure.

Abbreviations

T ₄	Thyroxine
T ₃	Triiodothyronine

The Riggs Model

Perhaps the most ambitious early effort of this type was the model of iodine metabolism developed by Douglas Riggs (1952). He presented a theoretical model that included ingestion of iodine as iodide, its absorption into the plasma, and transfer to the thyroid and other tissues, with loss through the kidneys, sweat and expired air. Within the thyroid gland, he described the organification of iodine and production of monoiodothyronine, diiodothyronine, and thyroxine (T₄) on the framework of thyroglobulin.

Riggs then simplified this structure into one which was accessible to experimental use, a modification of which is shown in Figure 19.1. In this model, iodide from food and water enters the body's iodide pool and is then distributed to either the thyroid gland or the kidneys for excretion in the urine. The thyroid organifies the iodine and releases the organic iodine (primarily as T₄) into the blood, and hence into the body pool of organic iodine. Except for a small amount excreted in the feces, the thyroid hormone in the periphery is metabolized and the resulting iodide re-enters the iodide pool.

At the time Riggs presented his model, the importance of triiodothyronine (T₃) had not yet been shown.

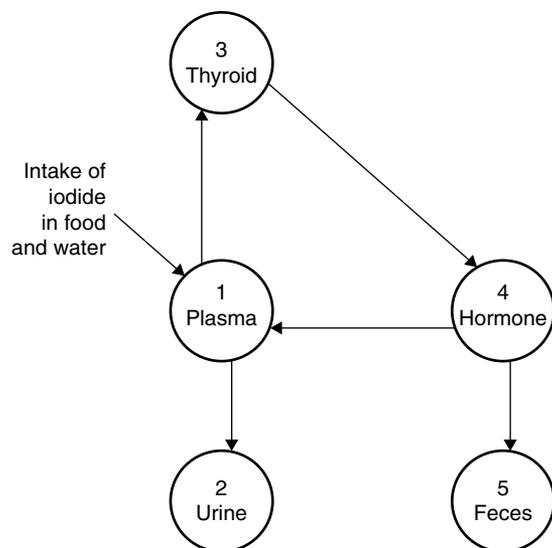


Figure 19.1 The simplified model of human iodine metabolism presented by Riggs (1952).

However, by combining all organified iodine compounds in his model, he accounted for its metabolism. The basic structure for understanding human iodine metabolism presented by Riggs remains helpful.

Models of Iodine within the Thyroid Gland

Many attempts have been made to describe the intrathyroidal metabolism of iodine in formal compartmental models, but the complexity of this metabolism and the paucity of data that are suitable for validating these models have led to limited success. A heroic early attempt was that of Berman *et al.* (1968). They fitted data from over 100 kinetic studies in persons with a variety of thyroid conditions, and found that their data best fit an intrathyroidal model similar to that shown in Figure 19.2. One can interpret the series of progressively delayed compartments

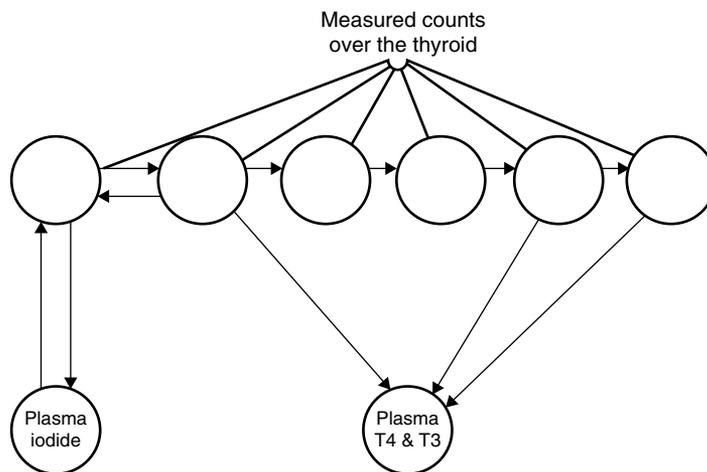


Figure 19.2 The thyroid portion of the comprehensive model of human iodine metabolism presented by Berman *et al.*, (1968). The thyroid is modeled as a series of compartments with progressively slower patterns of thyroid hormone manufacture and release.

in this model to reflect the known variety in the sizes and metabolic activity of the various thyroidal follicles. This model was used to fit data reflecting careful measurement of time–activity curves for plasma radioiodide, total plasma iodine, urinary excretion and external radioactivity over the thyroid gland. Despite the large amount of data, this model cannot be claimed to be unique. Other formulations might fit the data equally well. However, it is clear that a model of intrathyroidal iodine metabolism must take into account a marked variation in the rate of thyroid hormone formation and release within the thyroid gland.

Models of Peripheral Thyroid Hormone Distribution: Volume of Distribution

It was realized early that T4 and T3 were distributed widely into tissues in addition to the blood. Attempts were made to quantify their “volume of distribution,” the volume of plasma activity represented by the total amount of relevant nonthyroidal activity in the body. The early studies approaching this question (Ingbar and Freinkel, 1960) assumed that all of the activity could be presumed to be in a single compartment. In these studies, the plasma disappearance curves for the radioactivity from injected T4 or T3 were plotted on semilog paper and the monotonic portion of the time–log radioactivity curve was projected back to zero. The volume of distribution was taken to be the inverse of the zero intercept.

This technique worked quite well in measuring the T4 volume of distribution, but it led to an artificially high volume of distribution when T3 was studied (Figure 19.3), since it ignored the early data points in the disappearance curve. This was corrected by the introduction of the “noncompartmental” model that integrated the total area under the disappearance curve to calculate volume of distribution (Oppenheimer *et al.*, 1975).

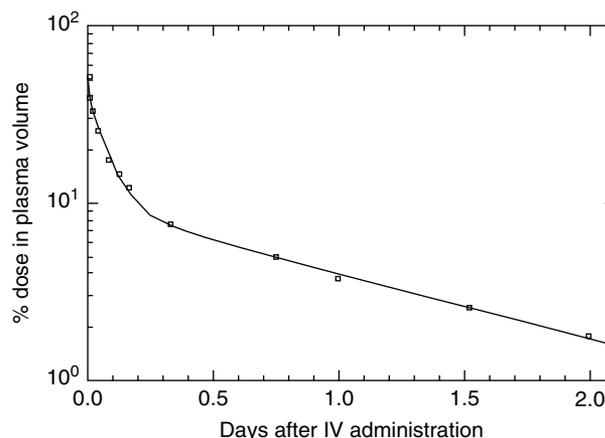


Figure 19.3 A time–activity study of human T3 metabolism. If the volume of distribution is calculated from a one-compartment model, the calculation will ignore the data points before 0.7 days. If the volume of distribution is calculated from a noncompartmental or 3-compartment model, all data points will be taken into account.

Studies that have included measurement of urinary and fecal excretion of radioactivity have helped to confirm these studies of total volumes of distribution of the thyroid hormones. When excreted radioactivity is subtracted from injected radioactivity and the result divided by plasma radioactivity (all in the same units and corrected for radioactive decay), the quotient is taken as the volume of distribution. The problem with this approach is that it is difficult to ensure complete collection of excreta.

Models of Thyroid Hormone Distribution that include Rates of Peripheral Tissue Metabolism

Studies of radioactive T4 and T3 showed that some tissues, notably the liver and kidneys, were much more active

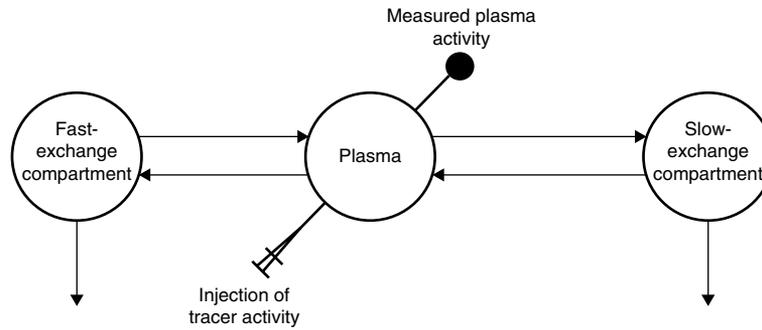


Figure 19.4 A 3-compartment model with metabolism shown from both fast- and slow-exchange compartments. This model cannot be solved uniquely without adding constraints to it.

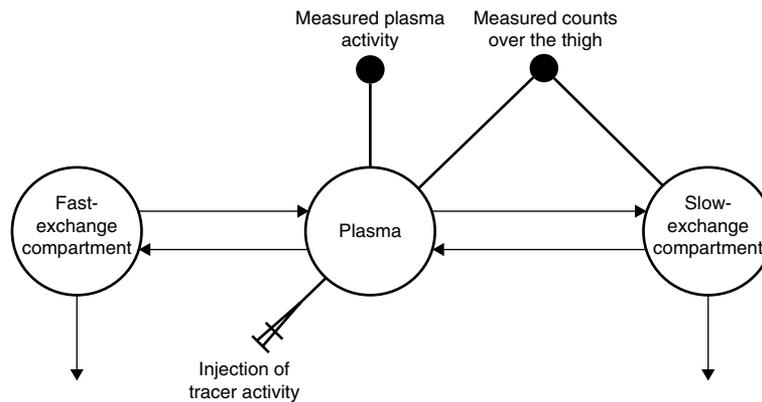


Figure 19.5 The same model as in Figure 19.4, but with counts over the thigh added to the data set. This model can be solved uniquely.

in their metabolism of the hormones than were other tissues, such as the skin. To formalize this knowledge, models were developed in which the plasma tracer hormone was distributed into separate compartments for those tissues with “fast” metabolism and those with “slow” metabolism (Figure 19.4). It was logical also to structure these models so that the hormone in each compartment left as iodide after metabolism. The problem with this model, if the only measured data are hormone-labeled radioactivity in the plasma after IV administration, is that the model is “underdetermined.” It will fit to different parameters depending on the distribution of the metabolic product between the two peripheral compartments. This problem can be addressed by arbitrarily setting a fixed ratio between the two exits or by assigning the entire metabolic exit to one compartment. These approaches are satisfactory when case-control studies are being done, but they do not add to our understanding of the relative importance of the different tissue compartments.

A better solution can be achieved by adding one or more additional data sets. For example, if counts over an extremity are included in the model fit, it will be possible to determine the shape of the disappearance curve from the slow-exchange compartment, since the extremity contains only blood and slow-exchange tissues such as skin, muscle

and fat (Figure 19.5). Solution with this information will “determine” the model. Even more confidence (but also more complexity) can be gained by also incorporating information about an area that contains fast-exchange tissue, such as the liver (Figure 19.6). External counts over the liver will reflect activity in plasma, in slow-exchange tissue, and in a fast-exchange tissue, the liver itself. Since the shapes of the plasma and slow-exchange disappearance curves are already known, it will now be possible to “strip off” the fast-exchange tissue data.

Iodide Kinetics

Iodide, inorganic iodine, also has a more complex distribution than the single compartment shown in Figure 19.1, the Riggs model. After IV administration, iodide is rapidly distributed not only to the thyroid and kidney, but also to other sites of active uptake, notably the salivary and gastric glands. The secreted iodide then resides in the stomach for varying times until it re-enters the small intestine to be reabsorbed into the plasma. This “gastrointestinal cycle” (Figure 19.7) can markedly affect the early peripheral distribution of injected radioiodide (Hays and Wegner, 1965). By solving this model using data from study of the plasma

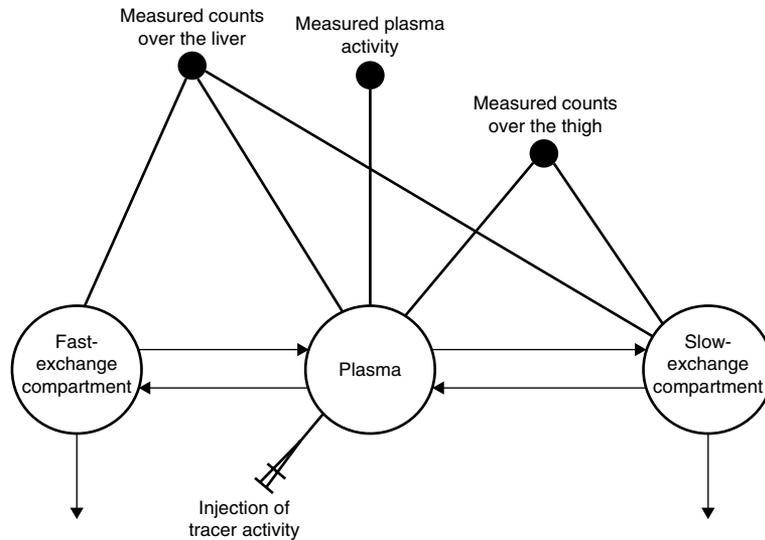


Figure 19.6 The same model as in Figures 19.4 and 19.5, but with data added from counts over the liver. This improves the estimation of kinetics in the fast-exchange compartment.

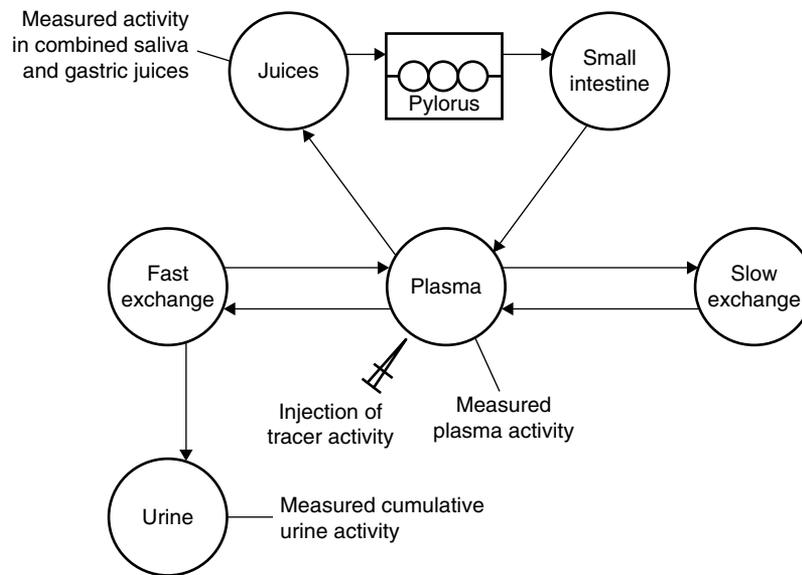


Figure 19.7 A model of iodide (inorganic iodine) metabolism, showing the gastrosalivary diversion pattern.

disappearance of radioiodide and of its appearance in the saliva, gastric juice, urine and thyroid gland, one can quantify the relative roles of the gastrosalivary diversion cycle, of thyroidal uptake, of renal excretion and of distribution into peripheral pools of radioiodide. From this model, the overall nonthyroidal volume of distribution of iodide (about 25 liters) can be calculated.

Usefulness of these Models

The models described here are simplifications. Actual physiology is much more complex. However, they provide

a theoretical structure on which to build more complex models. As more segments of human iodine physiology become quantified experimentally, it should become possible to support more complex models.

A model, when applied to tracer studies, provides an insight into internal processes that could not otherwise be measured without perturbing the organism, and thus interfering with its physiology. These studies have contributed greatly to our understanding of human iodine metabolism.

There are problems, however. In addition to experimental error, there is great normal variation among human subjects in their physiological parameters. For that reason,

Table 19.1 Published estimates of total iodine content in the human body

References	Estimate of human total body iodine (mg)
Salter (1940)	10–50
Riggs (1952)	9.3
Hamolsky (1965)	9–10
Margaria and DeCaro (1967)	50
Delange and Ermans (1996)	15–20
Venturi <i>et al.</i> , (2000)	30–50
Hays (2001)	15 (12–25)

it is preferable for a model to be fitted to data sets acquired simultaneously in the same subject. To apply data from an unrelated study introduces additional error; this problem is accentuated when the data are derived from nonhuman species. Even when the data sets are comparable, validation of a complex model becomes a challenge. Very likely we will continue to need to split up problems into segments that can be studied in an integral fashion, later combining such segments into a whole that approaches reality as closely as possible.

Application of the Modeling Approach: Determination of Human Total Body Iodine

The overall iodine content of the human body is of interest to students of the thyroid, but has eluded precise measurement. Iodine in the thyroid gland can now be measured with confidence using fluorescent scanning, its normal value ranging from 5 to 15 mg (Cavalieri and McDougall, 1996). However, the amount of iodine in the extrathyroidal spaces has been much more difficult to determine. Published estimates of total human iodine content are listed in Table 19.1.

The author used a modeling approach to estimate the iodine in human extrathyroidal spaces by analyzing the data from six normal young men who had undergone a long-term effort at equilibration of ^{125}I with stable iodine (Hays, 2001). Total body retention of ^{125}I was estimated from the difference between total intake and corrected excretion. Extrathyroidal ^{125}I was estimated as the difference between this total body retention and the measured ^{125}I content in the thyroid gland. As equilibrium approached, the ratio between extrathyroidal and total retained ^{125}I approached an asymptote calculated from a growth model. Assuming a thyroidal iodine content of 10 mg, the geometric mean estimate for total body iodine in these subjects was 15 mg (range 12–25 mg), as listed in Table 19.1.

Extrathyroidal iodine includes the measurable iodide and thyroid hormones in the plasma and in their peripheral

compartments, as estimated by mathematical models such as those discussed above. These account for approximately 1 mg of iodine (0.2 mg iodide, 0.6 mg T₄, and 0.2 mg T₃). Taken with thyroidal iodine of about 10 mg, they do not account for the totals estimated by various authors quoted in Table 19.1. Even though published estimates of total body iodine vary widely, they strongly suggest that there is a pool of iodine in the human body that is not readily accessible to direct measurement, iodine neither in the thyroid gland nor in the readily exchangeable thyroid hormone pools. This most likely consists of nonspecific protein binding of the iodine released after hormone metabolism (Hays, 2001).

One can expect that body iodine content will be low in iodine deficiency and high after administration of iodinated compounds, but no studies of these situations have come to the author's attention.

Previous direct measurements of human tissue iodine content, such as those that led to the estimate in Margaria's textbook (Margaria and DeCaro, 1967), probably reflected the iodine contamination present in most laboratories. This may explain their unusually high estimates and those of Venturi's group (Venturi *et al.*, 2000). More tissue assays of human iodine content using modern measurement techniques and avoiding the problems of iodine contamination are needed if we are to explain the nature of this nonthyroidal, nonhormonal iodine in the human body more clearly.

Meanwhile, estimating from the values in Table 19.1, a reasonable average value for total body iodine is 15 mg. If 10 mg are in the thyroid and 1 mg is in the peripheral thyroid hormone pools, approximately 4 mg can be inferred to be iodine bound nonspecifically to tissue proteins elsewhere in the periphery. These values can be expected to vary widely among subjects and to be influenced by many environmental and dietary factors, as well as by disease states.

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Iodine Transfer Through Mother's Milk: The Influence of Bromide

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Abstract

The influence of a high bromide intake in lactating rat dams on iodine and bromide transfer through mother's milk to the suckling and the effects on iodine metabolism are discussed. Moreover, the impact of high levels of bromide on the dam's performance in the course of the lactation period, and in particular on the well-being of their young, are described. Very high bromide intake in the dams caused a decrease in iodide accumulation in the mammary glands, and also an increase in iodide elimination through the kidneys. Two marked consequences caused by high bromide intake were observed in the dams: decline in the extent of diet and water consumption during the nursing period, and a conspicuous drop in the production rate of mother's milk. Bromide was transferred to the suckling through mother's milk. The observed pronounced decrease in iodine transfer to the young through mother's milk and/or an increase in the bromide concentration in milk caused a very significant decrease in the body weight increments in the pups. Enhanced bromide levels in the dams also adversely affected the thyroid gland of their young and induced hypothyroidism in them. The exact mechanism(s) of bromide interference with iodine metabolism and with postnatal developmental processes in the young remain(s), however, unclear.

Abbreviations

HPGe	High-purity germanium (detector)
INAA	Instrumental neutron activation analysis
RIA	Radioimmunoassay
tT3	Total triiodothyronine
tT4	Total thyroxine
U	International unit

Introduction

Iodine is one of the essential substances that are necessary for the proper development of young mammals. Since the intrathyroidal iodine stores of the neonate are low, and the neonate's glandular machinery turns over nearly 100% of its stores for its daily thyroid hormone production, the neonatal thyroid economy is extremely sensitive to fluctuations in the iodine supply from the mother (Glinoyer, 1997). Maternal care is, therefore, indispensable for the survival, growth and development of the young mammal for a certain period after birth. The length of this period depends on the degree of maturity of the infant. The young rat (like a human neonate), being born relatively immature, is entirely dependent on its mother, as mother's milk is the sole source of this element and of other substances that the young organism is incapable of producing. An adequate supply of iodine to the neonate is ensured by the iodide-concentrating mechanism in the mammary glands of the mother. In addition to being concentrated in the mammary glands, however, absorbed iodide is also accumulated in the thyroid gland, in the stomach, in a certain portion of the small intestine, in some of the salivary glands, as well as in the skin and hair of the rat (Gross, 1962; Pavelka, 2004). Several authors have recently shown that an enhanced bromide intake can markedly reduce iodide accumulation, not only in the thyroid (Van Leeuwen *et al.*, 1988; Buchberger *et al.*, 1990; Pavelka *et al.*, 1999), but also in the mammary gland (Lener *et al.*, 2000) and in the skin of the rat (Pavelka *et al.*, 2001). Van Leeuwen *et al.* (1983) examined the influence of bromide on the reproductive performance of rats in a three-generation reproduction study. At the highest dose (19.2 g NaBr/kg diet) complete infertility was observed, whereas at 4.8 g NaBr/kg diet fertility, as well as viability of the offspring was markedly reduced. At lower bromide doses no treatment-related changes were observed.

The effects of bromide on the reproductive performance appeared to be reversible. The impact of bromide treatment on the lactation of the dams was expressed as a lactation index, which was defined as the percentage of the young alive on day 21 from the pups alive on day 5 (Van Leeuwen *et al.*, 1983). As these authors did not provide any further data concerning the lactation of the dams, we decided to examine the impact of high bromide levels in the mother on iodine (and bromide) transfer to the suckling, and to study in greater detail the effects of high bromide intake in lactating rats on the performance of the dams and on the development of their young.

This concise overview summarizes some conclusions following from the results of our recent research on this subject; the outline also includes some relevant literature data.

Bromine (Bromide) Essentiality vs. Toxicity

The biological essentiality of bromine had been investigated repeatedly, but no reproducible data on the essentiality of this element could be derived from these findings. Because of its wide occurrence, it has not yet been possible to detect bromine deficiency symptoms in plants, animals and humans (Nielsen, 1986). Anke *et al.* (1989) performed bromine deficiency experiments with growing, pregnant and lactating goats. These authors reported that in the three replications of experiments, bromine-poor nutrition led to significantly reduced growth, a worse conception rate and a higher abortion rate, a decreased milk and milk fat production rate, a lower hemoglobin content and lower hematocrit, as well as a reduction in life expectancy of the mothers and their offspring. Nevertheless, the authors concluded that the results obtained so far did not allow any conclusive statement as to the essentiality of bromine (Anke *et al.*, 1989).

Acute toxicity, as well as short-term toxicity, of bromide appears to be low, at least in rodent species (for references, see Van Leeuwen and Sangster, 1987). In a 4-week toxicity study with rats fed with 300, 1200, 4800 or 19200 mg NaBr/kg diet, Van Logten *et al.* (1973) observed motor incoordination of the hind legs, depressed grooming and an increase in relative kidney weight only in the highest-dose group. In a 90-day experimental bromide intoxication study, Van Logten *et al.* (1974) observed, in addition, a significant growth retardation, particularly in male rats in the highest-dose group.

Transplacental distribution resulting in neonatal bromide intoxication has been repeatedly described in the literature (Pleasure and Blackburn, 1975). Rauws (1983) stated that the rat fetus appeared to be easily accessible to bromide, and that the elimination from the fetus might be retarded in comparison with the elimination from the

plasma and brain of the mother. Therefore, considerable concentrations of bromide in the fetus might be expected (Van Leeuwen and Sangster, 1987).

Although it is known that in humans therapeutic plasma-bromide concentrations range from 6 to 12 mmol/l (Wade, 1977), it is still questioned at what plasma bromide level toxic symptoms are to be expected. It is generally agreed that concentrations lower than 8–10 mmol/l are without apparent toxic effects. Definite symptoms can be expected at concentrations higher than 12 mmol/l, whereas at levels above 40 mmol/l some fatalities have occurred (Grensher *et al.*, 1983).

Influence of a High Bromide Intake in Lactating Rat Dams on Iodine and Bromide Transfer to the Suckling Through Mother's Milk

Transfer of iodine via mother's milk

An enhanced bromide intake in lactating rat dams caused a significant decrease in the extent of iodine transfer from dams to the suckling through mother's milk. Moreover, the decrease in iodine transfer was more pronounced in the young whose mothers drank water with higher bromide concentrations. This is documented in Figure 20.1, which shows the results of *in vivo* measurement of the amount of ^{131}I radioactivity transferred to the whole nests of eight pups (adjusted on the second day after delivery). The extent of iodine transfer was determined on day 16 after delivery, 3, 6 and 24 h after radioiodide application to the mother, and was expressed as the percentage of the administered dose of ^{131}I -iodide to the mother (Pavelka *et al.*, 2002).

Transfer of bromide via mother's milk

Figure 20.2 shows the results of a similar experiment, in which the transfer of ^{82}Br -bromide through mother's milk into the body of the suckling was followed (Vobecký *et al.*, 2005). As anticipated, radioactive bromide appeared in the body of the young in the course of the first 3 h after its application to the mother.

Figure 20.2 documents that bromide ions ingested by the dams moved easily into rat milk and were transferred via mother's milk to the suckling. The amount of bromide in mother's milk depended on the bromide concentration in the drinking water consumed by the dams.

Although bromide passed easily through mother's milk into the body of the young, this transfer occurred at a much slower rate than the transfer of iodide (cf., Figures 20.1 and 20.2). In the case of iodide, nearly 30% of ^{131}I radioactivity applied to the mother (in the form of radioiodide) appeared in the body of the young in the course

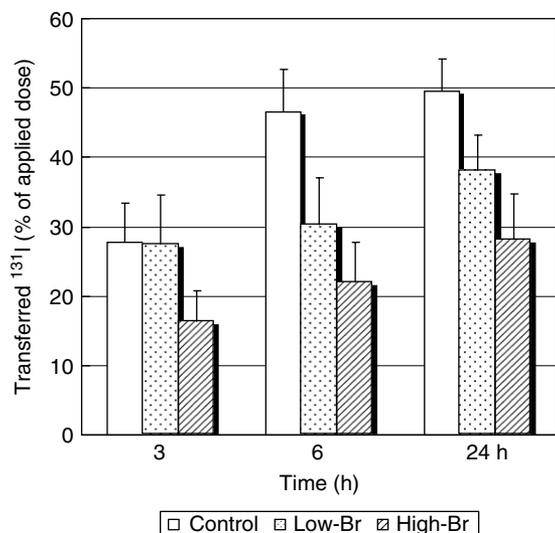


Figure 20.1 Dynamics and extent of ¹³¹I-iodine transfer from dams through mother's milk to the suckling (% of the dose applied to the dams), depending on the level of bromide intake in the dams. Three groups of female Wistar rats, each consisting of 20 members, were maintained, starting 2 weeks before mating throughout the lactation period, on a standard pelleted diet. Control dams drank distilled water, and rats of the low-Br and high-Br groups drank water with the addition of 1 g (i.e., 1000 ppm) and 5 g (i.e., 5000 ppm) bromide/l, respectively. On day 16 after delivery, the dams were given approximately 0.3 MBq ¹³¹I in the form of carrier-free iodide in 0.3 ml saline by subcutaneous injection. The whole-body radioactivity of the dams and that of the whole nests was measured *in vivo* at 3, 6 and 24 h after radiiodide application by using a gamma-spectrometric system equipped with a high-purity germanium (HPGe) detector. Adapted with permission from Pavelka *et al.* (2002, 2004).

of the first 3 h (Pavelka *et al.*, 2002). In contrast, in the case of bromide, this amount was lower than 3% at 3 h after the application of ⁸²Br-bromide to the mother, but it gradually increased during the next 22 h (Vobecký *et al.*, 2005). Our observation that bromide ingested by the dams readily moved into the milk and via mother's milk was transferred to the suckling was not unexpected with regard to the results of Disse *et al.* (1996). These authors found that bromide applied to pregnant rats in drinking water (2.5 g NaBr/l) in the period between the 5th and 15th days of gestation was transferred to embryos via placenta and later, up to 10 days after birth, even to the offspring via milk (although at decreasing concentrations).

Influence of dietary bromide on the elemental composition of mother's milk

Using the method of short-term instrumental neutron activation analysis (INAA) (Vobecký *et al.*, 2000), we simultaneously determined the concentration of bromine, chlorine and sodium in the samples of mother's milk taken

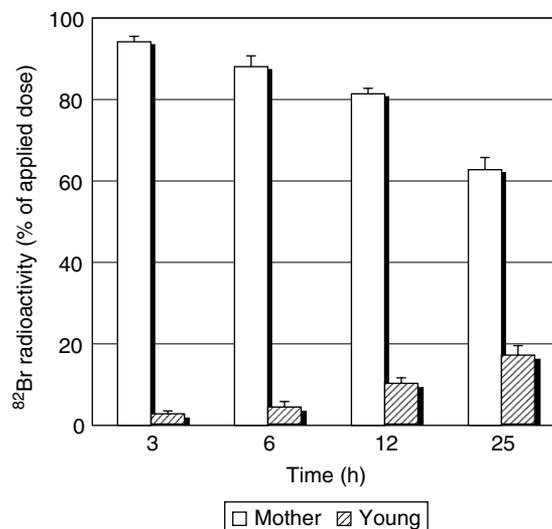


Figure 20.2 Time course of the transfer of ⁸²Br-bromide from dams to the young through mother's milk. Six lactating rats, each kept with a nest of eight young and maintained on a standard diet and tap water, were given approximately 2.3 MBq ⁸²Br in the form of potassium bromide (0.17 mg Br) in 0.3 ml saline by subcutaneous injection on day 12 after delivery. The whole-body radioactivity of the dams and that of the whole nests was measured *in vivo* at 3, 6, 12 and 25 h after radiobromide application by means of a gamma-spectrometric system equipped with an HPGe detector. Modified with permission from Vobecký *et al.* (2005).

from rat dams drinking water with various levels of bromide (Table 20.1). It can be seen from the results of these analyses that an enhanced bromide intake in the dams caused an increase in the sum of concentrations of both halogens, as well as in the concentration of sodium in mother's milk. Importantly, a considerable fraction of chloride was replaced with bromide in the milk of dams drinking water with the highest bromide concentration. With the addition of 5 g bromide/l (providing the mean daily dose of ca. 230 mg bromide/dam), bromide ions replaced about 54% of chloride in the milk, the sum of the molar concentrations of both halogens remaining unchanged. In contrast, the chloride concentration in the milk of dams with lower bromide intake did not change significantly (Vobecký *et al.*, 2005; see Table 20.1).

Effects of a High Bromide Intake in Lactating Rat Dams on Their Own Iodine Metabolism

We provided evidence for a marked interference of excessive bromide intake in adult male rats with their whole-body metabolism of iodine (cf. Chapter 61, this volume). Here, we extend our overview on the effects of a high bromide intake in lactating rat dams on their own iodine metabolism, and on the well-being of the afflicted young. There are two main routes of iodine elimination from the

body of a lactating female: through the mammary glands into milk and through the kidneys into urine. We have shown in groups of rat dams, kept either on a standard iodine-sufficient diet or on a special low-iodine diet, that the proportions of these two processes were changed by an enhanced bromide intake in the rats, even when the total iodine elimination was not affected (Lener *et al.*, 2000). An increase in iodide excretion into urine was observed in dams with high bromide intake. At the same time, very high bromide intake in the lactating dams caused a decrease in iodide concentration in the mammary glands, as in other tissues that are capable of iodine accumulation. The condition of mild iodine deficiency in the mothers apparently did not influence these effects. It appears that the observed decrease in ^{131}I -iodine transfer to the young was caused more probably by the decrease in iodide accumulation in the mammary glands, rather than merely by

the increase in iodide excretion through the kidneys (Lener *et al.*, 2000).

Impact of a High Bromide Intake in Lactating Rat Dams on Their Performance During the Lactation Period

Effect of dietary bromide on dams' food and water consumption

Very high intake of bromide in the dams (about 230 mg bromide/dam/day) caused a stagnation or even a mild decrease in the extent of their consumption of food and water during the nursing period, and a concomitant decrease in body weight gain (Table 20.2). This was in sharp contrast with control dams drinking distilled water or with

Table 20.1 Concentrations ($\mu\text{mol/g}$) of bromine, chlorine and sodium in mother's milk, depending on bromide content in the drinking water of rat dams

Bromide (g/l)	Br ($\mu\text{mol/g}$)	Cl ($\mu\text{mol/g}$)	Br+Cl ($\mu\text{mol/g}$)	Na ($\mu\text{mol/g}$)
0 (Control)	0.11 \pm 0.01	48.9 \pm 10.5	49.0 \pm 10.5	74.6 \pm 10.3
1 (Low-Br)	12.6 \pm 0.8	52.6 \pm 7.3	65.2 \pm 7.5	84.7 \pm 11.8
5 (High-Br)	34.1 \pm 5.5	29.3 \pm 1.7 ^{a,b}	63.4 \pm 7.1	87.2 \pm 5.3

Notes: Samples of mother's milk were obtained on postpartum day 11 and 16. Each mother was isolated from her litter for 8h. Then, the dams were injected subcutaneously with 1 U oxytocin and droplets of milk were pressed out manually from the nipples. Concentrations of bromine, chlorine and sodium in dried mother's milk were determined simultaneously by INAA. Values are means \pm SD, $n = 4-8$. Reprinted with permission from Vobecký *et al.* (2005).

^aSignificantly different from the control group ($p = 0.006$).

^bSignificantly different from the low-bromide group ($p = 0.029$).

Table 20.2 Mean daily consumption of food (g) and drinking water (ml) in the dams of control, low-bromide and high-bromide groups in the individual weeks of nursing period

Group	Week of nursing period			
	I	II	III	IV
Daily food consumption (g/dam)				
Control	33.9 \pm 1.3	58.3 \pm 4.9	72.1 \pm 6.4	89.9 \pm 8.5
Low-Br	24.8 \pm 0.8	53.0 \pm 4.6	62.9 \pm 3.4	84.0 \pm 6.4
High-Br	28.6 \pm 1.3	35.8 \pm 2.1	33.1 \pm 1.6	31.5 \pm 6.2
Daily water consumption (ml/dam)				
Control	51.8 \pm 5.9	83.4 \pm 13.9	109.8 \pm 17.2	152.7 \pm 21.7
Low-Br	45.7 \pm 3.2	72.8 \pm 7.6	92.9 \pm 14.8	137.1 \pm 20.1
High-Br	39.8 \pm 4.9	46.1 \pm 8.1	47.7 \pm 4.2	44.1 \pm 5.7

Notes: Experiments were performed on 30 female Wistar rats fed with a standard pelleted diet and tap water *ad libitum*. On the second day after delivery, the number of pups in each nest were adjusted to eight, and from 15 dams that gave birth on the same day one control and two experimental groups were formed. Control dams drank distilled water, and rats of the low-bromide and of the high-bromide groups drank water with the addition of 1 and 5 g NaBr/l, respectively. The consumption of diet and drinking water and the body weight of each dam and the corresponding nest were recorded at regular intervals. Values are means \pm SD, $n = 5$.

dams kept on a low bromide intake, in which a gradual increase in the consumption of food and drinking water in the course of the lactation period occurred regularly.

It should be stressed, however, that a decline in food consumption did not occur immediately after the first contact of the dams with water containing bromide, but in the course of the following 2–3 days. It is therefore evident that the reason for this change in eating behavior of the animals was not due to a change in the taste of drinking water (Vobecký *et al.*, 2005).

Influence of dietary bromide on the production rate of mother's milk

The other striking consequence of a very high bromide intake in the dams during the nursing period was a conspicuous drop in the production rate of mother's milk (Vobecký *et al.*, 2005; see Table 20.3).

One of the possible reasons for the observed marked decrease in the production of mother's milk in the dams with a high bromide intake could be a decreased stimulation of the mammary glands as a consequence of reduced consumption of mother's milk by their suckling. It is known that the intensity of lactation is regulated by the suckling young. Provided that a high concentration of bromide in mother's milk constituted a serious obstacle for the young to receive milk, a decline in the intensity of lactation, and consequently also in the consumption of food and water by the dams, would follow. Therefore, the observed anomaly in the mammary function of lactating dams of the high-bromide-intake group could be caused, paradoxically, by their own young.

Table 20.3 Production rate of mother's milk on different days of the lactation period, depending on the level of bromide intake in the dams

Group	Mean intake of bromide (mg/day/dam)	Milk (g/10h) on a day ^a	
		10	15
Control	0.24 ± 0.02	20.9 ± 3.4	21.8 ± 6.0
Low-Br	72.8 ± 7.6	17.6 ± 1.6	23.1 ± 1.8
High-Br	222.7 ± 28.7	7.4 ± 2.3 ^{b,c}	8.4 ± 5.3 ^{d,e}

Note: On the 10th and 15th day of lactation, each mother was isolated from her litter for 10h. Each dam was then weighed, injected subcutaneously with 1 U oxytocin, reunited with her litter, and permitted to nurse for 90 min. Then the young were again removed and each mother reweighed. The differences in the dams' weight were considered to be equal to the amount of mother's milk produced during the previous 10h. Reprinted with permission from Vobecký *et al.* (2005).

^aAverage values from at least two independent experiments; values are means ± SD, $n = 8-10$.

^bSignificantly different from the control group ($p < 0.0002$).

^cSignificantly different from the low-bromide group ($p = 0.0004$).

^dSignificantly different from the control group ($p = 0.0016$).

^eSignificantly different from the low-bromide group ($p = 0.0043$).

Impact of a High Bromide Intake in Lactating Rat Dams on the Well-being of Their Suckling

Effect of excessive bromide in the dams on the body weight gain in their young

A significant retardation of the body weight gain and, at the same time, a significant increase in the relative weight (mg/100 g body weight) of the thyroid glands was demonstrated in suckling rats whose mothers drank water with a high bromide concentration in the course of the first 16 days of the nursing period (Pavelka *et al.*, 2002). Because young rats do not begin to receive drinking water independently before the 18th day of life, this effect had to be mediated by mother's milk. There can be several explanations to this finding. Mother's milk might contain such concentrations of bromide that could impair the development of the young; or, very high bromide intake in the mothers might impair iodine transfer from the mother to the young and could cause hypothyroidism in the suckling. However, it is also possible that high bromide levels in the lactating dam could influence the secretion processes in the mammary glands and cause a decrease in the production rate of mother's milk. Hence, we examined in more detail some of the possible reasons for the observed adverse effects of high bromide levels in the mothers on the development of their young (Vobecký *et al.*, 2005). We found (Figure 20.3) that very high intake of bromide in the mothers during the nursing period (about 230 mg bromide/dam/day) caused a very significant decrease in the body weight increments in their suckling, starting on about the 4th day of life, in comparison with the control young (from mothers receiving about 0.2 mg bromide/dam/day) and with the young from mothers of the low-bromide group (about 70 mg bromide/dam/day). Only about one-half of the young of the high-bromide group survived, and their general condition was very poor. At the same time, the amount of bromide received daily by these young, in relation to the body weight, was about three times lower than that received by their mothers (Vobecký *et al.*, 2005).

Induction of hypothyroidism in the young by high bromide intake in the mothers

The impact of the enhanced bromide intake in the mothers, kept either on a standard iodine-sufficient diet (diet B) or on a low-iodine diet (diet A), on the relative weight of the thyroids of both the dams and the young is documented in Figure 20.4. In the dams, no significant bromide-related effects were found (Figure 20.4a). However, in the pups, there was a significant increase in the relative weight of the thyroids with increasing bromide intake in their mothers in both groups A and B (Figure 20.4b).

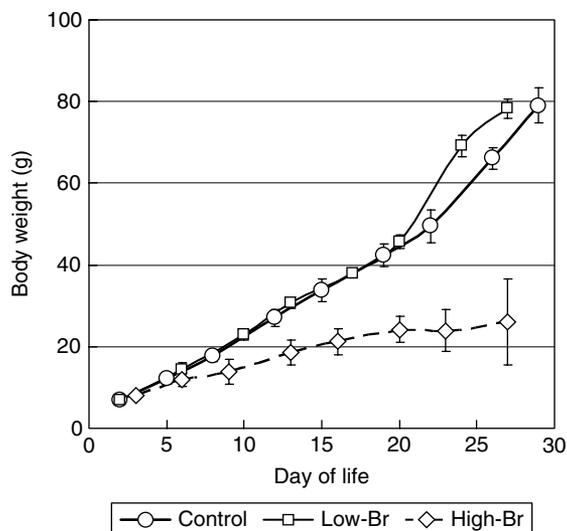


Figure 20.3 Body weight increments in the suckling in the course of the nursing period, depending on the level of bromide intake in their mothers. Three groups of rat dams, each comprised of 10 members, were maintained, starting 2 weeks before mating throughout the lactation period, on a standard diet *ad libitum*. Control dams drank distilled water, and rats of the low-Br and high-Br groups drank water with the addition of 1 g (i.e., 1000 ppm) and 5 g (i.e., 5000 ppm) bromide/l, respectively. On the second day after delivery, the number of pups in each nest was adjusted to eight. The body weight of each dam and the corresponding nest were recorded at regular intervals. Modified with permission from Vobecký *et al.* (2005).

The increase in the relative weight of the thyroids of the young, whose mothers drank water containing very high concentrations of bromide, indicates either a direct action of bromide or the institution of the condition of iodine deficiency in the young. Of course, the possibility that both mechanisms operate simultaneously cannot be excluded. If the iodide-concentrating mechanism in the mammary glands of the mother is impaired, e.g., by a high bromide level in the body of the mother, a decrease in iodine content in the mother's milk occurs. Direct support for the conclusion that the young were iodine-deficient comes from the results of radioimmunoassay (RIA) determination of total thyroxine (tT_4) and total triiodothyronine (tT_3) concentrations in the sera of the young whose mothers drank bromide-containing water, in comparison with the tT_4 and tT_3 concentrations in sera of the control pups whose mothers drank distilled water (Table 20.4).

The results of the two repeated series of experiments summarized in Table 20.4 document that a prolonged intake of high amounts of bromide in the lactating rat dams caused a marked hypothyroxinemia, both in their own body and in the bodies of their pups (Pavelka *et al.*, 2002). The effect of excess bromide was even more pronounced in the pups, as is evident from the dramatically decreased concentrations not only of tT_4 , but also of tT_3 in sera of the young whose mothers drank water with the highest bromide concentration. In contrast with the young, the changes in tT_3 concentration in the mother's sera caused by the high bromide intake are milder.

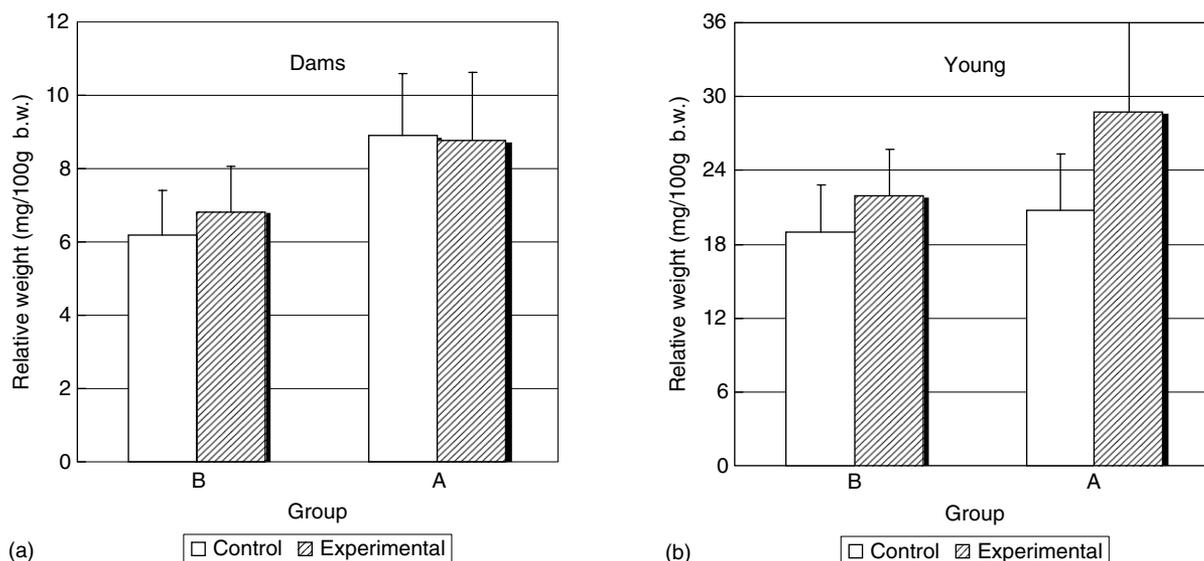


Figure 20.4 Relative weight (mg/100 g body weight) of the thyroids of (a) the dams; and (b) their young 17 days after delivery, as influenced by very high bromide intake in the mothers. Two groups of female Wistar rats, each consisting of 20 members, were maintained, starting 2 weeks before mating throughout the lactation period, either on a standard iodine-sufficient diet (diet B, Bergman) or on a special low-iodine diet (diet A, Altromin C1042). All the animals drank distilled water till the time of delivery. Then, the rats of both groups were further divided into two subgroups: dams of control groups drank distilled water, while those of experimental groups drank water with the addition of 5 g bromide/l. On day 17 after delivery, the rats were killed and the thyroids from (a) the dams; and (b) the young were collected and weighed.

Table 20.4 Concentrations of total thyroxine (tT₄) and total triiodothyronine (tT₃) in the blood sera of the dams and in the pooled sera of their pups, depending on the level of bromide intake in the dams

Group	Experiment 1		Experiment 2	
	tT ₄ (nmol/l)	tT ₃ (nmol/l)	tT ₄ (nmol/l)	tT ₃ (nmol/l)
Dams				
Control	53.3 ± 11.5	1.14 ± 0.16	25.7 ± 4.7	0.71 ± 0.24
Low-Br	26.2 ± 5.2	0.91 ± 0.26	17.4 ± 1.3	0.51 ± 0.13
High-Br	23.3 ± 1.9	1.07 ± 0.15	10.9 ± 2.2	0.46 ± 0.13
Pups				
Control	38.2 ± 3.7	1.25 ± 0.19	81.0 ± 12.3	1.28 ± 0.25
Low-Br	30.7 ± 3.6	1.08 ± 0.11	55.0 ± 13.0	0.99 ± 0.33
High-Br	15.3 ± 1.0	0.73	24.9 ± 5.7	0.58 ± 0.07

Notes: Concentrations of tT₄ and tT₃ in sera were determined by radioimmunoassay (RIA) using commercial RIA kits for rat sera or human sera. The effect of a high bromide intake in the dams was more pronounced in their pups, as is evident from markedly decreased concentrations not only of tT₄, but also of tT₃ in the sera of the young whose mothers drank water with the highest bromide concentration. Average values determined in duplicate in three repeated assays in samples from two independent experiments are shown. The values are means ±SD, *n* = 3–6. Modified with permission from Pavelka *et al.* (2002).

Consequently, the observed disturbance of the development of the young nursed by the dams drinking water with the highest bromide concentration could be the result of an impairment of the young's thyroid function.

Disse *et al.* (1996) also observed significant delays in the postnatal development in all bromide-treated animals. In the young, these authors recorded permanent deficits for body weight, brain weight and the protein content of brain tissue. The results of these authors, like ours, suggest that prenatal and perinatal exposure of rats to high or moderate concentrations of bromide might interfere with postnatal development, including the development of the brain. However, the exact mechanism(s) of bromide action on developmental processes remain(s) to be elucidated.

Summary Points

Very high bromide intake in lactating rat dams caused:

- a decrease in iodide accumulation in the mammary glands;
- an increase in iodide elimination through the kidneys; and
- a pronounced decrease in iodine transfer to the young through mother's milk.

Bromide ions ingested by lactating dams easily moved into rat milk and:

- were transferred via mother's milk to the suckling;
- caused stagnation in the extent of diet and water consumption in the course of the lactation period; and
- accounted for a significant drop in the production rate of mother's milk.

Consequences of a decreased iodine transfer and/or an increased bromide concentration in the mother's milk were:

- marked hypothyroxinemia both in the dams' bodies and in the bodies of their suckling;
- very significant decrease in the body weight increments in the pups;
- impaired development of the young due to impaired function of their thyroids and hypothyroidism.

The exact mechanism(s) of bromide interference with iodine metabolism and with postnatal developmental processes in the young remains unresolved.

Acknowledgments

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Cellular Iodine Transport: Body Distribution of the Human Sodium Iodide Symporter

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Abstract

Iodine plays an essential role in human development and the normal physiology of adults. Hence, absence of this critical element can lead to a number of different disease states in life. Because of its centrality of normal physiology, the body has developed a complex system of iodine absorption, concentration, storage and delivery. This system utilizes a number of different organ systems and ensures that iodine is available to a developing fetus, a young child, or a grown adult. In addition to its role in human physiology, the body produces a number of iodine-containing compounds that it then utilizes in its constant fight against infectious diseases. In this way, iodine helps minimize the possibility of bacterial infection in the upper digestive tract and the eyes. In order to accomplish these tasks, the body makes use of a specialized ion transporter, the sodium iodine symporter, to deliver iodine to the proper organs. This chapter examines the distribution of the symporter throughout the body. Special attention is paid to its biological significance, structure and function. Each organ important to the iodine handling process is examined for evidence of expression of the symporter. Throughout the discussion, a number of tools and techniques important to molecular biology are considered to demonstrate how specific organs utilize iodine. Each technique is briefly explained to provide the reader with a better understanding of how current scientific studies contribute to the understanding of iodine use.

Abbreviations

cAMP	Cyclic adenosine monophosphate
DNA	Deoxyribonucleic acid
hNIS	Human sodium iodide symporter
I ⁻	Iodide

mRNA	Messenger RNA
Na ⁺	Sodium
NIS	Sodium iodide symporter
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
rNIS	Rat sodium iodide symporter
RT-PCR	Reverse transcription-polymerase chain reaction
TSH	Thyroid-stimulating hormone

Introduction

Iodine is essential for the human body. Failure to have proper amounts of this element during development or in adulthood can lead to a host of medical problems, including mental retardation, cardiac complications, metabolic disturbances and mental health illness. Illnesses resulting from lack of iodine are common and have been known for centuries. Congenital hypothyroidism, initially referred to as cretinism, was described in the eighteenth century. Likewise, the large goiters in iodine-deprived individuals are well described and not uncommon in many countries. Because of the potential consequences of not having the proper amount of iodine, the body possesses a number of mechanisms by which it can absorb, collect, concentrate and excrete iodine in the form of its monovalent anion iodide. This system encompasses a large number of organ systems and different physiological processes, all to ensure that iodine processing and utilization take place properly.

Humans ingest iodine through food. In many countries, iodine fortification, normally through iodized salt, ensures proper iodine intake. The ingested iodine is then absorbed into the bloodstream through the gut. Once absorbed, the major location of iodine storage and utilization is the thyroid. Iodine is transported into the thyroid from the

bloodstream by a protein, the sodium iodide symporter (hereafter referred to as either the symporter or NIS). Definitions of some of the key terms used throughout this chapter are shown in [Table 21.1](#).

In the thyroid gland, iodine plays perhaps its most important role through incorporation into the thyroid hormone precursor thyroglobulin. It is through the diverse actions of thyroid hormones that iodine becomes important in the regulation of a number of different physiologic systems. Here, also, the role of the symporter has been studied extensively. Both molecular biology techniques and imaging studies have been used to describe the role that NIS plays in thyroid physiology.

A second major organ of iodine uptake is the breast. Iodine is essential for the developing child. If iodine is unavailable during development, the child can develop congenital hypothyroidism. This disease, marked by mental retardation and numerous physical deformities, clearly demonstrates the critical role of iodine in early life. The mother provides adequate amounts of iodine to the developing child through breast milk, which helps in preventing the disease. Delivery of iodine through the milk is a continuation of the process that started when the child was *in utero*; the mother provides the fetus with iodine through the concentrating properties of the symporter located in the placenta. The presence of NIS within both breast tissue and the placenta ensures that the proper amount of iodine will be present during development.

Table 21.1 Definitions of common terms in iodine physiology and transport

Sodium: An element essential for human life. Sodium (symbol Na on the periodic table, it has atomic number 11 and a mass of 23 Da). Proteins that transport elements in and out of the cells often use sodium as a cotransporter molecule, thereby taking advantage of chemical gradients that exist within the body.

Iodine and iodide: Iodine is the pure form of the element (symbol I on the periodic table, it has atomic number 53 and a mass of 127 Da). Iodine describes the chemical state of the element when it is not an ion, when it usually exists in its pure form as I₂. Iodide is the ionized form of iodine found within the body. In this chemical state, the element exists as an ion (it has a negative charge of -1) and can participate in a number of chemical and biological processes. It is the substrate for the Na⁺I⁻ symporter defined below.

Symporter: A protein found at cell membranes that actively transports multiple molecules into a cell simultaneously. The prefix “sym” means “together” or “with”; the root word “porter” is derived from the word “transporter.”

Sodium iodide symporter: A symporter that transports sodium and iodide simultaneously into a cell.

Notes: Knowledge of basic terms is essential to understanding iodine physiology and transport in the body. The Na⁺I⁻ symporter actively transports sodium and iodide, the ion form of iodide, into the cell.

In addition to its role in human physiology, iodine is an integral part of a number of compounds important for fighting infectious diseases. Secretion of these compounds in the upper digestive tract and eyes helps ensure that those parts of the body constantly exposed to outside contamination remain free of bacterial infection. Loss of these iodine-containing protective compounds could place people at risk of bacterial infections. The presence of the symporter at these locations is central to providing the needed iodine for immunologic protection.

Methods to Detect NIS Expression

Determining the presence of the symporter throughout the body utilizes a number of molecular biology and nuclear medicine techniques ([Figures 21.1](#) and [21.2](#)). One method involves harvesting the ribonucleic acid (RNA) from the tissues and measuring the amount of NIS messenger RNA (mRNA). This mRNA is the result of transcription of the deoxyribonucleic acid (DNA) strand, RNA processing and eventually translation by the ribosomes to form the mature NIS protein. RNA can be used directly to measure

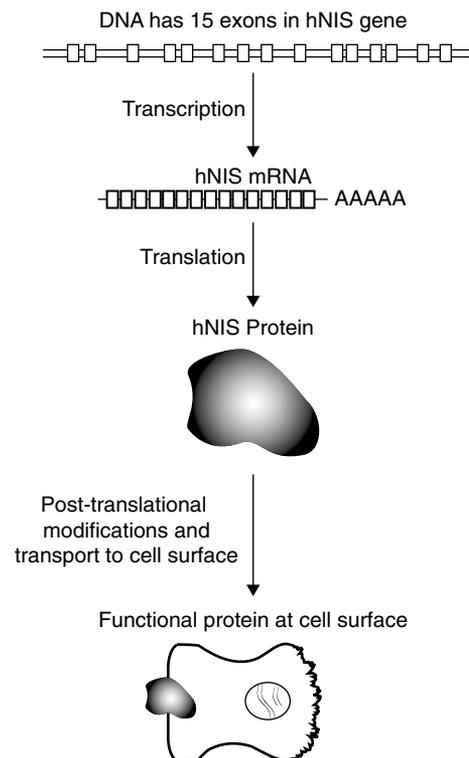


Figure 21.1 Placement of a functional sodium iodide symporter (NIS) at the cell's basolateral surface is a complex process involving a number of molecular biological steps. The gene is first translated into mRNA. The mRNA is then translated into a protein, which must then be properly folded, glycosylated and otherwise modified, and successfully trafficked to and inserted into the cell's basolateral plasma membrane.

NIS mRNA such as by northern blotting. Also, through the process of reverse transcription and a polymerase chain reaction (hereafter referred to as RT-PCR), a DNA strand identical to the RNA strand is formed and detected in a very sensitive assay. These RNA-based assays are two methods that can determine whether the symporter mRNA was present in a given tissue. Detection of symporter mRNA would indicate that the gene was being expressed.

Detection of the symporter protein can also take place through various means. In western blotting, proteins harvested from cells or tissues can be separated on a denaturing polyacrylamide gel, transferred to membranes, and then probed with specific antibodies directed toward the symporter protein that can indicate whether the symporter was present in that specific tissue. These specific antibodies may also be utilized in a process called immunohistochemistry. In this method, the tissue remains whole and is sectioned into thin slices for easy study under a microscope. Treatment of this tissue with the antibody directed against the symporter then allows for the detection of the protein within the tissue slice. A final method of determining symporter expression and iodine uptake makes use of the fact that some iodine isotopes are radioactive. The location of these radioactive isotopes can be determined by using cameras that detect iodine's radioactive emissions. This allows

for the visualization of the entire body to determine which sites actively take up iodine. However, it is specific to iodine and not the symporter. Therefore, it provides evidence as to where NIS *could* be located and does not exclude the possibility that iodine could be transported by a different protein.

Using these methods, detection of the symporter in a number of tissues in the body has been possible. Symporter expression has been detected in both the thyroid and a number of different tissues using RT-PCR and northern blot (Spitzweg *et al.*, 1999). Many nonthyroidal tissues actively take up iodide; these include salivary glands, choroid plexus, ciliary body of the eye, gastric mucosa, placenta and lactating mammary glands (Dohan *et al.*, 2003). This chapter explores the various locations throughout the body where iodine is important. However, examination of the distribution of iodine uptake and utilization must begin by first examining the sodium iodide symporter.

The Sodium Iodide Symporter

An intrinsic membrane protein, the sodium iodide symporter, facilitates the active accumulation of iodide in a cell. Located on human chromosome 19, the gene is interspersed with 14 introns. Western blotting of the NIS protein shows a major band corresponding to a molecular weight of 97 kDa. Upon translation, the symporter then undergoes a number of post-translational modifications including glycosylation. Proper folding and transportation to the cell surface results in a protein that spans the plasma membrane 13 times. Perturbations in any of these steps can result in a nonfunctioning symporter. A previous study has demonstrated that high levels of NIS mRNA do not correlate with NIS activity in cultured cells (Kogai *et al.*, 2000). The reason for this speaks to the complexity of the symporter itself. NIS function depends not only on the post-translational modifications it undergoes, but also on the polarity of the cells and their organization (Dohan *et al.*, 2003; Kogai *et al.*, 2000). NIS expression and function were improved when the cells organized themselves into the follicles reminiscent of those found in the thyroid gland, instead of a normal monolayer of cultured cells. The complexity and size of the symporter have been demonstrated by experiments utilizing both the human sodium iodide symporter (hNIS) and that from other animals, such as rat NIS (rNIS). The hNIS is much larger and more complex than rNIS. Cells transfected with rNIS had higher levels of functioning symporter at the cell surface, as measured by iodine uptake, compared to those transfected with hNIS (Zhang *et al.*, 2005). The differing complexity of these two related proteins may contribute to the different levels of functional symporter.

Once present on the cell surface, the symporter works by cotransporting a negatively charged iodide molecule and a positively charged sodium ion to the cell (Figure 21.3).

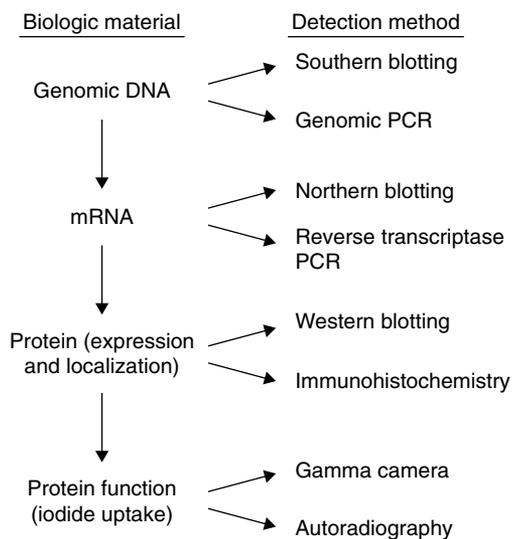


Figure 21.2 Detection strategies for the sodium iodide symporter (NIS) at each of the major biological steps along its synthesis and trafficking. Gene rearrangements, deletions, or amplifications can be measured at the level of genomic DNA by southern blotting or genomic PCR. Expression of the gene at the mRNA level can be detected by northern blotting or reverse transcriptase polymerase chain reaction (RT-PCR). Expression of the protein can be measured by western blotting or immunohistochemistry, the latter also being particularly useful for determining protein localization. Detection of a functional symporter can be determined by iodide uptake assays coupled with appropriate detection strategies, including autoradiography or gamma camera scintigraphy.

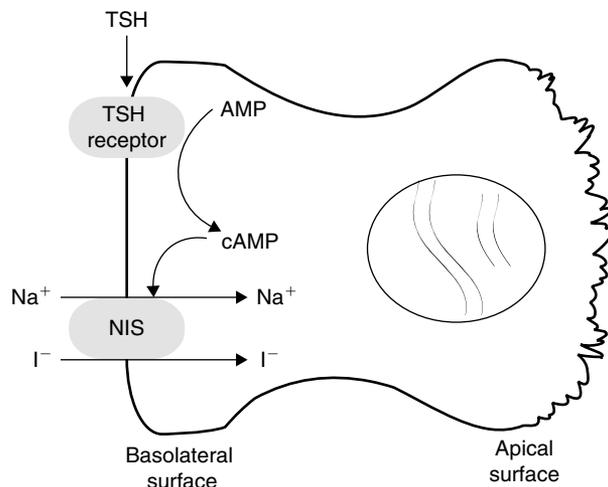


Figure 21.3 The symporter functions at the basolateral surface of the cell to bring iodide into it. Although a number of different hormones and molecules help regulate the sodium iodide symporter (NIS) expression, thyroid-stimulating hormone (TSH) is the most common regulator of the symporter. In this figure, TSH binds with its receptor, allowing for an increase in cAMP that in turn can power the symporter. Iodide, and two accompanying sodium ions, is transported into the cells through the function of the symporter.

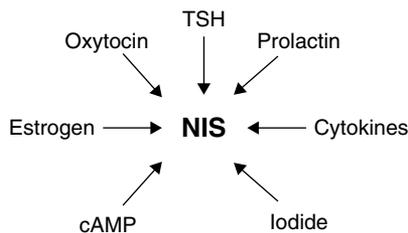


Figure 21.4 Regulation of the symporter occurs through a variety of hormones and molecules. This finding is consistent with its expression in diverse tissue types. NIS expression can be changed through the actions of thyroid-stimulating hormone (TSH), oxytocin, prolactin, estrogen, inflammatory cytokines, iodide, or cAMP levels.

NIS binds the sodium first followed by the iodide ion, which initiates a conformational change of the protein (Chung, 2002). The transport then takes place through an active, cyclic adenosine monophosphate (cAMP)-induced conformational change in the symporter. This transport mechanism in part utilizes a concentration gradient created by the ATP-dependent Na^+/K^+ exchange transporter located on the cell surface, which maintains a low intracellular concentration of sodium. Depending on the tissue type, iodine is then utilized in various biological processes, transported out of the cell, or organified into a protein/iodide molecule and stored for later use.

Complex mechanisms control NIS expression (Figure 21.4). Different tissue types often utilize different methods of controlling symporter expression. For example,

thyroid-stimulating hormone (TSH) plays an important role in NIS expression in the thyroid, while hormones such as estrogen are important in NIS expression in the breast. These various means will be discussed in relationship to the various organs. NIS expression can also be affected – many times through unknown mechanisms – by various disease states. In various forms of thyroid and breast cancer, NIS expression is lost relative to the normal cells of origin of the tumor for as-yet unknown reasons.

Thyroid Gland

The expression of the sodium iodide symporter is perhaps nowhere more important than in the thyroid gland. A complete review of the physiological importance of the thyroid is beyond the scope of this chapter. It is sufficient to say that the symporter provides the iodine needed for normal thyroid function. Once the symporter has been trafficked to the basolateral surface of the thyrocyte, it can transport iodine from the blood into the cell. Once inside the cells, iodine is transported to the apical membrane where it is organified through attachment to a tyrosine residue and incorporated into the thyroid hormone thyroglobulin. The thyroglobulin is then stored inside thyroid follicles as colloid, to be released into the bloodstream as thyroid hormones (thyroxine and triiodothyronine) via TSH stimulation.

TSH stimulates expression of NIS and drives its localization to the thyroid cell basolateral membrane (Dohan *et al.*, 2003). The importance of TSH stimulation in NIS regulation has been demonstrated by studies showing an absence of iodine uptake after suppression of TSH (Martino and Pinchera, 2000). TSH performs this function through activation of adenylate cyclase and increases in cAMP. Following transcription and translation, the protein is then transported to the cell surface. However, evidence suggests that localization of a functional symporter to the cell surface is a highly complex and regulated process which depends on cellular organization and cellular polarity (Kogai *et al.*, 2000). All of these factors are important in the process of determining the subcellular localization of the symporter, with some evidence suggesting that NIS may be absent from the cell surface but still present within membrane vesicles within the cell (Kaminsky *et al.*, 1994). Iodine can also help regulate NIS expression. This mechanism has been known for many years, and has been described as the Wolff-Chaikoff effect. Thyroid uptake of iodine is blocked through administration of high doses of the element. Although the exact details of this mechanism remain unknown, evidence suggests that high levels of iodine in the blood may inhibit TSH-mediated mechanisms of NIS expression (Panneels *et al.*, 1994). Blocking of TSH-regulated symporter expression can also occur through the action of various cytokines. Finally, estrogen may serve as another regulator of symporter levels. This

mechanism, to be discussed in greater detail later in the chapter, has been born out by the observation that there may be a connection between estrogen levels and susceptibility to goiters (Furlanetto *et al.*, 1999).

There is overwhelming evidence of the symporter in the thyroid. As early as the 1920s, the physician Plummer had observed that iodine was important to thyroid function. A further work has demonstrated that thyroid cells contain 20–40-fold higher intracellular concentrations of iodide than in the plasma (Carrasco, 1993). A number of studies have demonstrated the presence of NIS mRNA and NIS protein within the thyroid (Castro *et al.*, 1999; Dohan *et al.*, 2003). The presence of the symporter has been further confirmed through a host of imaging studies that have demonstrated the thyroid's ability to take up radioactive iodine. This is possible because the symporter can transport noniodine molecules into the cell. The affinity of NIS for several halides and pseudohalides enables the uptake of ^{99m}Tc -pertechnetate and ^{188}Re -perrhenate (Kotzerke *et al.*, 1998; Lin *et al.*, 2000). NIS-mediated uptake can also be competitively inhibited by the thiocyanate and perchlorate anions, the latter being of particular interest to environmental health science.

This unique ability of NIS to concentrate iodide and other ions has been utilized clinically in thyroid imaging and treatment of hyperthyroidism and thyroid cancer. Administration and detection of certain iodine isotopes can help determine whether the thyroid is taking up too much or too little iodine. These findings can often be suggestive of a thyroid malignancy. The presence of symporter in the thyroid gland also allows for the delivery of high doses of radioactive iodine almost exclusively to the thyroid, in order to ablate the thyroid tissue. This technique may be employed when a patient has hyperthyroidism or in order to kill malignant cells. Both the imaging and radioablative techniques make thorough use of the unique properties and location of the symporter in the thyroid gland.

Mammary Gland

Although the symporter's highest levels of expression occur in the thyroid gland, it is also detectable in a number of other tissues and organs (Table 21.2). Given the importance of proper thyroid function in human development, it is not surprising that the symporter would be located within the breast tissue, thereby allowing for delivery of iodine into breast milk for the baby's consumption. Iodine concentration in the breast milk also serves the purpose of providing antimicrobial protection. Iodine can be converted into a reactive compound with antimicrobial properties through the action of peroxidase enzymes (Geiszt *et al.*, 2003). This mechanism also confers antimicrobial properties to saliva and tears. These compounds are then secreted into the breast milk and protect the baby from infection.

Table 21.2 Major sites of sodium iodide symporter expression and iodide uptake in the human body

Thyroid
Lactating breast
Placenta
Gastric mucosa
Colonic mucosa
Parotid gland
Minor salivary glands
Lacrimal glands

Notes: The sodium iodide symporter plays an important role in a number of organs. In all of these organs, the symporter provides iodide for essential physiological processes. In addition, the regulation of the symporter is better understood in these organs.

As with the thyroid, molecular biology techniques have demonstrated that the symporter is responsible for the previously known iodine-concentrating ability of breast tissue. Detection of NIS mRNA in lactating breast tissue has shown that the gene is transcribed in this tissue (Perron *et al.*, 2001). Additional studies examining protein levels through immunohistochemistry, as well as imaging studies to detect iodine uptake, have demonstrated the presence of the symporter within breast tissue (Tazebay *et al.*, 2000).

NIS expression in breast tissue exclusively during lactation points to an estrogen-mediated mechanism of control. Suckling after birth can induce NIS expression, with additional studies demonstrating that the administration of hormones such as estrogen, prolactin and oxytocin also induce symporter expression (Wapnir *et al.*, 2004; Tazebay *et al.*, 2000). As in the thyroid, regulation of NIS expression in breast tissue is most likely a multifactorial process. Symporter expression can also be controlled through all-*trans*-retinoic acid. However, this mechanism depends on the presence of estrogen receptors (Alotaibi *et al.*, 2006).

Placenta

The presence of the symporter in placenta tissue highlights the importance of iodine during development. The presence of the symporter allows for the concentration of iodine by the placenta and delivery to the fetus. In this manner, the developing fetus has the iodine needed for use in thyroid gland and thyroid hormone production. Detection of symporter mRNA and protein through real-time RT-PCR and immunohistochemistry staining has demonstrated its presence in the placenta (Mitchell *et al.*, 2001; Di Cosmo *et al.*, 2006). Mechanisms controlling NIS expression in the placenta are not as well-understood as in the thyroid or breast. However, it appears that the placenta and thyroid may share some regulatory mechanisms for symporter expression. In the thyroid, the gene *pax8* is expressed and aids in TSH-mediated changes in the expression of other genes, such as the symporter. Evidence now suggests that *pax8* may also help regulate NIS

expression in the placenta (Ferretti *et al.*, 2005). However, the roles that pregnancy-exclusive hormones play in placental NIS expression remains to be described.

NIS Expression in the Stomach and Kidney

Given the importance of iodine in both human development and normal physiology, it would seem obvious that the symporter plays an important role in iodine absorption and excretion. Ingested iodine is primarily absorbed into the bloodstream through the duodenum – the upper portion of the small intestine – using a non-NIS mechanism. While transport of iodine into the blood would be expected, a number of additional studies have demonstrated iodine being transported from the bloodstream back into the lumen of the gut (Josefsson *et al.*, 2002). These findings correspond with a number of additional imaging studies, in both humans and animals, which have demonstrated iodine-concentrating properties in the digestive tract. They also correlate with evidence of the symporter expression in the gastric mucosa (Kotani *et al.*, 1998; Vayre *et al.*, 1999). As in the thyroid, the symporter is located along the basolateral surface of the cells. The exact function of iodine secretion *into* the gastrointestinal tract and *out of* the blood remains to be completely understood. Some have hypothesized that this may be part of a thyroid/gastric system to control circulating levels of iodine (Josefsson *et al.*, 2006), which would appear consistent with the previously described Wolff–Chaikoff phenomenon whereby circulating levels of iodine affect thyroid function and growth. Still others have posited that the reason for iodine secretion into the stomach may rest with iodine's potential antimicrobial properties (Spitzweg *et al.*, 1999). The control of NIS expression in the gut also appears to be distinct from other NIS-regulating mechanisms found elsewhere in the body, with some work demonstrating that TSH has no effect on NIS expression in the gut. Nor does it appear that regulatory molecules important to other gastric functions play a role in symporter expression in the gut (Josefsson *et al.*, 2006).

Blood iodine levels can also be controlled through excretion by the kidneys. Prior to detecting the symporter in kidney tissue, it has been known that iodine is accumulated and secreted in the kidney (Katz *et al.*, 1975). However, given the diverse filtering and secretion mechanisms found within the kidney, it was previously unknown whether NIS played an important role in this process. Examination of mRNA levels, western blotting to detect NIS protein and also immunohistochemistry of kidney tissue, have all revealed the presence of the symporter in this organ (Spitzweg *et al.*, 2001). These findings were then confirmed and extended by studies demonstrating iodine uptake by the kidneys. Aside from simply controlling the level of iodine circulating in the blood, symporter-mediated

iodine concentration within the kidney may serve an additional purpose. Here, also, iodine and various compounds containing the element, such as iodolactones and α -iodo-hexadecanal, may play a role in kidney physiology (Dugrillon, 1996; Panneels *et al.*, 1994). Despite the symporter's importance in this organ, the regulatory mechanism underlying its expression in the kidney remains to be described.

Salivary and Lacrimal Glands

Areas such as the eye and the mouth are repeatedly exposed to infectious organisms. The fact that these areas are not frequent victims of infection speaks to a complex system of immunological protection in these areas. These tissues employ not only antibodies, but also the use of small molecules with antimicrobial properties. Iodine and iodine-containing compounds likely play an important role in protecting the body against infection. As has been discussed previously, through the action of various enzymes iodine can form a number of active compounds important for fighting disease. In the presence of a peroxidase such as lactoperoxidase, and hydrogen peroxide, iodide is activated to hypoiodous acid (HOI), which is a potent antimicrobial. In addition, unincorporated iodine itself is an important disinfecting agent.

Studies employing immunohistochemistry on tissues from salivary and lacrimal glands have demonstrated the presence of the symporter (Spitzweg *et al.*, 1999; Jhiang *et al.*, 1998). To date, there appear to be no iodine uptake studies in lacrimal tissue. In addition, the mechanism controlling NIS expression there remains to be understood. Given the importance of having proper immunological defense of these areas, it is reasonable to hypothesize that the symporter may be constitutively expressed. It is also possible that in the salivary and lacrimal glands, symporter expression falls under the control of inflammatory cytokines. Such a system would seem consistent with the antimicrobial function iodine appears to be playing in these tissues.

Other Tissues

A number of additional experiments have demonstrated NIS expression in various tissues throughout the body. A complete list of these tissues can be found in [Table 21.3](#). This list is diverse, ranging from the pancreas, ovary, colon, heart and lung, to the adrenal and pituitary glands. In some instances, such as with the ovary and colon, the literature contains conflicting evidence of NIS expression (Spitzweg *et al.*, 1998; Perron *et al.*, 2001; Jhiang *et al.*, 1998; Tazebay *et al.*, 2000; Ajjan *et al.*, 1998; Smanik *et al.*, 1997). Evidence of symporter expression in thyroid and breast tissues ranges from detection of mRNA

Table 21.3 Minor sites of sodium iodide symporter expression and iodide uptake in the human body

Choroid plexus
Kidneys
Parotid
Thymus
Skin
Pituitary gland
Pancreas
Testis
Prostate
Adrenal gland
Lung
Heart
Nasopharyngeal mucosa
Extraocular muscles
Ovary

Notes: In addition to playing a prominent role in many organs, the sodium iodide symporter can also be found in a wide variety of other organs. In most of these locations, the symporter's function and regulation are unknown.

to protein detection and iodine uptake. Despite this, little evidence exists for many of these organs as to the regulation and function of the symporter.

Conclusion

Iodine plays an important role in human physiology, performing functions such as hormone synthesis as found in the thyroid, antimicrobial function as demonstrated in the breast and salivary glands, or its possible role in cellular physiology as hypothesized in the kidney. The sodium iodine symporter is central to regulating iodine uptake. Experiments examining mRNA levels, protein expression, and iodine uptake have demonstrated its presence in a host of organs and tissues. Much is already known of NIS function and regulation in organs such as the thyroid and breast. However, NIS expression in many parts of the body remains to be completely understood. Future experiments will help in better understanding the mechanism behind NIS regulation. Manipulation of NIS expression could aid in treating various diseases such as thyroid cancer or drug-resistant bacterial infections.

Summary Points

- Iodine is essential for human life. Failure to have proper amounts of this element during development and throughout life can lead to a number of disease states.
- The sodium iodide symporter transports iodine into the cell.
- The symporter is a large complex protein that is dependent on proper protein folding, posttranslational

modifications, protein trafficking, cellular polarity and cellular organization for its function.

- The symporter transports an iodide ion into the cell through an active process using sodium ions as cotransport molecules.
- The symporter is expressed in numerous organs of the body including the thyroid, stomach, breast, placenta, salivary glands and eye.
- The mechanisms controlling expression of the symporter are often tissue-dependent and rely on a number of different proteins and hormones.
- Disease states such as cancer can change the expression of the symporter.

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Sodium Iodide Symporter (NIS) in Gastric Mucosa: Gastric Iodide Secretion

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Abstract

Iodide is actively transported from the bloodstream into the gastric juice and some iodide accumulation occurs in the gastric wall, but no uptake of iodide takes place in the gastric lumen. The cDNA-sequence of the thyroid sodium-iodide symporter (NIS) was revealed in 1996 and, in the thyroid gland, iodide is actively transported into the thyrocyte by NIS. Later on NIS was also found to be present, in large amounts, in the gastric mucosa, where it is located basolaterally in the surface epithelial cells. Iodide transport over the gastric mucosa is attenuated by the selective competitive NIS inhibitor perchlorate, and also by ouabain, an inhibitor of Na^+/K^+ -ATPase, which powers NIS transport. Thus, gastric iodide secretion is to a large extent mediated by NIS. The regulation of gastric NIS expression is still unknown. The functional role of NIS in the gastric mucosa is uncertain, but several theories have been put forward. These include mediating recirculation of iodide, as well as securing the presence of iodide in the stomach for antimicrobial or antioxidative purposes. Gastric iodide secretion may also be a protecting mechanism against developing gastric cancer. Gastric NIS has further been suggested to be an important protein for transporting anions other than iodide, i.e., nitrate. In the future NIS expression, or lack thereof, may become a useful parameter in the diagnosis of gastric cancer. Gene transfer of NIS into cancer cells without NIS expression, as well as chemical induction of NIS expression, are methods under exploration. If means to regulate NIS expression in tumor cells are found, it may become possible to use radioiodine therapy in gastric cancer.

Abbreviations

BSA Bovine serum albumin
cDNA Complementary deoxyribonucleic acid

E17	Embryonic day 17
FITC	Fluorescein isothiocyanate
^{131}I ^{125}I	Radioactive isotopes of iodine
mRNA	Messenger ribonucleic acid
Na^+/K^+ -ATPase	Sodium-potassium adenosine triphosphatase
NIS	Sodium iodide symporter
RT-PCR	Reverse transcriptase-polymerase chain reaction
^{35}S -dATP	Radioactive sulfur isotope linked to deoxyadenosine triphosphate
SNAP	S-nitroso- <i>N</i> -acetyl-D, L-penicillamine
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
VIP	Vasoactive intestinal peptide

Introduction

The absorption, distribution and elimination of iodide as an essential, but in some geographical areas rare, nutrient have interested researchers for many years. When radioactive isotopes of iodide became readily available in the middle of the last century, the physiological role of iodide, as well as its therapeutic potential, became the focus of interest for several decades, as described in a review by [Brown-Grant \(1961\)](#). During this period of intensive research, it was established that iodide is absorbed from the small intestine, actively taken up by the thyroid, and incorporated as an essential constituent of thyroid hormone. Iodide is further found to be stored within the thyroid to meet future needs. Interestingly, a substantial iodide concentration, independent of acid secretion, was also found in the gastric juice. An intriguing discovery was that some accumulation of iodide could be detected in the gastric wall. The biological significance of this gastric iodide handling could not however, be discovered. A practical

consequence that came from this knowledge of iodide accumulation in gastric contents was the assumption of safety measures when handling vomit from patients receiving radioiodine therapy. The accumulation of iodide in the gastric wall and juice is also suspected of being responsible for the elevated incidence of, and mortality in, gastric cancer after ^{131}I -therapy (Hall *et al.*, 1992; Holm *et al.*, 1991).

Studies on bovines showed a recirculation of iodide, and this was suggested to be an important iodide-conserving mechanism (Miller *et al.*, 1975b). The functional role of iodide secretion into the gastric lumen has, however, remained elusive.

NIS Background

In the thyroid gland iodide is actively transported into the thyrocyte by the sodium iodide symporter (NIS). The transport of iodide against a gradient is powered by Na^+/K^+ -ATPase, and competitively inhibited by perchlorate (see review by Carrasco, 1993). With the revelation of the complementary deoxyribonucleic acid (cDNA)-sequence of rat-NIS (Dai *et al.*, 1996), soon followed by the sequencing of human NIS (Smanik *et al.*, 1996), a new era of intensive iodide research started. NIS was also soon identified in the gastric wall and the cDNA-sequence of human gastric NIS was revealed (Spitzweg *et al.*, 1998).

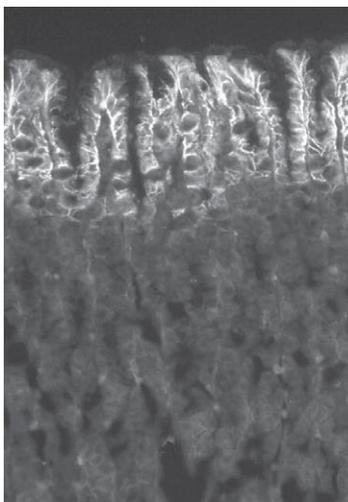
Gastric NIS Detection and Distribution

NIS protein is present in large amounts in the gastric mucosa, mainly located in the basolateral cell membranes of the epithelial surface cells. This has been demonstrated by immunohistochemistry with a polyclonal antiserum raised against human NIS in man (Vayre *et al.*, 1999), and with a polyclonal antiserum raised against rat-NIS in mouse, rat, guinea pig, pig and man (Josefsson *et al.*, 2002) (Figure 22.1). Expression of NIS was confirmed by demonstrating NIS messenger ribonucleic acid (mRNA) by *in situ* hybridization in mouse, rat and guinea pig (Josefsson *et al.*, 2002) (Figure 22.2), but the *in situ* probe used in these experiments unfortunately did not recognize NIS mRNA in porcine or human tissue. These findings are in accordance with the findings of Ajjan *et al.* (1998) who utilized southern blot and reverse transcriptase-polymerase chain reaction (RT-PCR) and found high levels (> 80% of thyroid level) of NIS mRNA in rat gastric mucosa.

NIS is located in the basolateral cell membranes of both the oxyntic and the pyloric portions of the gastric mucosa in rat, as well as in man (Josefsson *et al.*, 2002; Vayre *et al.*, 1999). In the rumen (*pars proventricularis*) of rat and mouse no NIS was found (Josefsson *et al.*, 2002), which is not surprising considering that this part of the rodent stomach is lined by squamous epithelium and not gastric



(a)



(b)

Figure 22.1 NIS in gastric mucosa. Sections of gastric mucosa from (a) the pyloric region of man; and (b) the oxyntic region of rat. Sections were stained by immunohistochemistry using a polyclonal antiserum raised in rabbit against a BSA-conjugated peptide corresponding to rat-NIS eight C-terminal amino acids. The site of the antigen-antibody reaction was revealed by FITC-labeled pig anti-rabbit IgG. Staining is intense in the basolateral cell membranes of the epithelial surface cells of human (a) and rat gastric mucosa (b). Magnification a $\times 160$ and b $\times 180$.

glandular mucosa as in the rest of the stomach. Apart from the abundant presence of NIS in the surface epithelial cells, NIS-immunoreactivity within parietal cells has also been described in mouse, guinea pig and man (Josefsson *et al.*, 2002; Spitzweg *et al.*, 1999). These findings could not however, be confirmed by *in situ* hybridization (Josefsson *et al.*, 2002), and thus the presence of authentic NIS in parietal cells is strongly questioned.

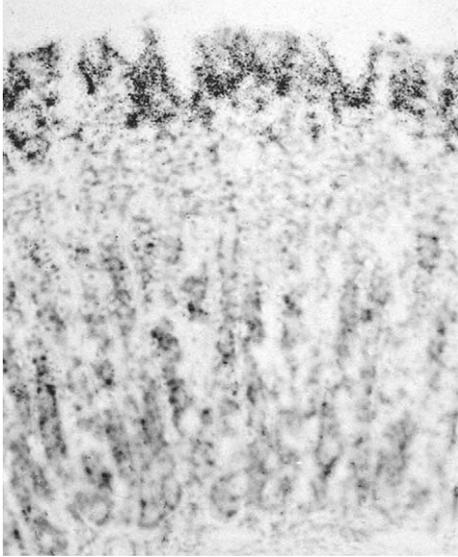


Figure 22.2 NIS mRNA in rat gastric mucosa. Section of rat stomach oxyntic mucosa autoradiographically labeled for NIS mRNA by a 33-mer oligonucleotide probe complementary to rat thyroid NIS mRNA 570–602 and 3'-endtailed with ^{35}S -dATP. Intense labeling (black silver grains) is seen in the gastric surface epithelium. Magnification $\times 200$.

Gastric NIS during Development

The presence and distribution of NIS and NIS mRNA expression have been explored in the rat gastric mucosa and thyroid during embryonic development and throughout the neonatal period (postnatal day 0–13) (Josefsson and Ekblad, unpublished). Gastric NIS was detected by immunohistochemistry and NIS mRNA by *in situ* hybridization. Expression of NIS in the gastric mucosa already occurs at embryonic day 17 (E17), which coincides with the appearance of NIS protein and NIS mRNA within the thyroid (Josefsson and Ekblad, unpublished). At this time-point gastric NIS-immunoreactivity is intense and located in the basolateral cell membranes of the epithelial surface cells. The topographic distribution and staining intensity noted in gastric mucosa at E17 persist during the later part of embryonic development and also throughout the neonatal period (Figure 22.3). Thus, the presence and expression of gastric NIS in pre- and postnatal rats are identical to those of adult rats. This is in contrast to the neonatal versus adult expression of thyroidal NIS which, although located to the basolateral cell membranes of the follicular cells, shows a patchy distribution in neonatal rats. In adult rats NIS is evenly distributed throughout the thyroid.

Gastric Iodide Transport Mediated by NIS

In vivo

Gastric iodide transport has been studied earlier *in vivo* in e.g. dog, rat (Brown-Grant, 1961) and bovines (Miller

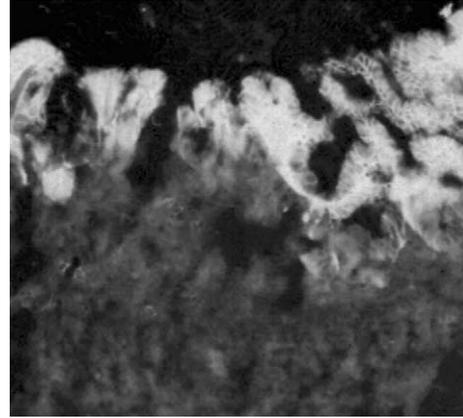


Figure 22.3 Gastric NIS in rat fetus. Section of oxyntic mucosa from a rat fetus at E19 stained by immunohistochemistry with a polyclonal antiserum raised against the eight C-terminal amino acids of rat-NIS. The site of the antigen–antibody reaction was revealed by FITC-labeled pig anti-rabbit IgG. Staining is intense in the basolateral cell membranes of the epithelial surface cells. Magnification $\times 200$.

et al., 1975a). The conclusions reached included that iodide is readily transported from the bloodstream into the gastric lumen, but not in the opposite direction, and that this transport is inhibited by thiocyanate, and even more effectively by perchlorate. In a recent study ^{125}I accumulation in thyroid was measured after oral or intravenous administration, respectively. In both groups animals with or without pyloric ligation were included. Thyroid ^{125}I accumulation was at least of the same magnitude after oral administration without pyloric ligation as after intravenous administration, but virtually no accumulation of ^{125}I was seen in the thyroid after oral administration with pyloric ligation (Josefsson *et al.*, 2002) (Table 22.1). In the group with pyloric ligation receiving ^{125}I intravenously, ^{125}I was measured in the gastric contents and after 60 min the amount of ^{125}I present in gastric lavage fluid ranged from 8.5% to 16% of the total administered dose, with the higher values in the group with pyloric ligation (Josefsson *et al.*, 2002) (Table 22.1). In conclusion, these *in vivo* experiments support the concept that iodide is actively secreted into the gastric lumen but not, to any significant degree, absorbed through the gastric mucosa.

In vitro

To be able to study iodide transport across gastric mucosa with better-controlled premises we developed an Ussing-chamber *in vitro* model (Figure 22.4) (Josefsson *et al.*, 2006). In this model we demonstrated considerable iodide transport from the serosal to the mucosal side, which was linear over time, while transport from the mucosal to the serosal side was negligible (Figure 22.5). The iodide

Table 22.1 Iodide uptake (percentage of total administered dose) in rat after oral or intravenous administration

Administration	Pyloric ligation	n	Thyroid	Blood (0.3ml)	Gastric lavage
Oral	No	3	3.5–4.2	0.35–0.38	
	Yes	4	≪0.5	0.004–0.2	
Intravenous	No	3	1.2–2.1	0.27–0.47	8.5–13
	Yes	3	1.1–2.7	0.31–0.54	11–16

Uptake of ^{125}I in the thyroid 1 h after oral or intravenous administration. Each route of administration was tested with and without pyloric ligation. After oral administration uptake was negligible with pyloric ligation. Values are ranges and expressed as a percentage of total administered dose.

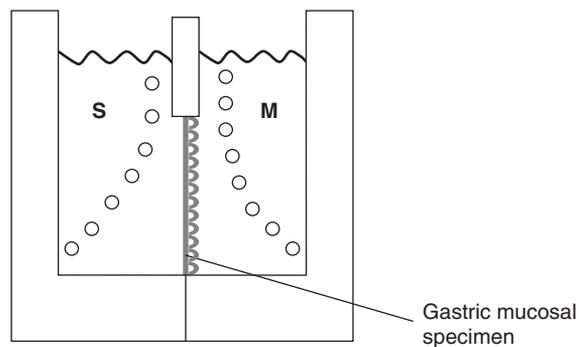


Figure 22.4 Schematic illustration of the Ussing-chamber. The chamber consists of two separate wells connected via an opening with a well-defined area (0.64 cm^2). Across the opening, the gastric mucosal specimen with the muscular layer stripped off is mounted. Both wells are filled with buffer solution and are continuously bubbled with carbogen (represented by small circles in the picture). The mounting of the specimen results in a polarized system with one serosal side (S) and one mucosal side (M). In order to measure transport over time, iodide is added to one side, and samples are then drawn from the other side at intervals.

transport from the serosal to the mucosal side was to a large extent, but not totally, inhibited by the selectively competitive NIS inhibitor perchlorate (Figure 22.6), indicating that NIS is responsible for this iodide transport. Further evidence supporting that NIS contributes in gastric iodide secretion is that transport was also attenuated by ouabain, an inhibitor of $\text{Na}^+/\text{K}^+-\text{ATPase}$ (Figure 22.6).

Regulation of Gastric NIS

Thyroid NIS expression is primarily regulated by thyroid-stimulating hormone (TSH), but also by other factors, e.g., iodide and cytokines as described in a review by Dohan *et al.* (2003). However, TSH is unable to change the rate by which iodide is transported over gastric mucosa both *in vivo* (see review by Brown-Grant, 1961) and *in vitro* (Josefsson *et al.*, 2006). So far, no known regulators either of gastric NIS expression or gastric NIS activity have been identified. In our Ussing-chamber model, besides TSH,

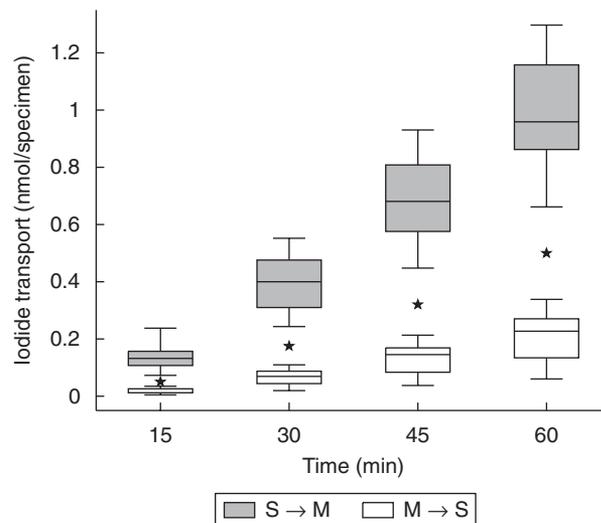


Figure 22.5 Direction of iodide transport across rat gastric mucosa in Ussing-chamber. Boxplots (medians, interquartile range and whiskers drawn to the extreme values) showing iodide transport from serosal to mucosal side (shaded boxes, $n = 11$) and from mucosal to serosal side (open boxes, $n = 8$). Values are nanomole transported per specimen (0.64 cm^2) after 15, 30, 45 and 60 min. Initial iodide concentration was 0.02 mM . $P < 0.001$ at all time points.

we also tested thyrotropin-releasing hormone (TRH), vasoactive intestinal peptide (VIP), histamine and the NO-donor S-nitroso-N-acetyl-D, L-penicillamine (SNAP), to investigate a possible neuroendocrine regulation of NIS activity, but none of these substances influenced the rate of gastric iodide secretion (Josefsson *et al.*, 2006). However, it must be emphasized that only acute regulatory effects can be studied in the Ussing-chamber *in vitro* model. The possibility of a neuroendocrine regulation of gastric NIS mRNA expression is still unexplored.

Thyroid NIS expression has been reported to be increased in fetuses in iodine-deficient rats (Schroder-van der Elst *et al.*, 2001), but no studies concerning the expression and regulation of gastric NIS during development have so far been performed.

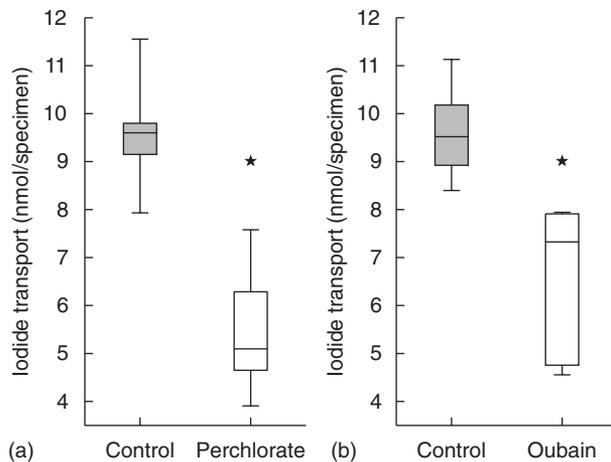


Figure 22.6 Attenuation of iodide transport in Ussing-chamber by perchlorate and ouabain. Rat gastric mucosa was tested. Boxplots (medians, interquartile range and whiskers drawn to the extreme values) showing iodide transport from the serosal to the mucosal side in rat gastric mucosa specimens in the presence of (a) perchlorate 20mM ($n = 7$) compared to control ($n = 6$); or (b) ouabain 500 μ M ($n = 5$) compared to control ($n = 5$). Initial iodide concentration was 0.2mM. Values are nanomole transported per specimen (0.64 cm²) after 60min. * $P < 0.01$ in a and b.

Functional Role of Gastric NIS

The presence of NIS in the gastric mucosa may serve several purposes. As NIS is abundantly expressed in the gastric mucosa of all studied mammals (Josefsson *et al.*, 2002), and also appears at the same gestational age in the embryonic development as thyroid NIS (unpublished observation by the authors, see section on “Gastric NIS during Development”), it is hard to believe that it is unimportant. The concept of recirculation as a means of iodide conservation, as previously mentioned, is only one possibility. One proposed hypothesis is that iodide acts as an antioxidant in the gastric lumen (Venturi and Venturi, 1999). Another possibility is that iodide has important antimicrobial effects in the gastric lumen (Majerus and Courtois, 1992). NIS can however, also transport other anions in addition to iodide, among which are nitrate (NO_3^-) (Wolff, 1998). The transport of NO_3^- is less efficient than that of iodide, but since plasma concentration of NO_3^- is normally much higher than that of iodide the total transport of NO_3^- may still be considerable. Studies on NIS transport of different anions have mostly been performed on thyroid-derived systems, like cells transfected with thyroid NIS or thyroid slices (see Eskandari *et al.*, 1997; Wolff, 1998 for reviews). Gastric NIS may have somewhat different transport properties, due to differences in post-translational modification, most probably divergent glycosylation (Tazebay *et al.*, 2000). NO_3^- is reduced to nitrite (NO_2^-) by bacterial enzymes and, in an acidic environment, then nonenzymatically reduced to nitric oxide (NO) (McKnight

et al., 1997; Weitzberg and Lundberg, 1998) – a powerful antimicrobial agent. Thus both iodide and NO_3^- may play important roles in our defense against microbes (Fite *et al.*, 2004). In addition, Fite *et al.* (2004) also indicate that the presence of iodide enhances the antimicrobial effect of NO. Interestingly, an entero-salivary recirculation of NO_3^- has been suggested by several groups (for an overview see Duncan *et al.*, 1997) and the salivary glands are, together with the thyroid, gastric mucosa and lactating mammary gland, the locations in which NIS is expressed in considerable amounts (Josefsson *et al.*, 2002; Spitzweg *et al.*, 1998).

Gastric Cancer and NIS

Gastric cancer is one of the most common neoplasms worldwide, and the diagnosis also carries a bad prognosis. Interestingly there are reports of gastric cancer being more prevalent in areas with iodine deficiency and possibly also with iodine excess (Venturi *et al.*, 2000). This indicates that the iodide secretion into the gastric lumen, mediated by NIS, may be an important factor in gastric carcinogenesis. In this context it is also interesting to note that NO_3^- , often suggested to be a risk factor for gastric cancer, is also transported by NIS, and that high levels of NO_3^- certainly would competitively reduce the iodide transport. A higher prevalence of thyroid disease (nontoxic goiter and autoimmune thyroid disease) in subjects with gastric cancer compared with matched controls has been reported (Kandemir *et al.*, 2005). A weakness in this report is that the authors do not provide any information on whether subjects with thyroid disease had received radioiodine therapy, which has previously been reported to elevate incidence of, as well as mortality in, gastric cancer (Hall *et al.*, 1992; Holm *et al.*, 1991).

Apart from the possible functional role of NIS-mediated iodide transport in gastric carcinogenesis, NIS expression is also interesting as a possible diagnostic tool for gastric cancer recurrence or metastasis, as indicated in a case report by Wu *et al.* (1984). On the other hand, Altorjay *et al.* (2007) found NIS expression to be absent or low in gastric carcinoma, and suggest that decreased NIS expression in gastric lesions could be used as a sign of malignancy. In the future it may be possible to use radioiodine accumulation by NIS for treatment of different types of cancer. To achieve this, ways to induce or enhance NIS expression in cancer cells must be explored. Gene transfer has been suggested as one means of inducing NIS expression (for a review see Dohan *et al.*, 2003) and chemical induction or enhancement of NIS expression by retinoic acid in cell lines has been reported in cell lines by Kogai *et al.* (2000).

Summary Points

- Iodide accumulation in gastric juice and within the gastric wall has been recognized since the middle of the last century.

- The sodium iodide-symporter (NIS) has been detected in gastric mucosa by immunohistochemistry, *in situ* hybridization and the combination of RT-PCR and southern blot.
- NIS is located in the basolateral cell membrane of the gastric mucosal surface cells.
- Gastric NIS is expressed in rats from E17 and abundantly expressed during the neonatal period.
- Iodide is actively transported from the bloodstream into the gastric lumen, but not in the opposite direction.
- Iodide secretion into the gastric lumen is mediated by NIS.
- The regulation of gastric NIS expression is still unknown.
- The physiological function of gastric NIS, as well as of gastric iodide transport, is poorly understood. Hypotheses include: antimicrobial effects of iodide, antioxidative effects of iodide, recirculation of iodide, and the transport of other anions such as NO_3^- by NIS.
- Gastric cancer, as well as thyroid disease, is more prevalent in areas of iodine deficiency.
- NIS expression might, in the future, be used in the diagnosis of gastric cancer metastasis or the absence of NIS expression as a sign of malignancy in gastric lesions.
- If NIS expression can be increased in cancer cells, i.e., by gene transfer or chemical induction, this could make radioiodine treatment possible not only in thyroid disease, but also in gastric cancer.

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Regulation and Enhancement of Endogenous Sodium Iodide Symporter Expression: NIS Regulatory Pathways in Thyroid and Breast Cancer

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Abstract

The sodium iodide symporter (NIS) gene is expressed at a high level in the thyroid gland and the lactating breast. Thyroid-stimulating hormone, or thyrotropin (TSH) is the major regulator of NIS expression in the normal thyroid and also stimulates NIS in more than 80% of differentiated thyroid cancers. TSH stimulates NIS gene expression through the proximal promoter and the NIS far upstream enhancer (NUE). The NUE requires at least two transcription factors, Pax-8 and CREB, for stimulation. Reduced expression of these transcription factors may contribute to the reduction in NIS expression that is seen in some differentiated thyroid cancer. High levels of endogenous TSH, or administration of recombinant TSH, are required to enhance the iodide uptake in thyroid cancer before radioiodide therapy. NIS gene expression in lactating breast tissue is primarily regulated by oxytocin and prolactin. A modest level of NIS expression has been reported in approximately 80% of cases of breast cancers. A number of strategies have been used to enhance NIS expression and radioiodide uptake in thyroid cancer and breast cancer. These include redifferentiation agents, such as retinoic acid and other nuclear hormone receptor ligands, as well as epigenetic modifiers. The results from application of these agents to *in vitro* models of thyroid and breast cancer will be described. Insights into NIS regulation, as well as agents that promote endogenous NIS expression, should lead to new approaches to using radioiodine in the treatment of thyroid and breast cancers.

Abbreviations

B-ZIP Basic-leucine zipper
 cAMP Cyclic AMP, or 3'-5'-cyclic adenosine monophosphate
 CRE cAMP-responsive element

CREB Cyclic AMP-responsive element binding protein
 CREM cAMP response element modulator
 Dex Dexamethasone
 DR2 Direct repeat-2
 DR-5 Direct repeat-5
 hCG Human chorionic gonadotropin
 HER2/neu Human epidermal growth factor receptor 2
 NIS Sodium iodide (Na⁺-I⁻) symporter
 NTF-1 NIS TSH-responsive factor-1
 NUE NIS far upstream enhancer
 Pax-8 Paired-domain containing transcription factor-8
 PI3K Phosphoinositide-3 kinase
 PKA Protein kinase-A
 PPAR γ Peroxisome proliferator-activated receptor- γ
 PyVT Polyoma virus middle T antigen
 RA Retinoic acid
 RAR Retinoic acid receptor
 RARE Retinoic acid response element
 Ref-1 Redox factor-1
 RET/PTC Rearranged in transformation/papillary thyroid carcinoma
 RXR Retinoid X receptor, 9-*cis* retinoic acid receptor
 RPL18a Ribosomal protein L18a
 T_g Thyroglobulin gene
 TPO Thyroperoxidase gene
 TSA Trichostatin A
 TSH Thyroid-stimulating hormone, or thyrotropin
 TSHR TSH receptor
 TTF-1 Homeodomain containing thyroid transcription factor-1

The primary regulator of sodium iodide symporter (NIS) expression in the thyroid is the thyroid-stimulating hormone, or thyrotropin (TSH). TSH stimulates adenylyl cyclase through the G-protein coupled TSH receptor (TSHR) resulting in accumulation of cyclic AMP (cAMP) in thyroid follicular cells. The elevation of endogenous cAMP induces NIS gene expression by stimulating factors that bind to specific regions of its promoter and the NIS upstream enhancer (NUE) (Kogai *et al.*, 2006). TSH also increases NIS protein half life and stimulates the trafficking of NIS to the plasma membrane in thyroid cells (Riedel *et al.*, 2001). Most well-differentiated thyroid cancers accumulate radioiodide in response to TSH through stimulation of the endogenous NIS gene.

The lactating mammary gland concentrates iodide to a degree similar to that seen in the thyroid, providing iodine for thyroid hormone synthesis to the developing infant. NIS is abundantly expressed in the lactating breast, and is also expressed at low levels in some breast cancer (Kogai *et al.*, 2006). Oxytocin and prolactin, but not TSH, are the primary stimulators of NIS expression in the lactating breast. Retinoids stimulate NIS expression in breast cancer cells, but suppress NIS expression in thyroid cells (Kogai *et al.*, 2006). Retinoids stimulate NIS expression at both the transcriptional and the post-transcriptional levels in breast cancer cells, but do not stimulate translocation of NIS protein to the plasma membrane (Kogai *et al.*, 2000b, 2004).

Structure of the Human and Rat NIS Gene

The difference in the factors that regulate NIS gene expression in thyroid and breast suggests that there are tissue-specific pathways involved. Many of these NIS regulatory factors have been identified, and are active in both human and rodent cell models (Kogai *et al.*, 2006). This suggests that the regulatory region(s) for NIS gene transcription are in sequences common to the human and rat NIS genes. This has been an important guide to mapping key NIS gene regulatory regions, focusing on regions with a high similarity in humans and rats.

The human NIS gene maps to 19p13.2-p12 (Smanik *et al.*, 1997) and contains 14 introns in 22116 bases, as measured from the first to the last exon. The rat NIS gene is 9260 bases, significantly smaller in size compared to the human gene, although the number of exons is the same and the full-length mRNA is similar in size. The next gene upstream of *NIS*, ribosomal protein L18a (*RPL18a*), is located 8657 bases in the human genome or 2130 bases in the rat genome upstream from the 1st exon of *NIS* (Figure 23.1). The similarity of the 5'-flanking region (from the NIS coding sequence to the *RPL18a* coding sequence) between humans and rats is only 11.8%. There are three regions with high homology between the human and the rat sequences in the 5'-flanking region of *NIS* (Figure 23.1),

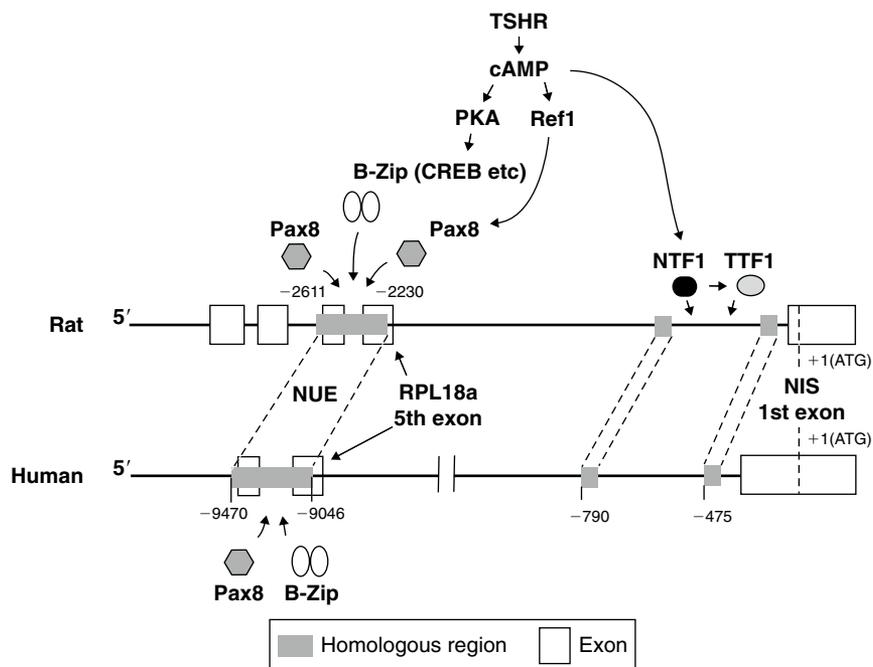


Figure 23.1 Comparison of the human and rat NIS gene structure. Three homologous regions (more than 60% homology) are shown in the 5'-flanking region. The TSH stimulatory signaling pathway to the NIS gene in thyroid cells is shown. NUE, NIS far upstream enhancer; RPL18a, ribosomal protein L18a; TSHR, TSH receptor; cAMP, cyclic AMP; CREB, cAMP responsive element binding protein; B-Zip, basic-leucine zipper transcription factor; Ref1, redox factor-1; Pax8, paired-domain containing transcription factor-8; TTF1, homeodomain containing thyroid transcription factor-1; NTF1, NIS TSH-responsive factor-1.

Pax-8 in the nucleus is likely required for the full activation of Pax-8. Decreased Ref-1 localization in the nuclei, however, has been observed in some thyroid cancer cells and tissues (Russo *et al.*, 2001). In addition, somatic rearrangements of the RET receptor (rearranged in transformation/papillary thyroid carcinoma, RET/PTC), frequently observed in papillary thyroid cancer, impair the activity of Pax-8 (De Vita *et al.*, 1998).

The PKA-dependent pathway is also down-regulated in some thyroid cancer cells. Reduced localization of PKA to the nucleus has been observed in PCCl3 rat thyroid cells constitutively expressing RET/PTC1 (Venkateswaran *et al.*, 2004). A study of specimens from 20 patients with thyroid cancer indicates that CREB mRNA and protein expression is significantly reduced in differentiated thyroid cancer tissue compared to normal tissue (Luciani *et al.*, 2003). The level of CREB expression, however, is not correlated with the NIS expression (Luciani *et al.*, 2003).

NIS Gene Regulation in Breast Cancer

NIS gene regulation in lactating breast tissue and breast cancer has been partially characterized. Oxytocin, a critical regulator of NIS in lactating mammary glands, activates the cAMP/PKA pathway and/or the inositol triphosphate- Ca^{2+} pathway. 8-bromoadenosine-cAMP or endogenous cAMP inducer, IBMX, and cholera toxin increase NIS expression in MCF-7 breast cancer cells, demonstrating a role for the cAMP pathway in mammary gland NIS expression (Knostman *et al.*, 2004).

Phosphoinositide-3 kinase (PI3K) is generally up-regulated in breast cancer. Transgenic mouse models of breast cancer, over-expressing HER2/neu (human epidermal growth factor receptor 2) or PyVT (Polyoma virus middle T antigen) targeted to breast tissue, express modest NIS (Tazebay *et al.*, 2000; Kogai *et al.*, 2004; Knostman *et al.*, 2004). HER2/neu and PyVT have a tyrosine kinase domain and activate PI3K. Expression of constitutively activating PI3K increases NIS expression in MCF-7 cells (Knostman *et al.*, 2004). The PI3K, therefore, could contribute to the NIS expression in some breast cancer.

Retinoic acid (RA) markedly induces NIS in some breast cancer cells, including MCF-7 (Kogai *et al.*, 2000b). RA-stimulated MCF-7 cells express three isoforms of retinoic acid receptor (RAR): α , β , and γ . An RAR β/γ agonist (AGN190168) is a more potent inducer of functional NIS expression than AGN195183 (RAR α agonist) and AGN194433 (RAR γ agonist), suggesting a central role of RAR β in NIS induction by retinoids (Kogai *et al.*, 2005). RAR with its ligand forms a heterodimer with retinoid X receptor (RXR), and acts as a *trans*-acting factor, stimulating a target gene through a retinoic acid response element (RARE). The consensus sequence of RARE contains two of the core motifs, 5'-PuG[G/T][T/A]CA-3', directly

repeating with a spacer of two bases or five bases (DR-2 or DR-5). The human NIS gene revealed two consensus DR-2 elements (AGGTCAGgAGTTCA) in the 1st intron. These putative DR-2 elements, however, do not respond to all-*trans* RA in MCF-7 cells. Interestingly, the DR-5, but not the DR-2, responds to the all-*trans* RA stimulation in MCF-7 cells, although no consensus sequence of the DR-5 RARE has been found around the human NIS gene (Kogai *et al.*, 2008). On the other hand, RA induces the cardiac homeobox transcription factor, Nkx-2.5, in MCF-7 cells. Since Nkx-2.5 stimulates the rat proximal promoter in MCF-7 cells, RA possibly regulates the NIS gene through Nkx-2.5 (Dentice *et al.*, 2004), but not through the RARE on the NIS gene.

Enhancement of the NIS Expression in Tissue Culture Cells

Extensive experience with ^{131}I treatment of thyroid cancer has demonstrated the importance of maximizing the magnitude of specific radioiodide uptake in tumors. The regulation of NIS expression in normal and malignant thyroid cells, therefore, has been widely investigated. Various agents have been recognized to influence NIS expression in thyroid cells, and are summarized in Table 23.1. Lactating mammary glands express functional NIS at a high level. Stimulation of NIS expression has been investigated in normal breast and in breast cancer. Many "redifferentiation agents" have been identified that induce NIS expression in breast cancer cells (Table 23.2).

TSH Receptor Stimulation in Thyroid Cells

TSH maintains the expression of NIS in the thyroid gland. TSH stimulates NIS mRNA and protein expression in rodent thyroid cell lines, FRTL-5 cells (Kogai *et al.*, 1997), PCCl3 cells (Trapasso *et al.*, 1999), and human primary thyroid cells (Kogai *et al.*, 2000a; Saito *et al.*, 1997). FRTL-5 cells require TSH to grow in monolayer for more than 10 days. Removal of TSH significantly decreases iodide uptake and NIS expression on plasma membrane in about 3 days (Riedel *et al.*, 2001). The addition of TSH to the quiescent FRTL-5 cells markedly induces the NIS mRNA expression within 6h, reaching the maximum in 24h (Kogai *et al.*, 1997). The maximum induction of iodide uptake, 20–30 fold above baseline, requires 60–72h of treatment with TSH (Figure 23.3a) (Kogai *et al.*, 1997). Translocation of NIS to the plasma membrane is also stimulated by TSH in FRTL-5 rat thyroid cells (Riedel *et al.*, 2001). Stimulators of the cyclic AMP pathway, forskolin and (Bu) $_2$ cAMP, mimic the effects of TSH on NIS expression, indicating that the effects of TSH are mediated through the cAMP pathway (Kogai *et al.*, 1997). Direct stimulation of TSHR by autoantibody in Graves'

Table 23.1 Agents that stimulate NIS expression in thyroid

Agent	Pharmacology	Experimental system	Iodide uptake	NIS mRNA	NIS protein
Bovine TSH	TSHR agonist	FRTL5 rat cell line	X	X	X
		PCCI3 rat cell line	X	X	
		WRT rat cell line			X
		Primary human thyroid cell culture (normal)	X	X	X
		KAT50 human cell line	X	X	
		Long-term culture of human thyroid (normal)	X	X	X
		Rat normal thyroid, <i>in vivo</i>			X
hCG	TSHR agonist	FRTL5 rat cell line	X	X	X
Forskolin	Adenylyl cyclase activator	FRTL5 rat cell line	X	X	X
		PCCI3 rat cell line		X	
Adenosine	A ₁ receptor agonist	Primary human thyroid cell culture (normal)	X	X	X
		FRTL5 rat cell line	X	X	X
All <i>trans</i> RA	RAR agonist	FTC133 and 238, follicular cancer cell lines		X	
Troglitazone	PPAR _γ ligand	FTC133, follicular cancer cell line		X	
		TPC1, papillary cancer cell line		X	
Depsipeptide	HDAC inhibitor	FTC133 and 236, follicular cancer cell lines	X	X	
		SW1736 and KAT4, anaplastic cancer cell lines	X	X	
		BHP18–21v, papillary cancer cell line	X	X	X
		BHP18–21v xenograft <i>in vivo</i>	X		
Trichostatin A	HDAC inhibitor	ARO, anaplastic cancer cell line	X	X	X
		TPC1, papillary cancer cell lines		X	
		FTC133, follicular cancer cell line		X	
		XTC1, Hurthle cell cancer cell line		X	
		BHP18–21v, papillary cancer cell line		X	X
Valproic acid	HDAC inhibitor	ARO, anaplastic cancer cell line		X	X
		NPA, papillary cancer cell line	X	X	X
5-azacytidine	Demethylation agent	ARO, anaplastic cancer cell line		X	X
		NPA, KAT5, KAT10, papillary cancer cell lines	X	X	
Clinical use					
hrTSH		Metastatic/recurrent cancer (50%–90%)	X		
13- <i>cis</i> RA	Pro-drug of tRA	Metastatic/recurrent cancer (0–42%)	X		

Note: Agents that significantly induce NIS are shown with an X. TSH, thyroid-stimulating hormone; TSHR, TSH receptor; hCG, human chorionic gonadotropin; RA, retinoic acid; RAR, retinoic acid receptor; PPAR, peroxisome proliferator-activated receptor; HDAC, histone deacetylase; hrTSH, human recombinant TSH. Reproduced from Kogai *et al.*, (2006). Copyright, Society for Endocrinology (2006).

Table 23.2 NIS expression stimulator in breast tissues

Agent	Experimental system	Iodide uptake ^a	NIS mRNA	NIS protein	Note
Oxytocin	Rat normal breast, <i>in vivo</i>	X		X	E ₂ required
	Cancer primary culture (3-D)		X		
Prolactin	Rat normal breast, <i>in vivo</i>	X		X	E ₂ required
	Mouse breast explant	X		X	
	Cancer primary culture (3-D)		X		
	MCF-7, ER+ cancer cell line	~10 ^b	X	X	
Estradiol	Rat normal breast, <i>in vivo</i>	X		X	
8-Bromo-cAMP, cholera toxin	MCF-7, ER+ cancer cell line	~3.3 ^b	X		
hCG	MCF-7, ER+ cancer cell line	~3.0 ^c			
Prostaglandin-E ₂	MCF-7, ER+ cancer cell line	~2.3 ^c			
Insulin/IGF-I/IGF-II	MCF-7, ER+ cancer cell line	11–14 ^b	X		
all <i>trans</i> RA	MCF-7, ER+ cancer cell line	~9.2 ^d	X	X	
	MCF-7 xenograft <i>in vivo</i>	X	X	X	
	MMTV-PyVT <i>in vivo</i>	X	X		
Dex + RA	MCF-7, ER+ cancer cell line	12–18 ^e	X		Synergistic effect with RA
AGN190168 (RAR _{β/γ} ligand)	MCF-7, ER+ cancer cell line	~9.3 ^f	X		Long duration
AGN194433 (RAR _γ agonist)	MCF-7, ER+ cancer cell line	~4.0 ^f			
AGN197496, 195183 (RAR _α ligands)	MCF-7, ER+ cancer cell line	~3.0 ^g , ~3.3 ^f	X		

(Continued)

Table 23.2 (Continued)

Agent	Experimental system	Iodide uptake ^a	NIS mRNA	NIS protein	Note
AGN195203, 194204, 196060 (RXR ligands)	MCF-7, ER+ cancer cell line	~2.5 ^g , ~6.0 ^f	X		
9- <i>cis</i> RA	MCF-7, ER+ cancer cell line	~14 ^g , ~9.0 ^d	X		
	T47D, ER+ cancer cell line		X		
	BT474, ER+ cancer cell line		X		
Troglitazone (PPAR γ ligand) + RA	MCF-7, ER+ cancer cell line	~9.6 ^h	X		Synergistic effect with RA

Note: X in column indicates significant induction by agent. Reproduced from Kogai *et al.*, (2006). Copyright, Society for Endocrinology (2006). Abbreviations: 3-D, three-dimensional; ER, estrogen receptor; E2, 17 β -estradiol; cAMP, 3'-5'-cyclic adenosine monophosphate; hCG, human chorionic gonadotropin; IGF, insulin-like growth factor; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid-X receptor; PPAR, peroxisome proliferator-activated receptor.

^aFold induction compared to without treatment is shown for data from MCF-7 cells.

^bThe induction at 12 h. Cells were maintained in DMEM with 0.2% FBS.

^cThe induction at 24 h (PGE2) or 48 h (hCG). Cells were maintained in DMEM: F12, 50:50 with 10% FBS.

^dThe duration is 36–72 h. Cells were maintained in MEM with 10% FBS or serum replacement.

^eThe duration is 2–4 days with 10⁻⁷M tRA or 2–5 days with 10⁻⁶ MAG190168. Cells were maintained in MEM with 10% FBS or serum replacement.

^fThe induction at 48 h. Cells were maintained in MEM with 10% FBS or serum replacement.

^gThe induction at 24 h.

^hThe induction at 24 h with 10⁻⁷M 9-*cis* RA.

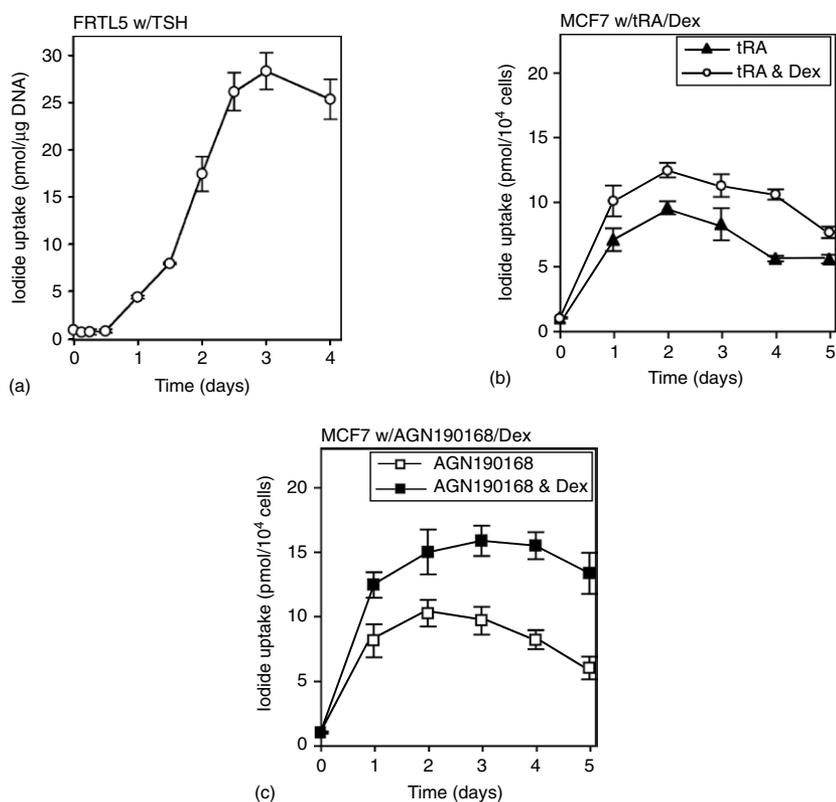


Figure 23.3 Induction of iodide uptake in FRTL-5 rat thyroid cells and MCF-7 breast cancer cells. Iodide uptake was measured with ¹²⁵I and 10 μ M of sodium iodide, and normalized with DNA content (a); or cell number (b and c). (a) TSH (1 mU/ml) significantly induces iodide uptake in FRTL-5 cells. Although the growth medium for FRTL-5 cells contains TSH, cells were grown without TSH for 8 days before the TSH treatment. Reproduced from Kogai *et al.*, (1997). Copyright 1997, The Endocrine Society. (b and c) Effects of long-term treatment with a retinoid, a pan-RAR agonist all-*trans* RA (tRA) or an RAR β/γ ligand tazarotene (AGN190168), and dexamethasone (Dex) on iodide uptake in MCF-7 cells. RAR stimulator markedly induces iodide uptake in MCF-7 cells. The duration and magnitude of the NIS induction by RA is markedly increased by the addition of Dex, especially in combination with an RAR β/γ ligand AGN190168. Reproduced from Kogai *et al.*, (2005). Copyright 2005, The Endocrine Society.

disease, constitutive activating mutations of TSHR in a hyperfunctioning thyroid adenoma, or the weak agonist human chorionic gonadotropin (hCG) (Hershman, 1999) activates the cAMP pathway and results in marked NIS expression.

More than 70% of differentiated thyroid cancer concentrates radioiodine after TSH stimulation (Robbins *et al.*, 1991; Jarzab *et al.*, 2003). Some differentiated thyroid cancer (approximately 10–20%), as well as anaplastic thyroid cancer, however, do not concentrate radioiodide, even after TSH stimulation (Robbins *et al.*, 1991). Since almost all differentiated thyroid cancer expresses TSHR (Brabant *et al.*, 1991), the absence of NIS induction in response to TSH is most likely due to defects in postreceptor signaling pathways. Recent studies have demonstrated the potential for NIS induction in poorly differentiated thyroid cancer by “redifferentiation” agents, such as nuclear receptor ligands, RA and peroxisome proliferator-activated receptor- γ (PPAR γ) ligands, and inhibitors of epigenetic modifications.

Effects of Redifferentiation Agents on Thyroid NIS

RA induces NIS mRNA expression in two follicular thyroid cancer cell lines, FTC-133 and FTC-238, but not in rat thyroid cells (Schmutzler *et al.*, 1997). Based on the findings, clinical trials have been conducted to evaluate the efficacy of RA for improving radioiodide uptake in recurrent/metastatic thyroid cancer. Twenty to 42% of aggressive differentiated thyroid cancer responds to RA treatment by an increase in radioiodide uptake (Kogai *et al.*, 2006). The studies, however, have not been randomized prospective studies of matched groups that would be necessary to confirm an effect of RA treatment.

Troglitazone, a PPAR γ ligand, also significantly increases the NIS mRNA in some differentiated thyroid cancer cell lines, FTC-133 and TPC-1 (Park *et al.*, 2005). Troglitazone inhibits cell proliferation and induces apoptosis in some papillary thyroid cancer cell lines *in vitro* and *in vivo* (Ohta *et al.*, 2001). Although troglitazone is no longer available for clinical use, a combination of PPAR γ agonist and radioiodide therapy might provide a synergistic inhibitory effect on some thyroid cancers.

Histone deacetylase inhibitors, depsipeptide (FR901228), trichostatin A (TSA), and valproic acid, increase NIS expression in thyroid cancer cell lines (Kitazono *et al.*, 2001; Kogai *et al.*, 2006). Depsipeptide significantly induces NIS mRNA and iodide uptake in follicular thyroid cancer cell lines (FTC 133 and FTC 236) and two anaplastic cancer cell lines (SW-1736 and KAT-4) at a low concentration (1 ng/ml) *in vitro* (Kitazono *et al.*, 2001). Pharmacokinetics of depsipeptide in patients have indicated that levels of more than 500 ng/ml are

achieved without significant toxicity, promising to obtain the NIS-inducible concentration in patients (Kitazono *et al.*, 2001).

The human NIS gene has three CpG-rich regions around the translation start site: the core promoter region (about 100 bp from the transcription start site), the 5'-untranslated region, and the coding region of the 1st exon (Venkataraman *et al.*, 1999). The demethylation agent, 5-azacytidine, restores NIS mRNA expression and iodide uptake in three papillary cancer cell lines, NPA, KAT-5, and KAT-10, but not in two follicular cancer cell lines, MRO and WRO (Venkataraman *et al.*, 1999). A correlation has been observed between the successful restoration of NIS expression by 5-azacytidine and demethylation of the 5'-untranslated region (Venkataraman *et al.*, 1999).

NIS Expression in Lactating Mammary Glands

NIS is predominantly expressed on the basolateral membrane of alveolar cells in mammary glands (Spitzweg *et al.*, 1998) and markedly induced during lactation (Tazebay *et al.*, 2000; Cho *et al.*, 2000). Treatment of the mice with the combination of oxytocin, prolactin and estradiol markedly induces NIS in mammary glands, while each hormone alone is not sufficient for NIS induction (Tazebay *et al.*, 2000). Basal levels of these three hormones are significantly increased in late pregnancy, and the lactogenic hormones, prolactin and oxytocin, are still elevated during the first few months of the postpartum period. The surge of oxytocin during lactation is required for maximum induction of NIS in mammary glands.

Enhancement of NIS Expression in Breast Cancer Cells

More than 80% of breast cancer tissues express NIS, although the fraction of tumors that functionally concentrate iodine is likely to be much lower (Wapnir *et al.*, 2003). Agents that stimulate NIS expression in breast cancer sufficient to concentrate radioiodide, therefore, have been considered as a potential therapy for some differentiated breast cancer (Boelaert and Franklyn, 2003). Recent *in vitro* studies have demonstrated significant induction of NIS in breast cancer cells by lactogenic hormones, insulin, and some nuclear receptor ligands, such as retinoids, PPAR γ ligands, and glucocorticoids (Kogai *et al.*, 2006) (Table 23.2).

Prolactin and oxytocin treatment induces NIS mRNA in some human breast cancer tissues cultured primarily on collagen gel, while the combination of these hormones does not produce an additive effect (Cho *et al.*, 2000). A significant induction of iodide uptake and NIS mRNA by prolactin has been reported in MCF-7 breast cancer cells

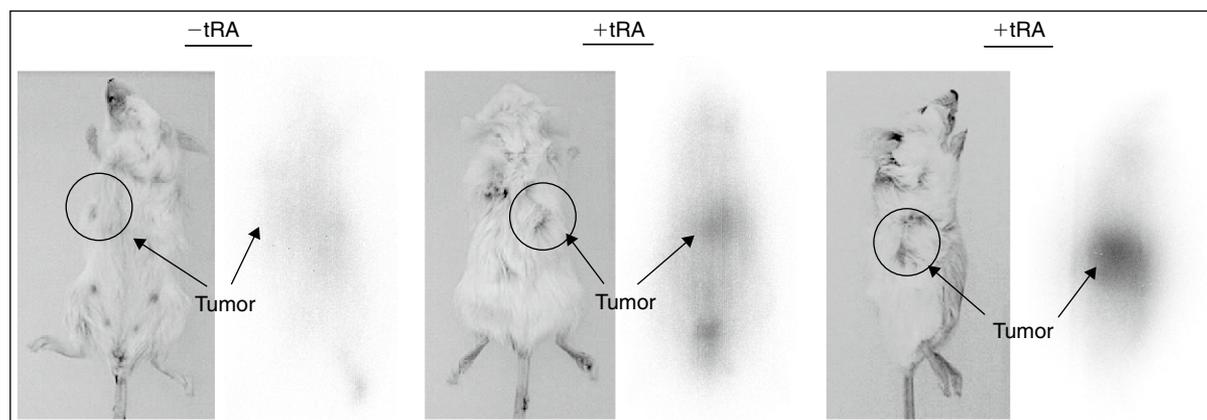


Figure 23.4 Imaging of the MCF-7 xenograft tumors with ^{125}I . In animals treated with systemic all-*trans* RA (tRA, 160 mg/kg/day) for 5 days, the tumor was visualized 2 h after the administration of ^{125}I . Reproduced from Kogai *et al.*, (2004) with permission from The American Association for Cancer Research.

(Arturi *et al.*, 2005). The duration of the prolactin induction, however, was relatively short with maximum iodide uptake at 12 h, but reduced at 24 h.

RAR agonists strongly induce functional NIS in MCF-7 cells. All-*trans* RA and the synthetic RAR ligands, TTNPB and tazarotene (AGN190168), markedly induce iodide uptake in MCF7 cells, up to 15-fold above baseline, which is sustained for more than 5 days (Figure 23.3b, c) (Kogai *et al.*, 2005). The induction is faster than that seen in FRTL-5 cells in response to TSH; NIS mRNA and iodide uptake reach the maximum around 12 h and 36–48 h (Figure 23.3b, c), respectively (Kogai *et al.*, 2000b). Tumor-selective induction of functional NIS and its mRNA expression has been confirmed in mouse breast cancer models, MCF-7 xenograft tumors (Figure 23.4), and transgenic mice over-expressing the PyVT oncogene in breast tissue (Kogai *et al.*, 2004). Isomers of all-*trans* RA, 9-*cis* RA, and 13-*cis* RA also significantly induce NIS expression in MCF-7 cells, as that seen with all-*trans* RA. Since the effect of RXR agonists on NIS expression is markedly reduced compared to that of RAR agonists, the effects of RA isomers is likely through RAR, with *trans* RA converted from these isomers by endogenous isomerases (Kogai *et al.*, 2005).

Glucocorticoid receptor ligands significantly increase RA-induced iodide uptake and NIS mRNA, and prolong the induction of iodide uptake (Figure 23.3b, c) (Kogai *et al.*, 2005; Dohan *et al.*, 2006; Unterholzner *et al.*, 2006). The addition of dexamethasone (Dex) reduces the median effective concentration (EC_{50}) of RA for the induction of iodide uptake to $\sim 7\%$ (Kogai *et al.*, 2005). The *in vivo* systemic dose of all-*trans* RA for maximum induction of NIS is quite high. The combination treatment with Dex, therefore, has potential to reduce the dose of RA, resulting in less toxicity *in vivo*. Interestingly, the duration of iodide uptake and NIS mRNA with an isoform-specific retinoid receptor agonist is significantly longer than that with all-*trans* RA,

especially in combination with Dex (Kogai *et al.*, 2005). A recent preliminary *in vivo* study, however, indicated the NIS induction by Dex in both normal and tumor tissues in a rodent breast cancer xenograft model, limiting its possible clinical application (Willhauck *et al.*, 2006). Further study is required for more specific induction of NIS in breast cancer.

NIS Expression in Placenta-Derived Cells

Placenta expresses NIS to transport iodide from the maternal circulation to the fetus. Endogenous NIS expression has been reported in two placental-derived choriocarcinoma cell lines, JAr (Mitchell *et al.*, 2001) and BrWo (Manley *et al.*, 2005). hCG increases the NIS mRNA expression and iodide uptake in JAr cells, partially through cAMP pathway (Arturi *et al.*, 2002).

Summary Points

- The primary cell signaling pathways that stimulate NIS expression differ in thyroid and lactating breast, although there is some overlap.
- The goal in thyroid cancer treatment is to optimize iodine uptake. A number of different strategies and agents have been used to stimulate NIS expression and iodine uptake in refractory thyroid cancer, although TSH remains the most potent stimulus.
- Basal NIS expression in breast cancer is relatively low, but RA and other compounds stimulate NIS expression and iodine uptake in some breast cancer cells.
- Improved understanding of NIS regulatory pathways in thyroid and breast cancer should lead to additional therapeutic options.

Acknowledgements

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Pendred's Syndrome: Deficiency in Iodide Transport

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Abstract

Pendred's syndrome (OMIM 274600) is an autosomal recessive disorder defined by the triad of congenital deafness, goiter, and a partial defect in iodide organification. Under conditions of normal iodide intake, patients with Pendred's syndrome are usually euthyroid, but if the nutritional iodide supply is scarce, overt hypothyroidism may develop. Pendred's syndrome is caused by mutations in the *PDS/SLC26A4* gene, which encodes the anion transporter pendrin. Pendrin is predominantly expressed in the inner ear, the thyroid, and the kidney. In thyroid follicular cells, pendrin localizes to the apical membrane. Functional studies suggest that it is involved in the efflux of iodide into the follicular lumen. Inactivating mutations of pendrin impair the efflux of iodide, and thereby explain the partial organification defect. In the kidney, pendrin acts as a chloride/bicarbonate exchanger. In the inner ear, pendrin is involved in anion and fluid transport, as well as in the maintenance of the endocochlear potential. The elucidation of the molecular basis of Pendred's syndrome is yet another illustration of how the characterization of the molecular basis of a Mendelian disorder can provide fundamental and often unexpected insights into physiology and disease.

Abbreviations

CFTR	Cystic fibrosis conductance regulator
CHO	Chinese hamster ovary cells
ClCn5	Chloride channel 5
ClO ₄ ⁻	Perchlorate
COS-7	Green monkey kidney cells
DEHAL1	Dehalogenase 1
DOCP	Deoxycorticosterone pivalate
DUOX2	Dual oxidase type 2
EVA	Enlarged vestibular aqueduct
FRTL-5	Fisher rat thyroid cell line 5
KCNJ10	Potassium channel

K_m	Michaelis constant
MDCK	Madin-Darby canine kidney cells
MIT/DIT mRNA	Monoiodotyrosine/diiodotyrosine Messenger RNA
NIS/SLC5A5	Sodium-iodide symporter/solute carrier family 5A5
OMIM	Online Mendelian inheritance in man
PAX8	Transcription factor PAX8
PCCL3	Rat thyroid cells
PCR	Polymerase chain reaction
<i>PDS/SLC26A4</i> (gene)	Pendred's syndrome (gene)
SLC26A4	Solute carrier family 26A4
STAS	Sulfate transporter and antisigma factor antagonist
T3	Triiodothyronine
T4	Thyroxine
TG	Thyroglobulin
TPO	Thyroid peroxidase
TR α /TR β	Thyroid hormone receptor α/β
TRPV5/ TRPV6	Calcium channels
TTF1	Thyroid transcription factor 1 (NKX2.1)
TTF2	Thyroid transcription factor 2 (FOXE1)

Introduction

Thyroid hormone is essential for normal development, growth, and function of numerous metabolic pathways. The synthesis of thyroid hormones requires a normally formed and functioning thyroid gland, as well as an adequate nutritional intake of iodide, which is essential for the synthesis of thyroid hormones. Thyroid hormone synthesis occurs in thyrocytes, which are arranged as follicles

(Figure 24.1) (Kopp, 2005). At the basolateral membrane, iodide is actively transported into thyroid follicular cells by the sodium–iodide symporter (solute carrier family 5A5; NIS/SLC5A5) (Dohan *et al.*, 2003). This transport of iodide by NIS is dependent on the sodium gradient

generated by the Na/K-ATPase (Wolff, 1964). At the apical membrane, iodide efflux into the follicular lumen is mediated, at least in part, by pendrin (PDS/SLC26A4) (Gillam *et al.*, 2004). Iodide is then oxidized by thyroperoxidase (TPO) in the presence of H_2O_2 within the follicle.

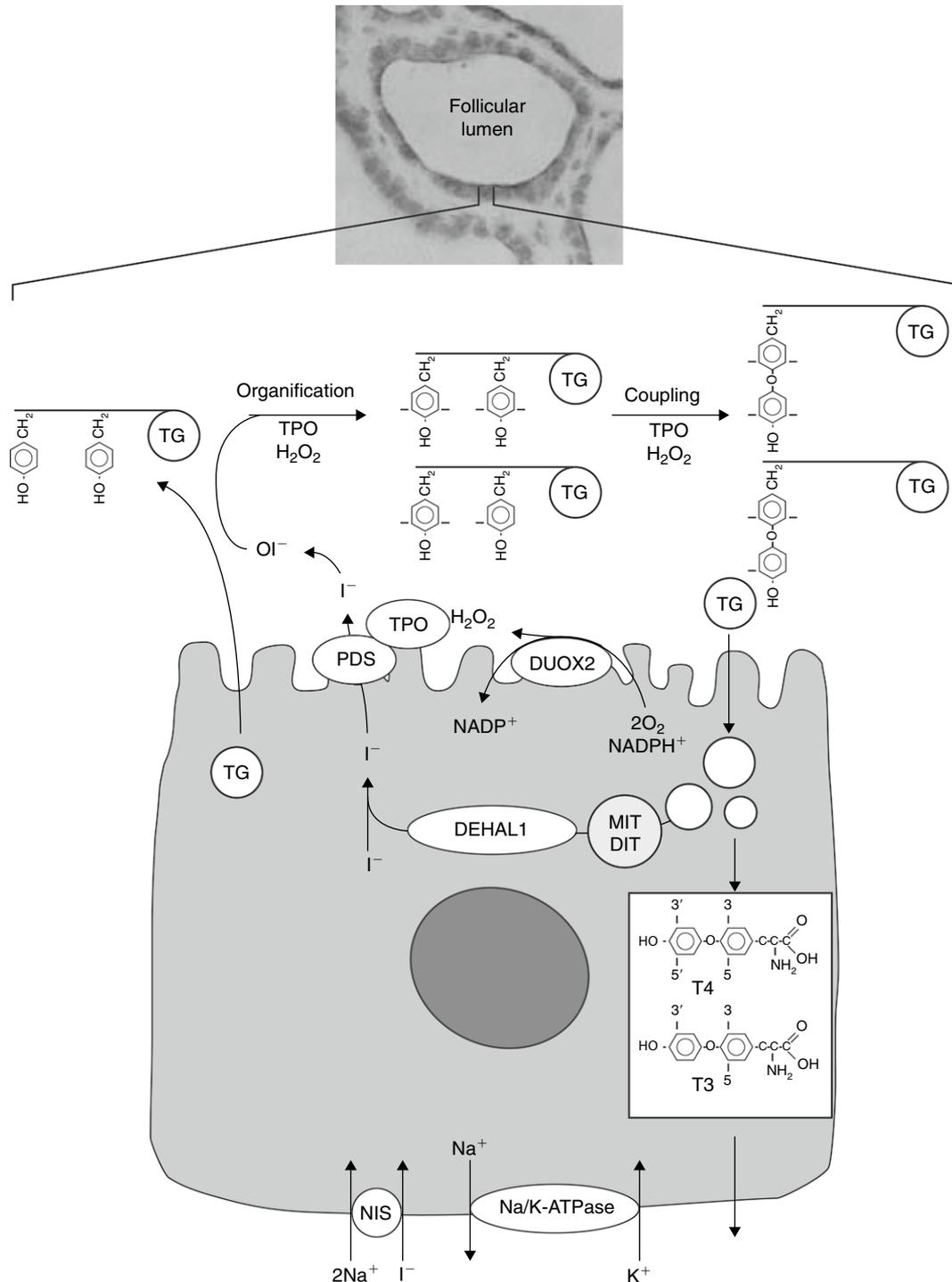


Figure 24.1 Thyroid follicle, thyroid cell, and thyroid hormone synthesis. NIS, sodium/iodide symporter; PDS, pendrin; TG, thyroglobulin; TPO, thyroperoxidase; DUOX2, dual oxidase type 2; MIT, monoiodotyrosine; DIT, diiodotyrosine; T4, thyroxine; T3, triiodothyronine; DEHAL1, dehalogenase 1.

The generation of H_2O_2 is catalyzed by the calcium-dependent NADPH oxidase, dual oxidase type 2 (DUOX2). The follicular lumen contains large amounts of thyroglobulin (TG), which serves as the matrix for the synthesis of T₄ and T₃ (Arvan and Di Jeso, 2005). In a first step, referred to as *organification*, TPO iodates selected tyrosyl residues on TG. This results in the formation of monoiodotyrosines and diiodotyrosines (MIT and DIT). In the subsequent *coupling* reaction, which is also catalyzed by TPO, two iodotyrosines are coupled to form either T₄ or T₃. Iodinated TG is then internalized into the follicular cell by micropinocytosis and macropinocytosis, and digested in lysosomes. MIT and DIT are deiodinated by an intracellular iodotyrosine dehalogenase 1 (DEHAL1) and the released iodide is recycled, whereas T₄ (~80%) and T₃ (~20%) are secreted into the bloodstream and transported to target tissues. After entering the cytosol in peripheral cells, T₄ is activated by monodeiodination to T₃ (Bianco *et al.*, 2002). T₃ then enters the nucleus, where it regulates gene transcription through the nuclear thyroid hormone receptors – thyroid hormone receptor α and thyroid hormone receptor β (TR α and TR β) (Oetting and Yen, 2007). The two receptors, having various isoforms, bind thyroid hormones with high affinity, but they differ in their developmental expression pattern and tissue distribution. Depending on the target gene, T₃ stimulates or inhibits gene expression. In addition to the genomic actions of thyroid hormone, nongenomic actions of physiological relevance are increasingly recognized (Oetting and Yen, 2007).

Iodide efflux at the apical membrane

Compared to the mechanisms mediating iodide uptake at the basolateral membrane, which is mediated by NIS (Dohan *et al.*, 2003), iodide efflux at the apical membrane is less well characterized. Electrophysiological studies performed with inverted plasma membrane vesicles suggested the existence of two apical iodide channels that could be involved in iodide efflux (Golstein *et al.*, 1992). One of these channels appears to display a high permeability and specificity for iodide ($K_m \sim 70 \mu M$), while the second channel has a much lower affinity for iodide ($K_m \sim 33 mM$) (Golstein *et al.*, 1992). The identity of these channels has not yet been determined at the molecular level. However, the demonstration of iodide transport by the anion channel pendrin, together with the clinical phenotype in patients with Pendred's syndrome, suggests that pendrin corresponds to one of the channels promoting apical iodide efflux (Everett *et al.*, 1997; Scott *et al.*, 1999; Taylor *et al.*, 2002; Yoshida *et al.*, 2002; Gillam *et al.*, 2004).

Pendred's syndrome

History and Definition The association of deafness and nonendemic goiter was first described by Pendred

(1896). Initial insights into the thyroid pathophysiology were gained in 1958 by recognizing that patients with Pendred's syndrome have a partial iodide organification defect (Morgans and Trotter, 1958). These observations led to the clinical definition of Pendred's syndrome as an autosomal recessive disorder with sensorineural deafness, goiter, and an impaired iodide organification (Fraser *et al.*, 1960; Medeiros-Neto and Stanbury, 1994; Kopp, 1999). Organification of iodide by the thyroid is determined by the perchlorate test (Baschieri *et al.*, 1963). The test is based on the fact that iodide is transported into thyroid cells by NIS, and then released into the follicular lumen by anion channels, including pendrin, before it is rapidly organified on tyrosyl residues of TG by TPO (Figure 24.1) (Kopp, 2005; Baschieri *et al.*, 1963). Anions such as perchlorate (ClO_4^-) inhibit NIS, and any intrathyroidal iodide that has not been incorporated into TG is rapidly released into the bloodstream at the basolateral membrane and cannot be transported back into thyrocytes. In the standard perchlorate test, the thyroidal counts are measured at frequent intervals after the administration of radioiodine, in order to determine the uptake into the thyroid gland. One hour later, 1 g of $KClO_4$ or $NaClO_4$ is administered and the amount of intrathyroidal radioiodine is monitored longitudinally. In individuals with normal iodide organification, there is no decrease in intrathyroidal counts, because the iodide has been incorporated into TG (Figure 24.2). In contrast, a loss of $\geq 10\%$ indicates an organification defect. Common causes include thyroiditis or congenital defects with abnormal efflux of iodide into the follicular lumen, such as Pendred's syndrome or dysfunction of TPO (Kopp, 2002). In the case of a complete organification defect, such as in patients with complete inactivation of TPO, there is

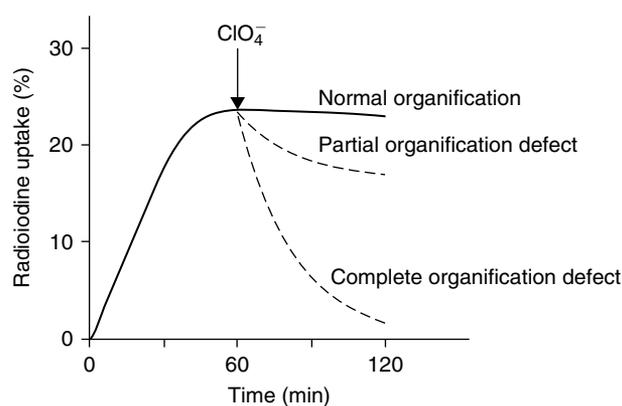


Figure 24.2 Perchlorate test. After iodide administration, the uptake in the thyroid is measured longitudinally. In individuals with normal iodide organification, there is no decrease in intrathyroidal counts after administration of perchlorate, a competitive inhibitor of NIS, because the iodide has been organified. A decrease of $\geq 10\%$ indicates an organification defect. The organification defect can be partial, e.g., in patients with Pendred's syndrome, or complete, e.g., in individuals with complete loss-of-function mutations of TPO.

no meaningful organification and the tracer is completely released from the gland (Figure 24.2). In the situation of a partial organification defect, such as in Pendred's syndrome or partially inactivating TPO mutations, the fraction of the tracer that has not been incorporated is released from the gland (Figure 24.2).

Clinical Presentation The obligatory clinical finding in patients with Pendred's syndrome is sensorineural hearing impairment. In most individuals, the deafness is prelingual and profound; more rarely the hearing impairment develops later in childhood and, in these instances, it is typically progressive (Reardon *et al.*, 1997; Cremers *et al.*, 1998). Patients with Pendred's syndrome have an enlargement of the endolymphatic system, which is most readily detected by documenting an enlarged vestibular aqueduct (EVA) (Phelps *et al.*, 1998; Fugazzola *et al.*, 2000; Pryor *et al.*, 2005). However, EVA can have multiple other causes (Pryor *et al.*, 2005). Pendred's syndrome is caused by biallelic mutations in the *SLC26A4* (solute carrier family 26A4) gene, which encodes pendrin. Patients with nonsyndromic EVA (deafness, no goiter) associated with *SLC26A4* mutations have, however, a positive perchlorate test (Usami *et al.*, 1999; Pryor *et al.*, 2005). Some patients with Pendred's syndrome also have a so-called Mondini defect, an unspecific malformation in which the cochlear turns are replaced by a rudimentary cochlea or a single cavity (Hartley and Phelps, 1997; Reardon *et al.*, 1997; Phelps *et al.*, 1998).

The thyroid enlargement in Pendred's syndrome typically develops during childhood, but there is substantial intrafamilial and regional variation (Fraser *et al.*, 1960;

Nilsson *et al.*, 1964; Fraser, 1965; Medeiros-Neto and Stanbury, 1994). The prevalence of goiters appears to be lower in patients living in iodine-replete regions, suggesting that nutritional iodide intake is a significant modifier of the phenotype (Gausden *et al.*, 1997; Sato *et al.*, 2001). At least, under conditions of adequate iodide intake, most individuals with Pendred's syndrome are clinically and biochemically euthyroid (Trotter, 1960; Nilsson *et al.*, 1964; Fraser, 1965; Medeiros-Neto and Stanbury, 1994). However, if nutritional iodide is scarce, patients with Pendred's syndrome may present with mild or overt hypothyroidism (Kopp *et al.*, 1999; Gonzalez Trevino *et al.*, 2001). Mice with targeted disruption of the *Slc26a4* gene (see below) do not display an enlarged thyroid or abnormal thyroid hormone levels (Everett *et al.*, 2001), but it remains unknown whether they are prone to develop a thyroidal phenotype under conditions of iodine deficiency. Taken together, the observations in patients and in the murine knockout model suggest that iodide can reach the follicular lumen independently of pendrin, either through a second iodide-transporting channel (Golstein *et al.*, 1992), or through unspecific (chloride) channels.

Pendred's syndrome may account for about 10% of all cases with syndromic deafness (Reardon *et al.*, 1997; Campbell *et al.*, 2001). It is caused by biallelic (homozygous or compound heterozygous) mutations in the *SLC26A4* gene. Biallelic *SLC26A4* gene mutations are also found in deaf individuals with familial EVA who have no goiter; they do, however, have an abnormal perchlorate test (Pryor *et al.*, 2005). *SLC26A4* gene mutations may be one of the most common causes of prelingual or progressive genetic hearing loss.

Table 24.1 Selected members of the Solute Carrier Family 26A

Gene symbol	Protein	Major expression	Anion transport	Chromosomal location	Human disease	OMIM#
SCL26A2 (DTD)	Diastrophic dysplasia	Ubiquitous	Sulfate Chloride Oxalate	5q32-q33.1	Diastrophic dysplasia Achondrogenesis IB Atelosteogenesis II	222600
SCL26A3 (DRA, CLD)	Down-regulated in adenoma	Ileum Colon Seminal vesicle	Sulfate Chloride Bicarbonate Hydroxide Oxalate	7q21-31	Congenital chloride diarrhea	214700
SCL26A4 (PDS)	Pendrin	Inner ear Thyroid Kidney	Chloride Bicarbonate Hydroxide Formate Iodide	7q21-31	Pendred's syndrome Familial enlarged vestibular aqueduct with positive perchlorate test without goiter	274600
SCL26A5 (PRES)	Prestin	Outer hair cells	Motor protein	7q21-31	Autosomal recessive deafness DFNB61	604943

Note: Comparison of expression pattern, function, chromosomal location and associated disorders of several members of the SLC26A family, which is also referred to as "Multifunctional SLC Family".

The PDS/SLC26A4 Gene After the linking of Pendred's syndrome to chromosome 7q22-31.1 by several groups (Sheffield *et al.*, 1996; Coyle *et al.*, 1996), the *SLC26A4* gene, originally referred to as *PDS* gene, was cloned in 1997 (Everett *et al.*, 1997). The SLC26A family contains several transporters of sulfate or other anions (Table 24.1), as well as a motor protein found in outer hair cells, prestin (SLC26A5) (Zheng *et al.*, 2000; Oliver *et al.*, 2001). The *SLC26A4* gene consists of 21 exons and is located in close vicinity to two other family members with a very similar genomic structure, prestin and DRA, an observation suggesting that they have evolved from a common ancestral gene (Everett *et al.*, 1997).

Pendrin Protein Structure Pendrin, the protein encoded by the *SLC26A4* gene, is a highly hydrophobic membrane protein consisting of 780 amino acids (Everett *et al.*, 1997). It is thought to have 12 transmembrane domains (Royaux *et al.*, 2000), and both the amino- and carboxyterminus are located intracellularly (Gillam *et al.*, 2004; Royaux *et al.*, 2000). In its intracellular carboxyterminus, pendrin contains a so-called sulfate transporter and antisigma factor antagonist (STAS) domain (Aravind and Koonin, 2001). It has been suggested that the STAS domain of SLC26 members, including pendrin, can interact with the regulatory domain of cystic fibrosis conductance regulator (CFTR) in certain epithelia (Ko *et al.*, 2002, 2004; Shcheynikov *et al.*, 2006).

Function of Pendrin in Thyroid Cells First insights into the functional role of pendrin were obtained in

Xenopus oocytes by demonstrating that it is able to mediate uptake of chloride and iodide (Scott *et al.*, 1999) and act as a chloride/formate exchanger (Scott and Karniski, 2000). The demonstration of pendrin-mediated iodide transport, the impaired iodide organification found in patients with Pendred's syndrome, and pendrin's localization at the apical membrane of thyrocytes (Royaux *et al.*, 2000; Bidart *et al.*, 2000) suggested that pendrin could mediate iodide transport into the follicular lumen (Figure 24.1) (Everett and Green, 1999).

The question whether pendrin is indeed involved in mediating apical iodide transport was addressed in a heterologous polarized cell system in order to *mimic* the situation found in thyrocytes (Gillam *et al.*, 2004). NIS and pendrin were introduced independently or simultaneously into polarized Madin-Darby canine kidney (MDCK) cells cultured in a bicameral system (Figure 24.3). The intracellular uptake of iodide added to the lower compartment, and the transport into the apical compartment, were then determined in the various experimental groups. Cells stably transfected with NIS showed a significant increase in intracellular iodide uptake compared to untransfected MDCK cells (Figure 24.3, left panel). The release into the apical chamber was higher than that in untransfected MDCK cells, most likely due to nonspecific transport across the apical membrane (Figure 24.3, right panel). In contrast, cells expressing NIS and pendrin showed significant iodide transport into the apical chamber, and, as a consequence of this vectorial transport, there was a significant drop in intracellular iodide content to levels observed in untransfected MDCK cells. Cells expressing only pendrin did

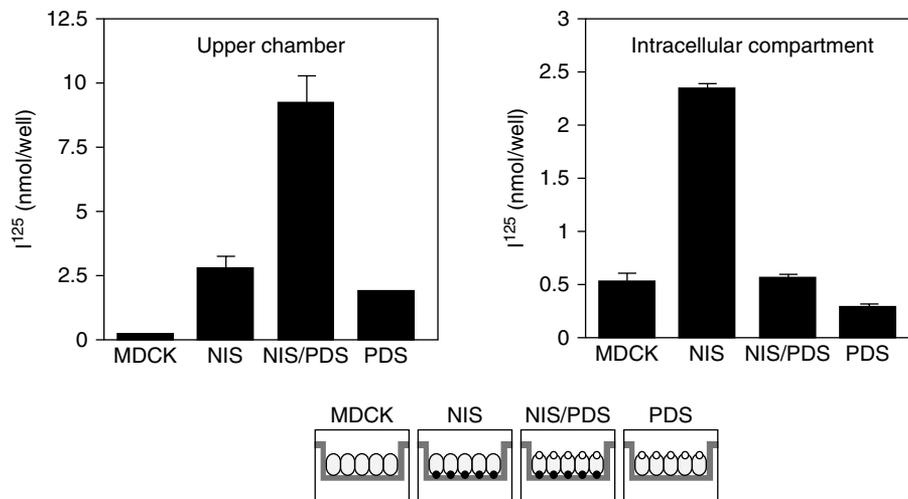


Figure 24.3 Iodide transport in polarized cells expressing NIS and pendrin. Polarized MDCK cells grown in a bicameral system expressing NIS, NIS and pendrin (PDS), or pendrin were exposed to radiolabeled iodide in the lower chamber. In cells expressing NIS, there is a significant increase in intracellular iodide. In contrast, the intracellular iodide is lower in cells expressing NIS and pendrin, because of an increased transport into the apical compartment. For further details see text. (Modified from Gillam *et al.* (2004) with permission from the Endocrine Society).

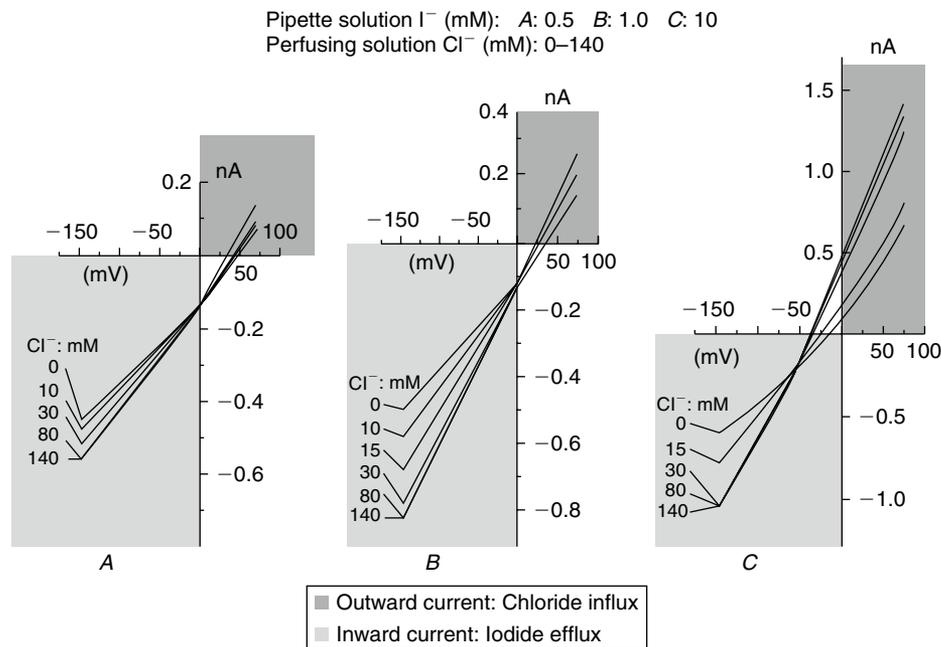


Figure 24.4 Electrophysiology experiment demonstrating pendrin-mediated iodide efflux. The x-axis shows the membrane potential, the y-axis the current. The current/voltage relation was determined using ramp pulses with the patch clamp technique. Pendrin-transfected COS cells were patch clamped with a pipette containing iodide between 0.5 and 10 mM, and perfused with an extracellular solution containing 0–140 mM chloride. Under these conditions, the inward current indicates the efflux of iodide and the outward current indicates the influx of chloride. Iodide efflux was detectable at 0.5 mM iodide, indicating that iodide efflux mediated by pendrin occurs under physiological concentrations of cytoplasmic iodide. The currents increased with increasing chloride concentrations, suggesting that chloride activates iodide efflux. (Modified from Yoshida *et al.* (2004) with permission from the authors and the Endocrine Society).

show lower intracellular iodide levels than untransfected MDCK cells, but levels were higher in the upper chamber. Taken together, the results support the concept that pendrin is involved in vectorial iodide transport at the apical membrane in polarized cells (Figure 24.3).

These observations are consistent with experiments in nonpolarized cells (Yoshida *et al.*, 2002; Taylor *et al.*, 2002; Gillam *et al.*, 2004, 2005). Using Chinese hamster ovary cells (CHO) stably expressing NIS without or with pendrin, the iodide release was found to be significantly higher in the pendrin-containing cells. The iodide efflux was dependent on continuous iodide uptake in order to maintain a high intracellular concentration (Yoshida *et al.*, 2002). Similarly, cells transfected with NIS alone demonstrate a time-dependent iodide efflux, whereas cells expressing wild-type pendrin and NIS exhibit a very rapid efflux of iodide (Gillam *et al.*, 2004). Electrophysiological studies using transfected COS-7 cells demonstrate that pendrin mediates iodide efflux, and that the currents are higher with increasing chloride concentrations, suggesting that chloride activates iodide efflux (Figure 24.4) (Yoshida *et al.*, 2004). Moreover, these experiments indicate that *iodide efflux/chloride influx* is more efficient than *chloride efflux/chloride influx* (Yoshida *et al.*, 2004).

Functional Consequences of Pendrin Mutations Mutations in the *SLC26A4* gene are very diverse and more than 100 alterations have been reported (for a relatively complete synopsis see: <http://www.healthcare.uiowa.edu/labs/pendredandbor/slcMutations.htm>). Missense mutations are the most common defects; a smaller number consist of nonsense and intronic mutations (Kopp, 1999). Only a relatively small number of these mutations have been tested functionally (Taylor *et al.*, 2002; Gillam *et al.*, 2004, 2005; Dossena *et al.*, 2006). The mutants lose their ability to mediate iodide efflux in cells coexpressing NIS, as demonstrated by an intracellular iodide concentration that is identical or similar to cells expressing only NIS (Figure 24.5) (Taylor *et al.*, 2002; Gillam *et al.*, 2004, 2005), a finding that is also demonstrable in time course experiments (Gillam *et al.*, 2004). A few mutants of pendrin have also been characterized with a fluorometric method that allows monitoring of the intracellular halide content and demonstrates that the mutants display a reduced anion transporting capability (Dossena *et al.*, 2006). Mechanistically, the loss of function of many of these mutations is due to retention of the mutated protein in intracellular compartments, most likely the endoplasmic reticulum (Rotman-Pikielny *et al.*, 2002; Schnyder *et al.*, 2005).

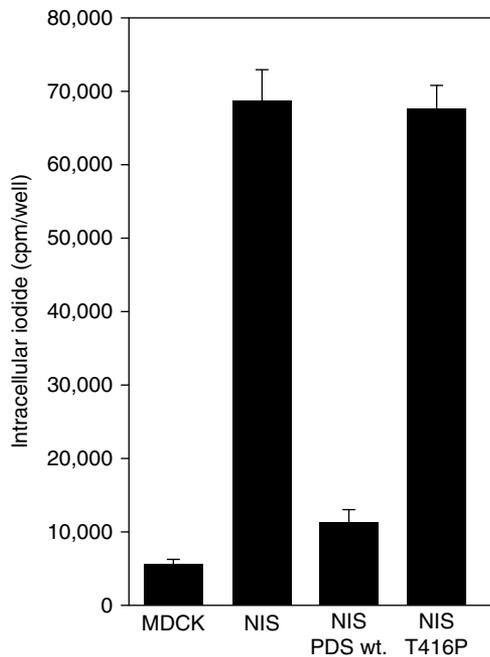


Figure 24.5 Functional consequence of a commonly found pendrin mutation on iodide efflux. Intracellular iodide content of cells expressing wild-type and mutant pendrin (PDS). In contrast to cells expressing NIS and wild-type pendrin, cotransfection of the pendrin mutant T416P does not result in a decrease in intracellular iodide accumulation in comparison to cells expressing NIS only. This indicates that the mutant loses the ability to mediate iodide efflux. Values are the means of triplicates \pm SE (Schnyder and Kopp, unpublished result).

Questions Concerning the Functional Role of Pendrin in Thyroid Cells

For a long time, the notion that iodide would simply cross the apical membrane because of the electrochemical gradient between the cytosol and the follicular lumen had been widespread (Wolff, 2005). However, autoradiography studies demonstrated that iodide first accumulates in the cytosol before it is transported into the follicle (Andros and Wollman, 1964). Moreover, apical iodide efflux is rapidly upregulated by thyroid-stimulating hormone (TSH) (Nilsson *et al.*, 1964, 1990). These observations, together with the impaired iodide organification in patients with Pendred's syndrome and studies performed with thyroid cell membrane vesicles (Golstein *et al.*, 1992), led to the concept that iodide crosses the apical membrane through an anion channel or transporter. The studies performed in polarized cells cultured in a bicameral system are consistent with the role of pendrin as an apical iodide transporter (Gillam *et al.*, 2004). However, it is also evident that individuals with biallelic mutations in the *SLC26A4* gene and a sufficiently high iodide intake may have no, or only a mild, thyroidal phenotype (Sato *et al.*, 2001). This indicates that iodide crosses the apical membrane independently of pendrin through another iodide channel or unspecific channels. This concept is

also supported by the absence of a thyroid phenotype in the *Slc26a4* knockout (Everett *et al.*, 2001), but, as mentioned, it is currently unknown whether these mice have an impaired iodide organification, and whether goiter development under conditions with scarce iodide intake would be more rapid compared to wild-type animals.

It has also been argued that the distinct proposed functional roles for pendrin in the thyroid, the kidney, and the inner ear (see below) are difficult to rationalize (Wolff, 2005). This led to the interesting hypothesis that pendrin might be a protein acting within a multiprotein complex that varies in its composition in different cell types, thereby leading to variable anion selectivity and regulation (Wolff, 2005).

Candidate Alternative Apical Iodide Transporters

As outlined above, iodide can cross the apical membrane independently of pendrin. It had been proposed that SLC5A8, a homolog of NIS, which was initially called human apical iodide transporter (hAIT), is also involved in apical iodide efflux (Rodriguez *et al.*, 2002). However, functional studies in our laboratory (Gillam and Kopp, unpublished), and functional studies by others in oocytes and polarized MDCK cells (Paroder *et al.*, 2006), did not show any evidence for iodide translocation by SLC5A8.

Interestingly, one group reported that the knockout of the chloride channel 5 (*ClCn5*) gene located on Xp11.22 leads to a thyroid phenotype that is reminiscent of Pendred's syndrome (van den Hove *et al.*, 2006). *ClCn5* is mutated in Dent's disease (Online Mendelian Inheritance in Man (OMIM 300009), an X-linked disorder characterized by hypercalcuric nephrolithiasis, proteinuria/aminoaciduria, glycosuria, and, in a subset of patients, hypophosphatemic rickets (Scheinman, 1998; Gambaro *et al.*, 2004). In the thyroid, *ClCn5* is also expressed at the apical membrane (van den Hove *et al.*, 2006). *ClCn5* Y/*CeCn5* Y⁻ mice have normal TSH and T₄ levels, but they show an accelerated goiter development, increased iodide uptake, and decreased organification of iodide into TG, compared to wild-type mice (van den Hove *et al.*, 2006). The mechanism of decreased iodide efflux and organification via *ClCn5* remains currently unclear (van den Hove *et al.*, 2006). It is conceivable that *ClCn5* might play a role, because the presence of chloride in the follicular lumen appears to be necessary for the efflux of iodide by pendrin (Yoshida *et al.*, 2004).

Regulation of Pendrin in Thyroid Cells

In contrast to many other thyroid-restricted genes, *SLC26A4* messenger RNA (mRNA) levels are not regulated by TSH in Fisher rat thyroid cell line 5 (*FRTL-5*) cells (Royaux *et al.*, 2000). It is, however, significantly upregulated by TG, the major component within the luminal space of the follicle, which forms the functional unit of the thyroid. This contrasts with the TG-mediated downregulation of other

thyroid-restricted genes such as the *TSHR*, *NIS* and *TPO*, *TG*, *PAX8*, *TTF1*, and *TTF2* (Suzuki *et al.*, 1999a–c; Royaux *et al.*, 2000; Suzuki and Kohn, 2006). Iodide on its own does not result in an alteration of *SLC26A4* gene expression (Suzuki and Kohn, 2006). Suppression of *NIS* expression by *TG* decreases iodide uptake *in vitro*, and the accumulation of *TG* in the follicular lumen correlates with low iodide uptake *in vivo* (Suzuki *et al.*, 1999c). The inverse relationship between the amount of follicular *TG* and the uptake of radioiodine *in vivo*, which is the consequence of differential gene regulation within thyroid follicular cells, could be of importance in the regulation of follicular function under conditions of constant TSH levels. Because of the significant upregulation of *SLC26A4* expression by *TG* it has been suggested that the regulation of its expression may be an important factor in determining the rate of iodine transport into follicular space at constant TSH levels (Suzuki and Kohn, 2006).

Similar to iodide uptake, iodide efflux is also stimulated by TSH. After treatment with TSH, iodide efflux increases rapidly in the poorly polarized *FRTL-5* cells (Weiss *et al.*, 1984), as well as in polarized porcine thyrocytes (Nilsson *et al.*, 1990; Nilsson *et al.*, 1992). In primary porcine thyrocytes grown in a transwell system, bidirectional measurements indicate that TSH stimulates iodide efflux at the apical membrane, while leaving efflux in the basal direction unchanged (Nilsson *et al.*, 1990). Hence, this rapid effect of TSH facilitates the vectorial transport of iodide into the follicular lumen. Exposure of rat PCCL3 cells to TSH leads to a rapid increase in pendrin protein abundance in the membrane (Pesce and Kopp, 2007). Whether this is associated with increased iodide efflux is currently unknown.

Pendrin in the Kidney

In addition to its expression in the thyroid and the inner ear, *SLC26A4* mRNA is readily found in the kidney (Everett *et al.*, 1997), in particular in the renal cortex, and nephron segment RT-polymerase chain reaction (PCR) led to the detection of positive signals in the cortical collecting duct (Soleimani *et al.*, 2001). Within the cortical collecting duct, pendrin localizes to the apical brush border membrane in type B, and in non-A/non-B intercalated cells (Royaux *et al.*, 2001; Soleimani *et al.*, 2001). Type B intercalated cells are secreting hydroxide, whereas type A intercalated cells are hydrogen-secreting cells (Wall, 2005). It remains unclear whether non-A/non-B intercalated cells interconvert between type A and type B cells.

Functional studies in transfected HEK-293 cells revealed that pendrin can function as an exchanger of chloride with bicarbonate, hydroxide, and formate (Soleimani *et al.*, 2001). Chloride/formate exchange has also been documented in pendrin-expressing oocytes (Scott and Karniski, 2000). Renal tubules isolated from alkali-loaded

wild-type mice secrete bicarbonate, whereas tubules from alkali-loaded *Slc26a4* $-/-$ mice fail to secrete bicarbonate (Royaux *et al.*, 2001). These results confirm that pendrin can function as an apical exchanger of chloride with bicarbonate in type B intercalated cells. Patients with Pendred's syndrome have no apparent abnormalities in acid–base metabolism, possibly due to the presence of other chloride–base exchangers, but detailed analyses under conditions of metabolic alkalosis have not been performed. In mice, pendrin is upregulated with aldosterone analogs and with chloride restriction (Verlander *et al.*, 2003, 2006). Remarkably, *Slc26a4* knockout mice do not develop weight gain and hypertension after treatment with deoxycorticosterone pivalate (DOCP), an aldosterone analog (Verlander *et al.*, 2003). In response to salt restriction, which leads to an increase in aldosterone, urinary volume and urinary excretion of chloride are greater in *Slc26a4* $-/-$ mice than in wild-type mice. Lastly, *Slc26a4* $-/-$ mice, have higher serum bicarbonate levels than wild-type mice, due to an impaired ability to excrete hydroxide equivalents in response to salt restriction or DOCP treatment. These observations suggest that *SLC26A4* contributes to the regulation of blood pressure and arterial pH through renal regulation of net acid and chloride excretion (Verlander *et al.*, 2003, 2006).

Pendrin in the Inner Ear

The most prominent and obligatory clinical sign in patients with Pendred's syndrome is sensorineural hearing impairment (Kopp, 1999). The majority, if not all, patients with deafness associated with mutations in the *SLC26A4* gene appear to have an enlargement of the endolymphatic sac and duct (Phelps *et al.*, 1998; Reardon *et al.*, 2000). The so-called Mondini cochlea, the replacement of the cochlea by a single cavity or a rudimentary structure, is a less frequently observed malformation (Phelps *et al.*, 1998; Fugazzola *et al.*, 2000; Gonzalez Trevino *et al.*, 2001). Mice with targeted disruption of the *Slc26a4* gene are completely deaf, and also display signs of vestibular dysfunction (Everett *et al.*, 2001). The inner ear appears to develop normally until embryonic day 15, but subsequently there is a progressive dilatation of the endolymphatic system, severe degeneration of sensory cells and malformation of otoconia and otoconial membranes (Everett *et al.*, 2001).

In the developing mouse, *Slc26a4* mRNA is predominantly detectable in the endolymphatic duct and sac, in areas of the utricle and saccule, and in the external cochlear sulcus region by *in situ* hybridization (Everett *et al.*, 1999). This expression pattern involves several regions that are important for endolymphatic fluid resorption. More recent studies reveal that *Slc26a4* $-/-$ mice lack the endocochlear potential, which is normally generated by the basolateral potassium channel KCNJ10 located in intermediate cells, because they do not express this channel

(Wangemann *et al.*, 2004, 2007). In addition, the endolymphatic calcium concentration is elevated in the *Slc26a4*^{-/-} mouse through inactivation of two apical calcium channels (TRPV5 and TRPV6) (Nakaya *et al.*, 2007).

Pendrin in Other Tissues

In addition to its expression in the inner ear, the thyroid, and the kidney, pendrin expression has been reported in several other tissues.

In the placenta, immunohistochemical analysis suggested that the NIS protein is present on the entire membrane of the cytotrophoblast, whereas pendrin is mainly located at the brush border membrane of syncytiotrophoblast cells facing the maternal side (Bidart *et al.*, 2000). The functional significance of these two anion transporters in the placenta is currently unknown.

Iodide is secreted into the milk and the concentrations are significantly higher than the levels found in maternal plasma (Delange *et al.*, 1986). The concentration of iodide in alveolar epithelial cells occurs through NIS, which is expressed at the basolateral membrane (Dohan *et al.*, 2003). Pendrin expression has also been documented in the lactating mammary gland using immunoblotting (Rillema and Hill, 2003), but its subcellular location and physiological significance have not been addressed in further detail. It is also unknown whether patients with Pendred's syndrome or *Slc26a4*^{-/-} mice have an impaired secretion of iodide into the milk.

Very low levels of *PDS* mRNA expression have been reported in tissues such as lung, endometrium, prostate, and testis (Lacroix *et al.*, 2004). Pendrin protein expression has also been documented in Sertoli cells and the lung (Lacroix *et al.*, 2004; Pedemonte *et al.*, 2007). It is currently unknown whether pendrin has a physiological role in these tissues. In the lung, pendrin protein expression is upregulated by interleukin 4 (IL-4) and it has been proposed to play a role in the transport of thiocyanate (Pedemonte *et al.*, 2007).

Summary Points

- Pendred's syndrome is an autosomal recessive disorder characterized by sensorineural deafness, goiter, and impaired iodide organification.
- Pendred's syndrome is caused by biallelic mutations in the anion transporter *SLC26A4* gene, originally referred to as *PDS* gene, which encodes pendrin.
- Pendrin is an exchanger of chloride and bicarbonate, and it is involved in apical efflux of iodide in thyroid follicular cells.
- In addition to pendrin, at least one other apical iodide channel can mediate apical iodide efflux in thyroid follicular cells.
- Pendrin is expressed in type B intercalated cells in the renal collecting duct and involved in chloride/bicarbonate exchange. Pendrin knockout mice have a decreased rise in blood pressure in response to a high salt diet or treatment with aldosterone analogs.
- In the inner ear, pendrin is essential for generation of the endocochlear potential. In the absence of functional pendrin, the endolymphatic system undergoes a progressive enlargement that results in severe degeneration of sensory cells and otoconia.

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Autoregulation of Thyroid Growth and Function by Iodine: Independent Regulation of the Thyroid Gland by Iodocompounds

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Abstract

The daily intake of iodine varies throughout the world from about 50 µg to about 1 mg per day, and the thyroid function remain normal without changes in circulating TSH, if there is no underlying thyroid disease. This might be explained by the fact of that iodide can modulate its own organification, and also influence growth and apoptosis of thyroid cells. It has been shown that iodide is not only the essential substrate for thyroid hormone synthesis, but it is also incorporated into thyroid cell membrane lipids. These iodolipids are able to modulate the action of TSH and growth factors on thyroid cell function and growth. Therefore, the thyroid gland regulates its own function and growth depending on the availability of iodine and the formation of iodolipids. So far, the best characterized iodocompounds are 2-iodohexadecanal (2-IHDA) and the δ-iodolactones. 2-IHDA inhibits adenylate-cyclase activity and NADPH-dependent H₂O₂ generation, and therefore cAMP-dependent iodine uptake and organification. The family of iodolactones are also well-characterized, they are mainly responsible for the regulation of growth; δ-iodolactone also induces apoptosis of porcine and human thyroid cells *in vitro*, and might be responsible for goiter involution.

Abbreviations

2-IHDA	2-Iodohexadecanal
cAMP	Cyclic adenosine monophosphate
δ-iodolactone	6-Iodo-5-hydroxy-8,11, 14-eicosatrienoic acid δ-lactone
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
GC-MS	Gas chromatography-mass spectrometry
IP3	Inositol-1,4,5-triphosphate

MMI	Methimazole
NADH	Nicotinamide adenine dinucleotide hydride
NIS	Sodium iodine symporter
PTU	Propylthiouracil
RNA	Ribonucleic acid
TGFβ	Transforming growth factor
TSH	Thyroid-stimulating hormone

Introduction

The main cause of endemic goiter is iodine deficiency, and thyroid growth is presumed to be regulated by thyroid-stimulating hormone (TSH). However, in slightly iodine deficient areas TSH levels are found to be normal (Pisarev *et al.*, 1970) or even decreased compared to areas with normal iodine intake (Gutekunst *et al.*, 1986). It had been suggested that the iodine-deficient thyroid is more sensitive to TSH, as shown by Bray (1968) in rats. This hypothesis however, has been re-evaluated, not only by measuring thyroid weight and volume, but also by measuring the deoxyribonucleic acid (DNA) content per volume (Stübner *et al.*, 1987), allowing the differentiation between hyperplasia and hypertrophy of the thyroid. It could be demonstrated that thyroid volume increases with decreasing iodine content of the gland, but proliferation, measured by DNA content of the thyroid, only occurs if the iodine concentration within the thyroid is below 0.15 µg/mg of thyroid tissue. If the iodine content is above this threshold, the thyroid weight increases, but the DNA content remains constant. This can be explained by the fact that during iodine depletion, the first event is hypertrophy of the cells, followed by hyperplasia when the iodine content falls below the threshold of 0.15 µg/mg thyroid tissue. Hyperplasia of the thyroid tissue exponentially increases below this threshold, even if TSH is within the normal range. Plasma TSH levels correlate directly with the thyroid weight, indicating that hypertrophy of the gland is directly dependent

on, TSH stimulation, but not on the proliferation of thyroid cells. Therefore, a direct influence of the iodine content of the thyroid cells on proliferation mediated by iodocompounds has to be postulated, which might be more important than TSH in the regulation of hyperplasia (Stübner *et al.*, 1987).

One of the acute effects of excess iodide is to impair iodide organification (Wolff–Chaikoff effect). However, since afterwards the iodide transport mechanism is also inhibited, the accumulation of putative iodinated compounds involved in this inhibition decreases and the gland escapes the Wolff–Chaikoff effect (Wolff, 1989). Additional inhibitory actions of excess iodide include hormone secretion, thyroid blood flow, glucose and amino acid transport, and protein and ribonucleic acid (RNA) biosynthesis (Pisarev and Gärtner, 2000). Since most of the inhibitory effects of iodide are reversed by thionamides like methimazole (MMI) and propylthiouracil (PTU), it was proposed that an organic iodocompound might be the intermediate in this autoregulatory mechanism.

Within the last two decades, iodocompounds that are generated depending on iodine supply have been identified and further examined, which could explain these autoregulatory mechanisms exerted by iodine (Figure 25.1).

Thyroid Iodolipids

Iodocompounds other than thyroid hormones were detected in thyroid homogenates, and iodolipids have been known to occur from radioiodine incorporation studies since the early 1950s (Taurog *et al.*, 1957). Their physiological role was unknown, but was suggested to be involved in thyroid autoregulation.

Specific compounds were identified when new highly sensitive methods, such as gas chromatography-mass

spectrometry (GC-MS) and nuclear magnetic resonance were used. When an exogenous fatty acid, such as arachidonic acid or docosahexaenoic acid, was supplemented, the formation of iodinated derivatives could be demonstrated in rat thyroid slices (Boeynaems and Hubbard, 1980). The main product of the conversion from arachidonic acid has been identified as 6-iodo-5-hydroxy-8,11,14-eicosatrienoic acid δ -lactone (δ -iodolactone, for formula see Figure 25.2) *in vitro* in porcine thyroid follicles, as well as *in vivo* in human thyroid tissue derived from patients treated with high doses of iodine before surgery (Dugrillon *et al.*, 1994).

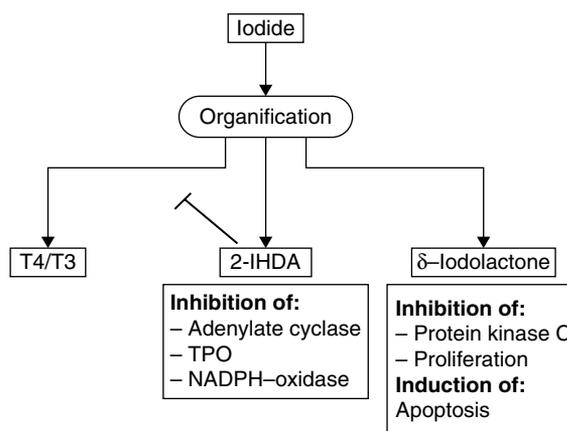


Figure 25.1 Iodinated compounds regulating thyroid function and growth. Besides thyroid hormones, two main iodocompounds are generated within the thyroid cells. 2-Iodoheptadecanal (2-IHDA) inhibits adenylate-cyclase activity and NADPH-dependent H_2O_2 generation. Δ -Iodolactone inhibits proliferation through inhibition of protein kinase C activity and induces apoptosis in thyroid cells.

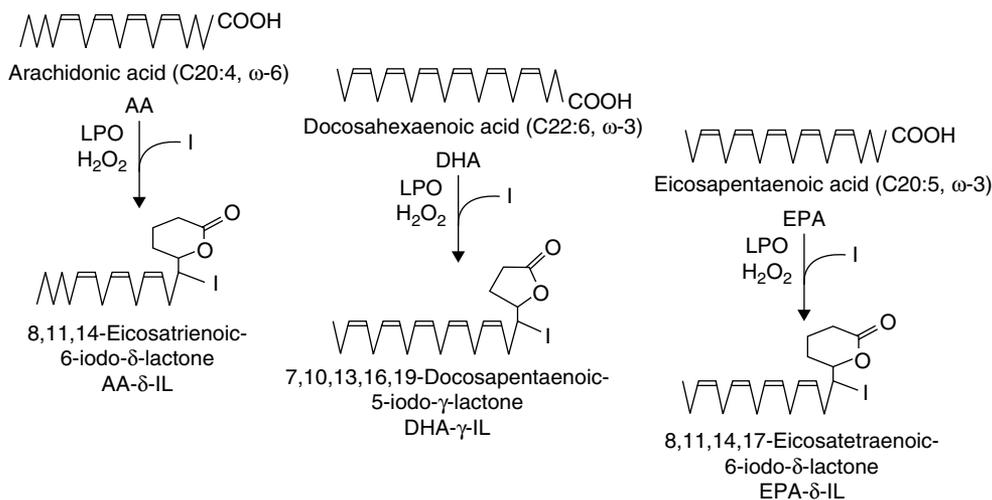


Figure 25.2 Formulas of iodolactones, derived from arachidonic acid, docosahexaenoic acid, and eicosapentaenoic acid. Adapted from Gärtner *et al.*, (1996).

According to Pereira *et al.* (1990) 2-iodohexadecanal (2-IHDA) could be identified as the major iodolipid in the horse thyroid gland. This major compound has probably been seen earlier as a polar nonphosphatide lipid by investigators using thin-layer chromatography (Taurog *et al.*, 1957). This compound is probably formed by the addition of iodine to the vinyl ether group of plasmalogens.

Iodolipid Effects: δ -Iodolactone

The synthesis of δ -iodolactone was first described by Boeynaems and Hubbard (1980) by incubating arachidonic acid with lactoperoxidase and H_2O_2 *in vitro*. The δ -iodolactone can then be purified by silica gel chromatography and characterized by GC-MS spectroscopy (Dugrillon *et al.*, 1990). The effect of δ -iodolactone has been further investigated in isolated porcine thyroid follicles *ex vivo*. Δ -Iodolactone has no effect on cyclic adenosine monophosphate (cAMP) formation in porcine thyroid follicles, and seems to be exclusively involved in cAMP-independent growth control. It could be shown that δ -iodolactone inhibited epidermal growth factor (EGF)-induced proliferation of isolated porcine follicles dose-dependently, without the requirement of further organification comparable to iodine, but in about 50-fold lower concentrations (Dugrillon and Gärtner, 1995). Also, in FRTL-5 cells δ -iodolactone inhibits thyroid cells proliferation (Pisarev *et al.*, 1992). Δ -Iodolactone inhibits inositol-1,4,5-triphosphate (IP3) formation induced by EGF, but not on TSH-induced IP3 formation, and its specificity was proven since γ -iodolactone had no effect on either cell proliferation or IP3 formation. These results demonstrate an action of δ -iodolactone at the calcium-dependent signal transduction mechanism modulating thyroid cell proliferation induced by EGF. Δ -Iodolactone acts in the porcine cells proximal to the generation of IP3, whereas the TSH-dependent signal transduction seems to be unaltered. Also, in human thyroid cells in culture, δ -iodolactone inhibited phorbol ester-stimulated cell proliferation, thus indicating that it has an inhibitory effect at the protein kinase C pathway (Dugrillon *et al.*, 1994). Therefore, it has been postulated as the specific inhibitory mediator of iodide on growth factor-induced thyroid cell proliferation (Figure 25.1).

In human thyroid homogenate, derived from a patient with Graves' disease, treated with high doses of iodine before surgery, δ -iodolactone could be isolated and characterized by GC-MS spectroscopy and was identical to that synthesized *in vitro*, and was shown to inhibit porcine thyroid cell proliferation induced by EGF (Dugrillon *et al.*, 1994). Iodolactones can also be generated from eicosapentaenoic acid and docosahexaenoic acid *in vitro* using the same method as δ -iodolactone. The δ -iodolactone derived from eicosapentaenoic acid is more active in growth inhibition than δ -iodolactone from arachidonic acid, but the

δ -iodolactone from docosahexaenoic acid was ineffective (Gärtner *et al.*, 1996) (Figure 25.3). This also indicates the specificity of different iodolactones, and might explain why fish and iodine consumption is important for maintaining normal thyroid volume.

During goitrogenesis, endothelial growth precedes the proliferation of thyroid cells. Thyroid follicles *ex vivo* also produce a paracrine endothelial growth factor (fibroblast growth factor, FGF-1) which stimulates fibroblast and endothelial growth. This is inhibited by both iodine and δ -iodolactone (Greil *et al.*, 1989). These findings explain the inhibitory effect of iodine on goitrogenesis, which obviously is also mediated by δ -iodolactone. In addition, transforming growth factor (TGF β 1)-expression on RNA and protein level is increased by iodine and δ -iodolactone (Gärtner *et al.*, 1997). As TGF β 1 inhibits growth of thyroid cells, this might be an additional factor inhibiting growth of thyroid cells by δ -iodolactone.

We could also recently show that both iodide and δ -iodolactone induce apoptosis, but no necrosis was demonstrated by electron microscopy in porcine thyroid follicles *ex vivo* in a three dimensional tissue culture (Langer *et al.*, 2003). The follicular structure remained normal; the apoptotic bodies were extruded out of the follicles. Apoptosis was already induced by 0.05 μ M of δ -iodolactone, whereas 20 μ M of iodide was necessary to obtain a comparable effect, which was inhibited by MMI. Withdrawal of TSH did not induce apoptosis and did not change the follicular structure. Interestingly, the induction of apoptosis was lowered by preincubating human thyroid follicles with low concentrations of selenium (10 and 100 nM), which induced glutathionperoxidase activity. This might explain that the induction of apoptosis is mediated by free oxygen radicals and the mitochondrial pathway (Lehmann *et al.*, 2006).

Recently, it has been shown that iodine, as well as δ -iodolactone, also inhibits the proliferation of mammary breast cancer cells (MCF-7) (Aceves *et al.*, 2005). These cells express sodium iodine symporter (NIS) and lactoperoxidase, and therefore exhibit comparable machinery to metabolize iodine to δ -iodolactones like thyroid cells. This is a new and exciting field, which might have implications for further examination into the role of iodine and δ -iodolactone in the treatment of breast cancer (Arroyo-Helguera *et al.*, 2006). In fact Japanese women, with their high daily iodine intake, have a five-fold lower incidence of breast cancer; breast cancer was treated in ancient years with high doses of iodine-containing algae.

Thus, δ -iodolactone seems to be generated within the thyroid cell membrane depending on iodine supply, and inhibits not only cAMP-independent growth, but also induces apoptosis and seems to be involved in goiter involution induced by iodine. The same δ -iodolactone might also inhibit mammary breast cancer cells, and therefore seems not to be unique to the thyroid, but to all iodine trapping tissues.

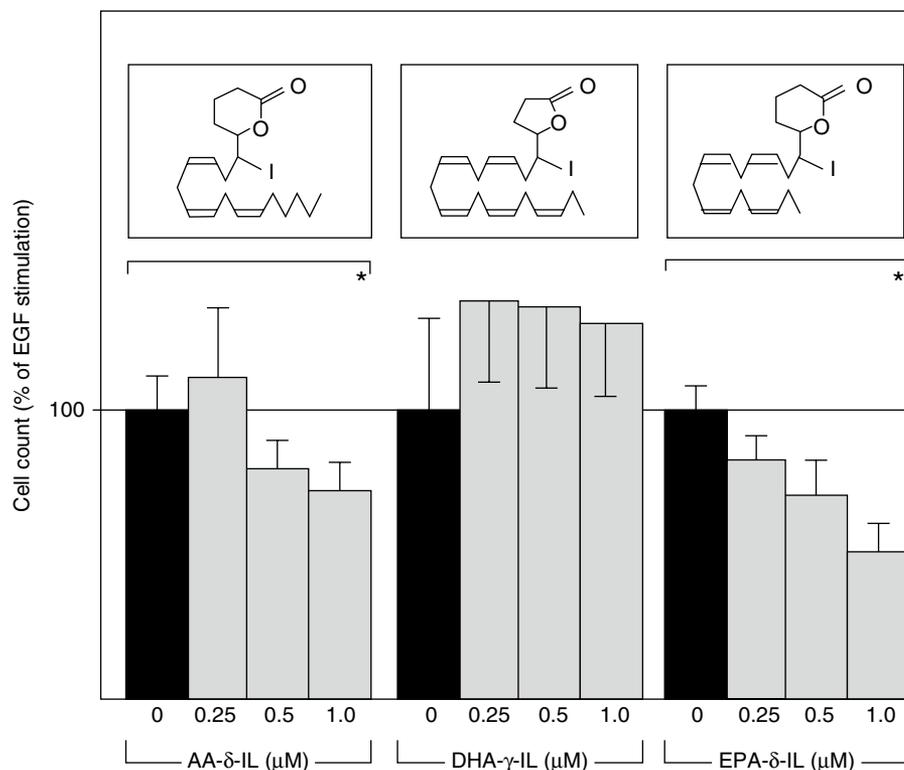


Figure 25.3 Different effects of iodolactones on EGF-induced growth of porcine thyroid follicular cells. The iodolactone derived from arachidonic acid is less effective than the iodolactone derived from eicosapentaenoic acid. The iodolactone from docosahexaenoic acid has no effect on cell proliferation. Adapted from Gärtner *et al.*, (1996).

Iodolipid Effects: 2-Iodoheptadecanal

2-IDHA, which has been identified as the major iodolipid in the horse thyroid *in vitro* (Pereira *et al.*, 1990), has been shown to inhibit NADPH-dependent H_2O_2 generation *in vitro* (Ohayon *et al.*, 1994), in dog thyroid cells (Panneels *et al.*, 1994b) and also to inhibit the activity of human adenylate-cyclase activity (Panneels *et al.*, 1994a) (Figure 25.1). Therefore, this compound may be responsible for the well-known Wolff–Chaikoff effect by inhibition of NADPH oxidase, as well as thyroid peroxidase. Further inhibitory effects, at higher concentrations of 2-IHDA, were observed on TSH-induced cAMP and on carbachol-induced IP3 formation. However, no growth suppressing effect could be demonstrated by measuring the 3H -thymidine incorporation into canine thyroid cells (Panneels *et al.*, 1994a, 1996).

Summary Points

- Iodine has a direct effect on thyroid function and growth, independent of pituitary TSH regulation *in vivo*, as well as *in vitro*. Because the action of iodine is inhibited by compounds inhibiting peroxidase activity such as MMI or PTU, and thyroid hormones have no direct effect on thyroid growth and function, other

iodinated compounds must be responsible for the direct iodine effect.

- Two iodolipid families have been shown to be mediators of the effects of iodine in thyroid autoregulation: iodinated derivatives of polyunsaturated fatty acids (iodolactones) and iodoaldehydes derived from plasmenylethanolamine.
- Δ -Iodolactone has been identified in human thyroid tissue pretreated with high doses of iodine, it has also been isolated from porcine thyroid follicles *ex vivo*. It inhibits proliferation in micromolar concentrations and also induces apoptosis, thus it might be responsible for autoregulatory growth and thyroid involution induced by iodine.
- Growth of mammary breast cancer cells, which also express the same NIS as thyroid cells, is inhibited by iodine and also δ -iodolactone. Thus, a sufficient iodine supply has an important role, not only for thyroid function and growth, but also for the mammary gland.
- 2-IHDA, inhibits nicotinamide adenine dinucleotide hydride (NADH)-dependent H_2O_2 generation *in vitro*, as well as *in vivo*. It also inhibits adenylate-cyclase activity, and therefore is supposed to mediate the well-known Wolff–Chaikoff effect.
- 2-IHDA also seems to be the iodinated compound which inhibits the well-known cAMP-dependent thyroid-specific

functions of the thyroid. It seems to be the iodinated compound which decreases thyroid-specific functions during high iodine supply.

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Is Iodine an Antioxidant and Antiproliferative Agent for the Mammary and Prostate Glands?

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Abstract

Iodine is a well-known micronutrient that is essential for the synthesis of thyroid hormones in all vertebrates, as well as a promoter of metamorphosis or transformation of life stages (pupa to larvae, larvae to adult, sessile to free life, etc.) in several invertebrates. This paper reviews the evidence showing iodine to be an antioxidant and antiproliferative agent that may contribute to the integrity of normal mammary and prostate glands. Seaweed is an important dietary component in Asian communities and a rich source of several chemical forms of iodine. The high consumption of this element (25 times more than in the Occident) correlates with a low incidence of benign and cancerous breast and prostate disease in the Japanese population. In animal and human studies, molecular iodine (I_2) supplements suppress the development and size of both benign and malignant neoplasias and significantly reduce cellular lipoperoxidation. Iodine, in addition to its incorporation into thyroid hormones, is bound in the thyroid to antiproliferative iodolipids called iodolactones, which may also play a role in the proliferative control of neoplastic growth in mammary and prostate glands. We propose that an I_2 supplement should be considered as an adjuvant in breast and prostate cancer therapy.

Abbreviations

6-IL	6-Iodolactone
DMBA	7,12-Dimethylbenz[α]anthracene
DHT	Dihydrotestosterone
LPO	Lactoperoxidase
MNU	<i>N</i> -methyl- <i>N</i> -nitrosourea
NIS	Sodium iodide symporter
PPAR	Peroxisome proliferator-activated receptor
ROS	Reactive oxygen species

T_3	Triiodothyronine
T_4	Thyroxine
TPO	Thyroperoxidase

Introduction

Iodine was first described by Courtois in 1811, after seaweed ashes treated with sulfuric acid produced a purple vapor that condensed into purple crystals, but it was not until 1927 that Sir Charles Harrington reported that the major thyroid hormone, thyroxine, contained covalently bound iodine (Emsley, 2001). Most of the investigations regarding the status of iodine in humans and animals have been focused on the role of iodine in thyroid function. Relatively little attention has been given to its extrathyroidal roles. Recently, some groups have postulated that iodide may have an ancestral antioxidant function in all iodide-concentrating cells, from primitive algae to more recent vertebrates (Cann *et al.*, 2000; Venturi, 2001; Smyth, 2003). In these cells, iodide acts as an electron donor in the presence of H_2O_2 and peroxidase; the resulting iodine atom is a free radical that readily iodates tyrosine, histidine and certain specific lipids. In fact, iodine can attach to double bonds of some polyunsaturated fatty acids in cellular membranes, making them less reactive with oxygen radicals (Cocchi and Venturi, 2000). Moreover, it has been demonstrated that iodine distribution in the organism depends on the chemical form of iodine ingested (Thrall and Bull, 1990), and that molecular iodine (I_2) is not reduced to iodide (I^-) before it is absorbed systemically from the gastrointestinal tract (Thrall *et al.*, 1992). Indeed, in iodine deficiency conditions, I^- appears to be more efficient than I_2 in restoring the thyroid gland to normal from a goitrous state, whereas I_2 is distinctly more effective in diminishing mammary dysplasia and atypia secondary to iodine deficiency (Eskin *et al.*, 1995; Ghent

et al., 1993; Kessler, 2004). In this paper, we review the different reports related to nonthyroidal iodine functions described in normal and neoplastic mammary gland, and present data that led us to propose that this protective effect could also be demonstrated in the prostate gland.

Mammary and Prostate Similarities

Mammary and prostate glands have many similarities with regard to appearance, physiology and pathology (Lopez-Otin and Diamandis, 1998). Both glands appeared at the same time during evolution, because when animals developed a breast, they became mammals and also developed a prostate. Both glands contain receptors for estrogen, androgen and progesterone. Reviews have pointed out that many prostate tissue markers, such as prostate-specific antigens, are also present in the breast, where they are also under androgenic regulation (Lopez-Otin and Diamandis, 1998; Coffey, 2001). In humans, the appearance of tumors and the age-adjusted incidence and mortality rates of cancer in the two glands appear to be correlated. The annual, age-adjusted death rates are almost identical at approximately 25 per 100000, and both glands also have a high incidence of benign disease and cancer, as well as early preneoplastic lesions. Both cancers metastasize to the bone and cause osteoblastic lesions. Both tumors require gonads for development and can be treated by hormonal manipulation. In addition, under normal conditions, both glands are capable of iodine uptake, and both decrease the machinery to internalize or generate iodine locally when they become pathological (Spitzweg *et al.*, 1998; Wapnir *et al.*, 2003; Anguiano *et al.*, 2006).

Iodine in Normal Tissues

A large body of data has demonstrated that several tissues share with the thyroid gland the capacity to actively accumulate iodide, including salivary glands, gastric mucosa, lactating mammary gland, the choroid plexus, ciliary body of the eye, lacrimal gland, thymus, skin, placenta, ovary, uterus, prostate and so on, and may either maintain or lose this ability under pathological conditions. The iodide transport system in these extrathyroidal tissues reveals several functional similarities to its thyroid counterpart, such as inhibition by thiocyanate and perchlorate (KClO_4^-), suggesting the presence of the specific iodine transporter called the sodium iodide symporter (NIS). However, only some of these organs express the enzymatic machinery to oxidize I^- to I_2 , which is bound to cell components and exhibits physiological effects. Data from our laboratory demonstrate that several tissues from the reproductive tract such as prostate and epididymis, are also able to take up and generate iodine (Anguiano *et al.*, 2008). As shown

in Figure 26.1, thyroid, mammary gland and prostate can accumulate both types of iodine; it also shows that thyroid, lactating mammary and prostate glands exhibit a significant uptake of I^- , which is internalized by NIS (inhibited by KClO_4^-). In thyroid and lactating mammary gland, I_2 uptake is three times less than thyroid, and only about half of this I_2 capture is inhibited by KClO_4^- . In contrast, in nubile animals, mammary tissue and prostate captured 300 times less iodine than thyroid and four times less than lactating mammary gland, and NIS does not participate in their internalization. These findings strongly support previous data showing that this chemical form of iodine contributes to the maintenance of the normal integrity of the mammary gland. Eskin *et al.* (1995) showed that iodine deficiency alters the structure and function of the mammary gland of virgin rats, and that I_2 is effective in diminishing ductal hyperplasia and perilobular fibrosis secondary to this iodine deficiency. Similarly, I_2 treatment of patients with benign breast disease is accompanied by a significant bilateral reduction in breast size, in addition to causing a remission of disease symptoms, which is not observed when I^- or protein-bound iodide is administered (Ghent *et al.*, 1993; Kessler, 2004). Detailed descriptions of these trials can be found in Chapter 82 of this book. The low incidence of benign and malignant breast and prostate disease in the Japanese population has been associated with seaweeds, which are widely consumed in Asian countries and contain high quantities of iodine in several chemical forms, i.e., I^- , I_2 , iodate (IO_3^-) and protein-bound iodine; average iodine consumption in the Japanese population is $5280\mu\text{g}/\text{day}$ versus 166 and $209\mu\text{g}/\text{day}$ in the UK and USA, respectively (Kupper *et al.*, 1998; Hou *et al.*, 1997; Teas *et al.*, 2004). The importance of I_2 as an oxidized chemical form of iodine agrees with our recent demonstration (Alfaro-Hernandez, 2004) that the addition of I_2 , but not potassium iodide (KI), to mammary gland homogenates from virgin rats significantly decreases lipoperoxidation measured by the thiobarbituric acid reaction and expressed as malondialdehyde. The inability of I^- to decrease lipoperoxidation may be explained by the absence of lactoperoxidase (LPO) in mammary glands from virgin rats, which is only present during pregnancy and lactation (Strum, 1978). LPO is a homolog of thyroperoxidase (TPO); both enzymes are able to oxidize I^- in order to bind iodine covalently to proteins or lipids. A specific iodination species generated from LPO activity has not yet been identified, but several candidates exist, such as I_2 , I^+ (iodinium ion), $(\text{I}^0)\text{I}\cdot$ (iodine free radical), and IO^- (hypoiodite) (Smyth, 2003). Another possible source of oxidized iodine is the deiodination of thyroid hormones. Mammary gland expresses two different deiodinase enzymes that locally convert the prohormone thyroxine (T_4) into the active thyroid hormone, triiodothyronine (T_3). This conversion results in variable intracellular concentrations of free iodine: higher levels during puberty,

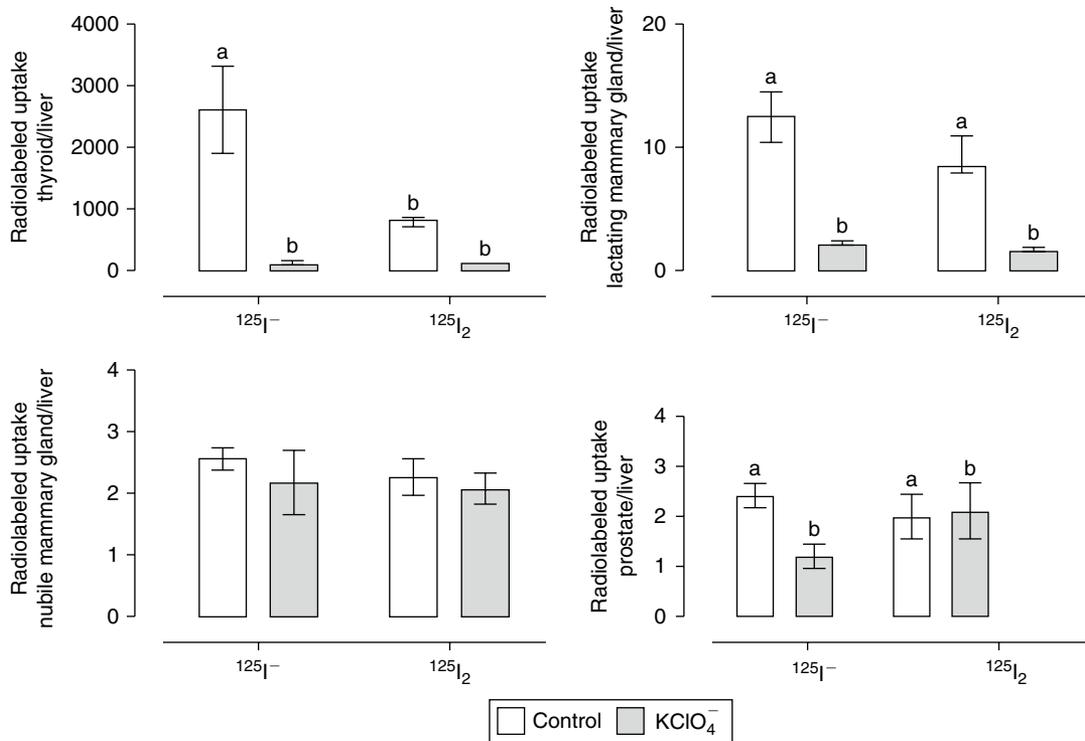


Figure 26.1 Effect of perchlorate (KClO_4^-) on $^{125}\text{I}^-$ and $^{125}\text{I}_2$ uptake. Rats were i.p. injected with KClO_4^- (20 mg/kg); 2 h later $50\ \mu\text{Ci}$ of either $^{125}\text{I}^-$ or $^{125}\text{I}_2$ was injected, and the animals were sacrificed 1.0 h after the radioactivity administration. Data were normalized as radioactivity uptake (cpm) compared to liver (nonuptake organ) and expressed as cpm tissue/cpm liver. Thyroids were obtained from female and male rats. Results are expressed as the mean \pm SD, $n = 7$ rats/group. Data were analyzed with one-way ANOVA, and differences between means were evaluated by the Tuckey test. Different letters indicate significant differences between groups ($p < 0.05$).

pregnancy and lactation mediated by deiodinase type 1, and lower but relatively constant levels in virgin or postpartum conditions catalyzed by deiodinase type 2 (Aceves *et al.*, 1995). Although the chemical form of iodine that results from deiodination has not been determined, it possibly corresponds to a different and perhaps more reactive form than I^- . Thus, we hypothesize that iodine generated by LPO activity is bound to an abundant and specific protein (e.g., thyroglobulin in the thyroid and casein in lactating mammary gland), whereas I_2 or another oxidized form of iodine, obtained by deiodination or in the diet, binds to lipids and/or other membrane or nuclear components, and acts as an antioxidant and/or antiproliferative agent (Aceves *et al.*, 2005). This notion is supported by our finding that in the tumoral mammary cell line MCF-7, I_2 but not I^- supplement, is accompanied by antiproliferative effects and the appearance of iodinated proteins and lipids (Arroyo-Helguera *et al.*, 2006).

Reproductive Factors and Cancer Risk

Epidemiological studies indicate sexual reproductive history as the most important risk factor for breast cancer.

Early age at menarche, late age at menopause and nulliparity increase the risk of a woman for developing breast cancer. Conversely, late age at menarche, early age at menopause and early age at first pregnancy decrease this risk (Seidman *et al.*, 1982). In this regard, Wynne-Edwards (2000) postulated that the modern increased risk in breast cancer is the result of recent cultural and reproductive changes in women's lives. These include: (a) an increased exposure to endogenous estrogen, which is a weak carcinogen (via early menarche, low parity, abbreviated breast feeding and pharmaceutical hormone manipulation) and (b) an increased risk of breast cancer by altering the proportion of our lives spent in developmental stages of breast tissue with a high underlying rate of mitotic cell division (cells of breast tissue that has never undergone differentiation to produce milk divide 20 times more often than cells that have acquired the terminal phenotype) (Helzlsouer and Couzi, 1995; Pihan and Doxsey, 1999). Table 26.1 summarizes, with an evolutionary perspective, the primordial changes that occur in the modern Western woman in comparison with the Aboriginal population and the hypothetical natural ancestral woman. Except for the high ovulation number, each of the other demographic variables is a known risk factor for breast cancer.

The role of the effects of iodine and estrogen has not been analyzed, but it has been observed that during pregnancy and lactation, hormonal stimulation of the mammary gland leads to glandular differentiation, dramatically enhancing both iodide absorption and local generation of free iodine by deiodination (Aceves *et al.*, 1995; Shah *et al.*, 1986). A high iodine concentration in breast tissue may also explain the reduction in modularity and tissue density that are often observed following pregnancy and lactation (Haagensen, 1971). Thus, a link may exist between elevated breast iodine content during pregnancy/lactation and the subsequent reduction in breast cancer risk. Reproductive periods may protect against breast cancer, given the lobuloalveolar differentiation present during these stages (Russo and Russo, 1997). Cann *et al.* (2000) have proposed that increased iodine content may also play a pivotal role in this differentiation process. For example, most studies in Asia have found lactation to help prevent subsequent development of breast cancer in both pre- and postmenopausal women (Yang *et al.*, 1977). In contrast, studies conducted in North America and Europe have generally shown breastfeeding to be protective only in premenopausal women (Newcomb *et al.*, 1994), or not at all (Michels *et al.*, 1996). Thus, recent cultural and reproductive changes and/or the different levels of iodine intake may be responsible for these differences.

Analyses of these parameters in prostate cancer are again scarce. It is well-established that age and hormonal environment exert dramatic effects on the morphology and function of the prostate gland. It was observed that estrogen shows a marked synergy with androgen, inducing a greater than four-fold increase in total prostate weight and DNA content. This enhancement of prostate growth requires the specific combination of estrogen with a 5 α -reduced steroid, such as dihydrotestosterone (DHT). In contrast, testosterone plus estrogen does not enhance canine prostate growth beyond that of the normal prostate. Treating a castrated dog only with estrogen causes the basal cells to develop into squamous cell metaplasia;

however, if androgens are added to the estrogen, these estrogenic effects are suppressed, resulting in either normal glandular growth with testosterone or glandular hyperplasia with DHT. It is believed that the changing estrogen–androgen ratio that occurs with aging may promote abnormal growth of the prostate (Coffey and Walsh, 1990). In addition, a recent study of several sexual factors related to prostate cancer showed that a reduced ejaculatory output in otherwise normal males is associated with an increased risk of prostate cancer, especially if this commences in early adulthood (Giles *et al.*, 2003). Again, in this gland the increase in life span and the modern lifestyle that delay sexual activity could account for the increase in cancer. All of these findings agree with recent data from our group that adult prostate tissue expresses deiodinase type 1, which may generate high concentrations of T3 and free iodine, and that this enzyme is stimulated by sex hormones and prolactin (Figure 26.2), as well as by thyroid hormones (Anguiano *et al.*, 2006). In addition, preliminary data suggests that this activity almost disappears in old animals, but if sexual activity continues deiodinase type I activity remains at levels characteristic of young males (López-Juárez *et al.*, 2004).

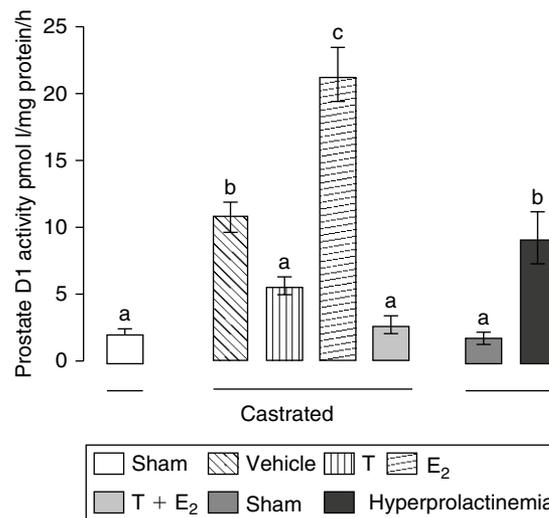


Figure 26.2 Effects of castration and hormone replacement or hyperprolactinemia on prostate D1 activity. For sex hormone replacement, rats were bilaterally castrated via the scrotal route and treated with supraphysiological doses of testosterone (1.0mg) and/or estrogen (20 μ g). Hormones were administered by slow delivery in oil via s.c. Hyperprolactinemia was induced by implanting one pituitary under the kidney capsule of animals whose pituitary remained intact. D1 activity was measured by the radiolabeled iodide release method ($n = 5$ rats/group). Data were analyzed with one-way ANOVA, and differences between means were evaluated by the Tuckey test. Different letters indicate significant differences between groups, $p < 0.05$. D1, type 1 deiodinase; T, testosterone; E₂, 17 β -estradiol. Data adapted from Anguiano *et al.*, (2006).

Table 26.1 Evolutive etiology of woman's reproductive behavior

Primordial changes	Ancestral	Aborigines ^a	Modern
Menarche (age)	16	16.1	12.1
First partum (age)	17	19.5	24
Lapse between menarche/ first partum (years)	1	3.4	11
Child	10	5.9	1.8
Number of ovulations in 50 years	80	160	450

Source: Data adapted from Wynne-Edwards (2000).

^aEstimates from Kung, Aché, Agta, Inuit, Aborigine Hazda, Hiwi, and Efe women, which are also populations with significant low or null mammary cancer incidence.

Iodine in Neoplastic Glands

In rat mammary carcinomas induced by 7,12 dimethylbenz[α]anthracene (DMBA), supplementation with Lugol's solution (mixture of I^- and I_2) exerts a suppressive effect on the development and size of the neoplasias (Kato *et al.*, 1994). This suppressive effect is enhanced when the Lugol treatment is combined with progesterone (medroxyprogesterone acetate). The suppressed tumors were found to have significantly higher iodine content than unsuppressed tumors, with uptake was apparently enhanced by progesterone (Funahashi *et al.*, 1996). The enhancement of iodine uptake by progesterone has been observed in other hormone-dependent tissues, including the uterus and the ovary (Brown-Grant and Rogers, 1972). Data generated in our laboratory (García-Solís *et al.*, 2005) have shown that chronic administration of I_2 exhibits a potent protective effect (70%) against mammary cancer induced by the carcinogen *N*-methyl-*N*-nitrosourea (MNU). This effect is exerted only by I_2 , not by KI or T4. The cancellation of I_2 treatment is accompanied by the development of latent mammary cancers that do not progress to overt

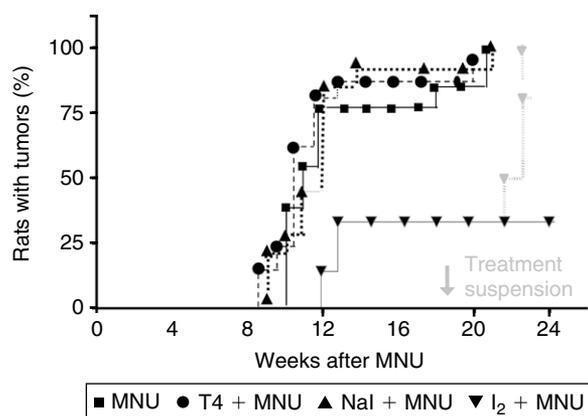


Figure 26.3 Effect of I_2 on the incidence of mammary cancer induced by MNU. Five-week-old rats were treated for 24 weeks with 0.05% potassium iodide (KI), molecular iodine (I_2), or 3 mg/ml thyroxine (T4) in the drinking water. At 7 weeks of age, rats were injected (i.p.) with one dose of 50 mg/kg MNU. In one I_2 -supplemented group, the supplement was suspended on the 16th week after MNU treatment (arrow). Data adapted from García-Solís *et al.*, (2005).

cancers, suggesting that I_2 acts by decreasing carcinogenesis at the promotion level (Figure 26.3). Moreover, this protective effect of I_2 was accompanied by a significant reduction in lipoperoxidation and an increase in catalase activity (Table 26.2). These findings are important in relation to the notion that reactive oxygen species (ROS), such as single oxygen (O_2), superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), are interrelated in the etiology of cancer (Morales *et al.*, 1990; Cook *et al.*, 2004). Human tumor cells produce a substantial amount of H_2O_2 (Szatrowski and Nathan, 1991). ROS have a wide range of cellular and molecular effects resulting in mutagenicity, cytotoxicity and changes in gene expression. G–C base pairs in CpG dinucleotide sequences are a common site for point mutations in p53 tumor suppressor gene closely related to breast cancer (Lane, 1994). Cellular genes are usually converted into oncogenes, particularly *ras* family oncogenes in codons 12 and 13. It has been demonstrated that these G–C sites are the main targets of oxidative damage (Bos, 1988). In this respect, our data suggest that the antioxidant effect of I_2 could operate by two mechanisms: (1) I_2 competes with ROS for various cellular components, or it neutralizes OH radicals by the formation of hypoiodous acid (HOI); and/or (2) I_2 acts indirectly to increase the expression or activity of antioxidant enzymatic machinery.

Another important effect of iodine on the thyroid is its ability to diminish the hypervascularity and hyperplastic characteristics of the diffuse goiter in Graves' disease. This phenomenon is widely used to facilitate surgical therapy of this disorder, and although its molecular mechanism is uncertain, it has been postulated that iodine might be oxidized to a more reactive form that binds to organic components which, in turn, interfere with metabolic or molecular processes necessary for the maintenance of hyperplasia (Mutaku *et al.*, 2002; Pisarev and Gartner, 2000). Vitale *et al.* (2000) showed that an excess of KI induces apoptosis in cultured thyroid cells, but if TPO activity is blocked with propylthiouracil, the apoptotic effect of KI is eliminated. Furthermore, Zhang *et al.* (2003), using lung cancer cells transfected with NIS or NIS/TPO, observed that only in NIS/TPO-transfected cells does KI excess induce apoptosis, indicating that I^- from KI needs to be oxidized to have a cytotoxic effect.

Table 26.2 Lipoperoxidation rate and catalase activity in normal and tumoral mammary glands

Rate/activity	Normal		Tumor	
	Control	I_2	Control	I_2
Lipoperoxidation (TBARS/mg · protein)	0.5 ± 0.08	0.3 ± 0.08	0.5 ± 0.1	0.15 ± 0.03*
Catalase (μ mol/mg · protein)	3210 ± 90	5214 ± 470*	530 ± 98	1894 ± 148*

Notes: Data are expressed as mean ± SD ($n = 5$).

* $p < 0.05$ vs. control.

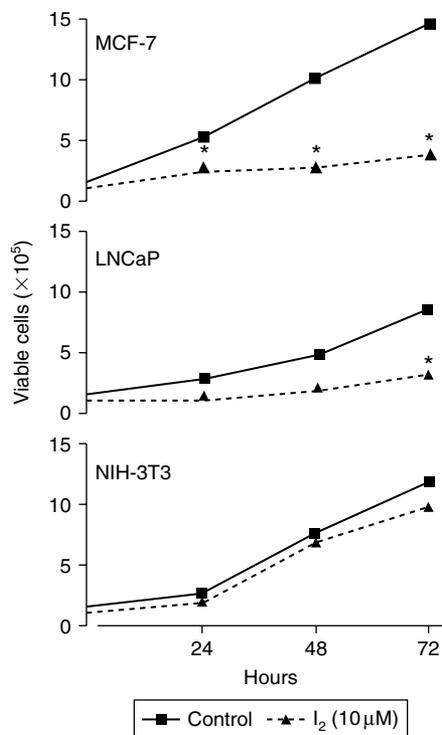


Figure 26.4 Antiproliferative effect of I_2 in human cell lines. After the indicated times, aliquots were removed and the cell number was determined by direct counting using trypan blue exclusion. Data are expressed as mean \pm SD ($n = 5$); * $p < 0.05$ vs. control cells. MCF-7, mammary cancer; LNCaP, prostate cancer; NIH-3T3, normal fibroblast.

The postulated mechanism by which iodine induces apoptosis is via the formation of specific iodinated arachidonic acid (AA) derivatives, such as 6-iodolactone (6-IL) or iodoheptadecanal (Dugrillon *et al.*, 1990; Pisarev *et al.*, 1994; Langer *et al.*, 2003). 6-IL is capable of inhibiting *in vitro* thyroid cell proliferation and inducing apoptosis (Dugrillon *et al.*, 1990; Langer *et al.*, 2003). Our data (Figure 26.4) show that I_2 administration significantly decreases the cellular proliferative rate in a tumor cell line from mammary (MCF-7) or prostate (LNCaP) gland, but not in normal cells (fibroblast NIH3T3), suggesting that tumoral cells contain specific components that can be iodinated and then can exert an antiproliferative effect. Similarly, the increase in DNA content induced by sex hormones in the prostate hyperplasia model is prevented with I_2 supplementation, without any harmful effect observed in control tissue (Figure 26.5). Although the specific iodocomponent has not yet been characterized, several studies have reported elevated prostaglandin levels in breast and prostate cancer, but not in normal glands (Tan *et al.*, 1974; Bennett *et al.*, 1977; Rolland *et al.*, 1980; Pham *et al.*, 2004). Prostaglandins are produced from AA by the enzyme cyclooxygenase, indicating the presence of high levels of AA in breast and prostate tumors. It is possible that these high levels of AA, and the iodolipids formed from them, may explain the specific effect of I_2 in tumoral cells.

In the thyroid gland, iodine treatment arrested the cell cycle at the G0/G1 and G2/M phases (Tramontano *et al.*,

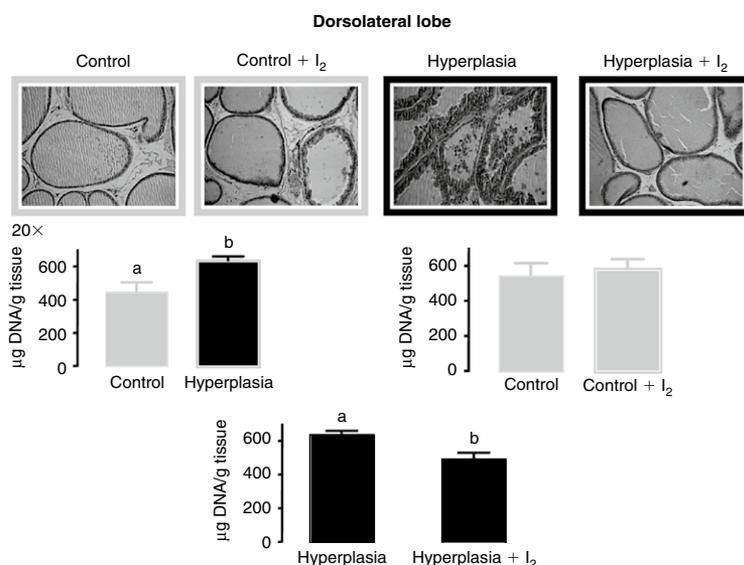


Figure 26.5 Iodine (I_2) supplementation prevents prostatic hyperplasia and reduces the DNA content in the dorsolateral lobe. Six-week-old rats were treated for 6 weeks with 0.05% of I_2 in the drinking water. At 8 weeks of age, the rats were castrated and treated (s.c.) daily for 3 weeks with supraphysiological doses of testosterone (1.0 mg) and estrogen (20 μg). Paraffin sections were stained with hematoxylin and eosin (magnification 20 \times). DNA was extracted with phenol/chloroform followed by ethanol precipitation. Data are expressed as mean \pm SD ($n = 5$); $p < 0.05$ vs. control.

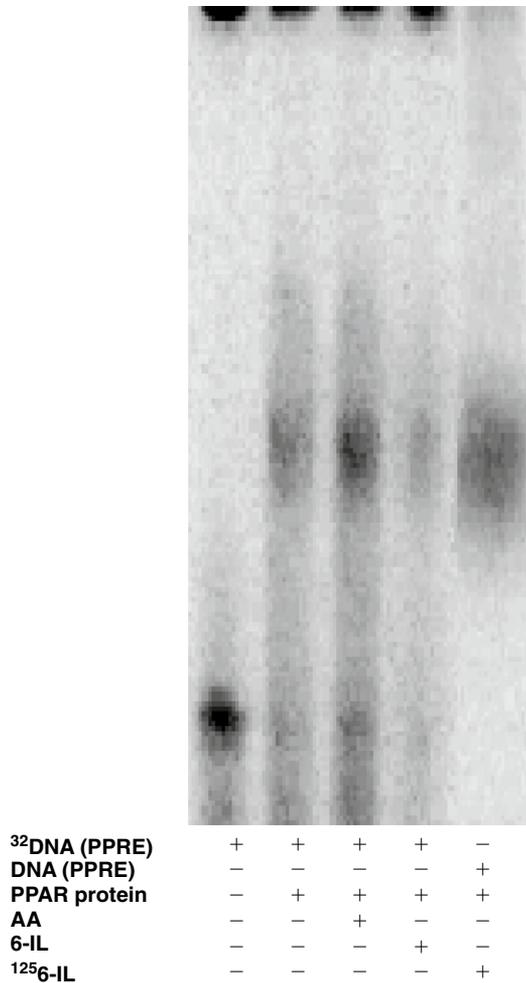


Figure 26.6 Affinity of PPAR receptors for 6-IL. The figure shows a representative autoradiograph from a gel mobility shift assay. Sequences of oligonucleotides for the PPAR receptor response element (PPRE) were: sense 5'GATCCAGGGAAAAGGT CATCAGGGAAA AGGTCAGTGGGAAAAGGTCAC-3', and antisense 5'TCGAGTGACCTTTCCCT AGTGACCTTTTCCTGATGACCTTTTCCTG-3. Cytoplasmic extracts from the MCF-7 cells containing proteins (10 μg) were incubated with ³²P-radiolabeled PPRE or nonradiolabeled PPRE as indicated, alone or in combination with arachidonic acid (AA), nonradiolabeled iodolactone (6-IL), or radiolabeled iodolactone (¹²⁵I-6-IL).

1989; Smerdely *et al.*, 1993). Other studies have shown an apoptotic effect induced by iodine excess in cultured thyrocytes. This effect neither involved changes in the antitumor protein p53, nor required expression of apoptosis-related proteins such as Bax, Bcl2, or Bcl XL (Vitale *et al.*, 2000). With regard to the mammary gland, studies in our laboratory have shown that chronic administration of I₂, but not I⁻, has a potent antineoplastic effect at the promotional level of mammary cancer without altering p53 expression (García-Solís *et al.*, 2005). In addition, recent reports showed that in human breast cancer cell lines these effects may be mediated by the activation of a complex

signaling cascade that included bax/bcl2- caspases, as well as the AIF-PARP-1 pathways (Shrivastava *et al.*, 2006; Arroyo-Helguera *et al.*, 2008). The toxicity of 6-IL toward thyroid, mammary gland, or prostate cells at the molecular level has not yet been fully documented, although the involvement of different apoptotic pathways has been hypothesized (Dugrillon *et al.*, 1990). In this regard, we proposed that the peroxisome proliferator-activated receptor (PPAR) is an excellent candidate to mediate 6-IL toxicity. PPARs, originally related only to the regulation of lipid metabolism, are widely expressed; they form part of the nuclear receptor family that binds thyroid hormones, steroids and vitamins (Kliwer *et al.*, 1994). PPARs have been implicated in mechanisms involved in cellular differentiation, proliferation and apoptosis (Shen and Brown, 2003). Polyunsaturated fatty acids, such as linoleic acid, eicosanoids and AA (one of the targets of oxidized iodine), have been identified as endogenous PPAR ligands (Kliwer *et al.*, 1994). The data summarized in Figure 26.6 indicate that 6-IL is able to bind PPAR receptors with high affinity, suggesting that this ligand-dependent transcription factor can participate in the antiproliferative I₂ effect.

In conclusion, it is possible that iodine in vertebrates acts in the following different ways:

- as an antioxidant by competing with free radicals for membrane lipids, proteins and DNA, or by increasing the expression or activity of antioxidant enzymes to help stabilize the cells. This antioxidant action can be exerted through oxidized iodine species (I^{*}) obtained in the diet or by local deiodination;
- as inducers, through PPAR receptors, of antiproliferative and apoptotic mechanisms after incorporation into iodolipids; and
- as a constitutive part of thyroid hormones.

As we and other authors have demonstrated, a chronic I₂ diet supplement is not accompanied by any harmful secondary effects on the health of humans or animals (body weight, thyroid economy, reproductive cycle). Thus, we propose that I₂ supplementation should be considered for use in clinical trials of breast and prostate cancer therapies.

Summary Points

- Mammary and prostate glands take up iodine in several chemical forms.
- Iodine exerts a potent antioxidant effect in several organs.
- Molecular iodine exhibits an antiproliferative and apoptotic effect in pathological, but not in normal mammary or prostate, epithelium.
- The antiproliferative effect of iodine might be mediated by the formation of iodolipids.

Acknowledgments

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Uncertainty of Iodine Particulate Deposition in the Respiratory Tract

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Abstract

Inhalation intakes of iodine are possible from workplace exposures or releases to the environment, and potential health risks are an outcome. Radioactive iodine releases to the environment that affect the general public and deliver radiation dose are of particular concern. Inhalation is a prominent pathway for radiation dose contributions from environmental or occupational intakes of radioactive iodine. The ability to model and predict the kinetics of iodine in the body can be used for internal dosimetric assessments of the radiation dose delivered to various tissues. Inhalation models are necessary for estimates of iodine uptake and deposition that may result in radiation exposure among workers or members of the public. The estimates of radiation dose via internal modeling have significant uncertainty, due to the variability in parameters involved in model calculations. The uncertainty in internal dosimetry can be a considerable component of the overall uncertainty in radiation dose assessment. In the United States, ICRP 30 is the lung model of regulatory concern and, therefore, the more commonly employed. The International Commission on Radiological Protection developed and issued the "New Lung Model" (ICRP, 66) in 1994. ICRP 66 incorporates recent knowledge and research into lung deposition, retention and clearance. The frequency distributions of regional deposition fraction are best described by a normal distribution for the BB and extrathoracic regions. The bb and AI regions are best described by a log-normal distribution. Breathing rate, diameter of the trachea and particle mass density have the greatest influence on deposition in all regions of the lung. The resulting distribution and characteristics of each region contribute to the total uncertainty of particulate deposition, and hence the overall uncertainty of inhalation dose estimates. Research into the more sensitive parameters and their distributions may lead to reduction in the uncertainty of the deposition model of ICRP 66.

Abbreviations

AI	alveolar-interstitial
AMAD	activity median aerodynamic diameter
BB	bronchial
bb	bronchiolar
BR	breathing rate
cm	centimeter
d	airway diameter
d(ae)	aerodynamic diameter
ET	extrathoracic
F(m)	fraction inhaled through the mouth
F(N)	fraction inhaled through the nose
FRC	functional residual capacity
I	iodine
ICRP	international commission on radiation protection
kev	kilo electron-volts
NRC	nuclear regulatory commission
p	density
U	windspeed
um	micrometer
V	volume
V(d)	anatomical deadspace
V(t)	tidal volume
X	particle shape factor

Introduction

Iodine is known to be extracted and concentrated in the thyroid gland for the purpose of hormone production. Inhalation intakes of iodine can occur through various mechanisms, leading to potential health risks, especially in the case of radioactive iodine, which when absorbed into the bloodstream may result in significant radiation dose to the respiratory tract and other organs. The major concern is the development of a thyroid carcinoma.

There are a number of iodine isotopes that are radioactive, with some being more hazardous than others. From a radiological perspective, the radioisotope ^{131}I is the most harmful and poses the greatest health risk. ^{131}I emits multiple beta particles and photons that contribute to the radiation dose. The most important beta particle, which predominantly delivers radiation dose to local tissues, has a mean energy of 192 keV.

Inhalation is a prominent pathway for radiation dose contributions from environmental or occupational intake of radioactive iodine. The ability to model and predict the kinetics of iodine in the body can be used for internal dosimetric assessments that predict the radiation dose delivered to various tissues. These assessments may be used to assess risk to the individual from inhalation intakes of radioactive iodine, which may occur through two distinct processes depending on its physical form. Iodine bound to particulates may be deposited in the respiratory tract, and iodine gas may be taken up by various tissues in the respiratory system during the breathing cycle. Inhalation models are necessary for estimates of iodine uptake and deposition that may result in radiation dose to workers or members of the public.

Inhalation models are important in estimating internal dose from contaminants in the atmosphere, such as iodine. Occupational radiation workers and members of the public might have been exposed to small quantities of various radionuclides, via the inhalation pathway, from releases at Department of Energy (DOE) sites (Heeb *et al.*, 1996; Kantelo *et al.*, 1993; Murphy *et al.*, 1991; Shipler *et al.*, 1996), and during the incident at Chernobyl (Pitkevich *et al.*, 1996). The estimates of radiation dose via internal modeling have significant uncertainty, due to the variability in parameters involved in model calculations. This can be a considerable component of the overall uncertainty in radiation dose assessment. An important calculational step in any lung model is the estimation of deposition fractions of particulates in various regions of the lung. Clearance via absorption, sequestration and surface transport, and thus the amount of material transferred to successive compartments or tissues, depends on regional deposition fractions of the particulate material. Therefore, variability in regional deposition within lung compartments may significantly contribute to the overall uncertainty of the lung model.

ICRP Report No. 66 (ICRP 66) incorporates recent knowledge and research into lung deposition, retention and clearance. In addition, the ICRP 66 methodology offers the ability to input data characteristic of specific materials. The model may be applied throughout the world; it has the ability to consider the effects of modifying factors (smoking, pollution and disease) on lung deposition, clearance and inhalation, and is more consistent with morphological, physiological, and biological characteristics of the respiratory tract (ICRP, 1994).

The uncertainty of regional deposition depends on physiological and anatomical input parameters of individuals, as

well as characteristics of the particulate material. Parameters describing particulates introduce uncertainty into estimates of regional deposition fractions, due to their inherent variability. As the anatomical and physiological input parameters are age-dependent, the uncertainty in regional deposition for various age groups should be determined independently. There is also significant variability in input parameters based on gender; therefore, adult males and females should be evaluated separately. Individuals are partitioned into eight age- and gender-dependent groups: adult males, adult females, 15-year-old males, 15-year-old females, 10-year-old children, 5-year-old children, 1-year-old children and 3-month-old infants. The differences between prepubescent males and females are assumed to be insignificant; therefore, children under the age of 11 will not be segregated into gender groups.

Particulate Deposition in the Respiratory Tract

Environmental intakes of particulate material may be from a range of particle sizes, but the mean typical value for these intakes is $1\ \mu\text{m}$ activity median aerodynamic diameter (AMAD) particles. Fractional deposition estimates of $1\ \mu\text{m}$ AMAD particles within regions of the lung were determined by multiple Latin hypercube estimates following a simulated intake of particulate material via inhalation. Latin hypercube estimates were determined by segregating input distributions into intervals of equal probability, with all intervals being sampled an equal number of times. Inhalability and regional deposition efficiency for inhalation and exhalation were estimated to determine total regional deposition. Numerical integration of the resultant deposition fractions was performed over the input distribution of particle aerodynamic diameter, thus yielding an estimate of the deposition fraction for $1\ \mu\text{m}$ AMAD aerosols. The integrated deposition fraction estimates using default values in ICRP 66 were compared to the published ICRP 66 values in Annex F (ICRP, 1994). Estimates of deposition fraction were within four percent of the ICRP values, hence demonstrating reasonable agreement and establishing a method of quality assurance.

The ICRP 66 model employs 10 input parameters for the determination of regional deposition fraction. These input parameters were varied according to their characteristic distributions, in an effort to perform uncertainty analysis of regional deposition fraction. The parameter particle aerodynamic diameter (d_{ac}) (Table 27.1), is dependent on the AMAD distribution of the aerosol, and cannot be varied with respect to the uncertainty analysis. The remaining nine parameters (Table 27.1) are independent and are varied to determine the uncertainty in regional deposition fraction estimates. The resulting estimates are fitted to known distributions and described by their characteristic parameters. Statistical analyses were performed on input

Table 27.1 Input parameter distribution assignments^a

Symbol	Parameter (units)	Distribution type ^b	Mean ^c	Standard deviation ^d
U	Windspeed ($\text{m}\cdot\text{s}^{-1}$)	U	–	0, 10
d_{ae}	Aerodynamic particle size (μm)	LN	1.00	2.47
χ	Particle shape factor	T	1.5	1.1, 1.9
ρ	Particle mass density ($\text{g}\cdot\text{cm}^3$)	T	3.0	1.0, 10.0
			<i>Male</i>	<i>Female</i>
d_0	Diameter of trachea (cm)	N	1.65 (0.067)	1.53 (0.06)
d_9	Diameter of airway at generation 9 (cm)	N	0.165 (0.007)	0.159 (0.006)
d_{16}	Diameter of airway at generation 16 (cm)	N	0.051 (0.002)	0.048 (0.002)
BR	Breathing rate ($\text{m}^3\cdot\text{h}^{-1}$)	N	1.74 (0.67)	1.37 (0.45)
V_d	Anatomical dead space (ml)	N	146 (25.5)	124 (21.0)
FRC	Functional residual capacity (ml)	N	3300 (600)	2680 (500)
			<i>15-year male</i>	<i>15-year female</i>
d_0	Diameter of trachea (cm)	N	1.59 (0.068)	1.52 (0.065)
d_9	Diameter of airway at generation 9 (cm)	N	0.161 (0.007)	0.156 (0.007)
d_{16}	Diameter of airway at generation 16 (cm)	N	0.047 (0.002)	0.045 (0.002)
BR	Breathing rate ($\text{m}^3\cdot\text{h}^{-1}$)	N	1.51 (0.51)	1.41 (0.45)
V_d	Anatomical dead space (ml)	N	130 (22)	114 (19)
FRC	Functional residual capacity (ml)	N	2680 (562)	2330 (488)
			<i>10-year-old</i>	<i>5-year-old</i>
d_0	Diameter of trachea (cm)	N	1.31 (0.06)	1.06 (0.049)
d_9	Diameter of airway at generation 9 (cm)	N	0.143 (0.007)	0.127 (0.006)
d_{16}	Diameter of airway at generation 16 (cm)	N	0.039 (0.002)	0.031 (0.001)
BR	Breathing rate ($\text{m}^3\cdot\text{h}^{-1}$)	N	1.21 (0.41)	0.65 (0.19)
V_d	Anatomical dead space (ml)	N	78 (14.9)	46 (8.5)
FRC	Functional residual capacity (ml)	N	1480 (311)	767 (161)
			<i>1-year-old</i>	<i>3-month-old</i>
d_0	Diameter of trachea (cm)	N	0.75 (0.028)	0.62 (0.027)
d_9	Diameter of airway at generation 9 (cm)	N	0.107 (0.004)	0.099 (0.004)
d_{16}	Diameter of airway at generation 16 (cm)	N	0.022 (0.001)	0.020 (0.001)
BR	Breathing rate ($\text{m}^3\cdot\text{h}^{-1}$)	N	0.40 (0.11)	0.22 (0.06)
V_d	Anatomical dead space (ml)	N	20 (3.7)	14 (2.6)
FRC	Functional residual capacity (ml)	N	244 (26)	148 (28)

^aReferences are given in the text.

^bDistributions: N = normal; LN = log-normal; T = triangular; U = uniform.

^cArithmetic mean for normal distributions, geometric mean for log-normal distributions, and the mode for triangular distributions.

^dStandard deviation for normal distributions, geometric standard deviation for log-normal distributions, and minimum and maximum for triangular and uniform distributions.

parameter distributions to determine their sensitivity to fractional deposition. The fundamental methodology used is consistent with the ICRP 66 lung model (ICRP, 1994).

Nose breathers versus mouth breathers

The ICRP 66 model is based on the assumption that individuals are classified in one of two breathing categories – nose or mouth breathers. Nose breathing predominates as the typical breathing pattern, but mouth breathing is also significant within a typical population (ICRP, 1994). The fraction of air inhaled through the nose is assumed to be unity for nose breathers, except when breathing rates (BRs) reach the levels required for heavy exercise. The fraction of air inhaled through the nose and mouth is divided equally at BRs required for heavy exercise in the nose breather.

For a mouth breather, air is inhaled through the mouth and nose at all levels of respiration. However, the amount

of air inhaled through the nasal and buccal pathways varies with BR for the mouth breather. The fraction of air inhaled through the nose is assumed to be 0.7 for mouth breathers at resting levels of activity. As activity level and BR increases, the fraction of air inhaled through the nose decreases to 0.4 for light exercise and 0.3 for heavy exercise. BRs vary with the level of physiological exercise and are age- and gender-dependent (ICRP, 1994). Therefore, a relationship between the fractions of air inspired through the nasal and buccal pathways, as a function of BR, has been determined in this work for each age and gender group.

Inhalability

Inhalability is defined as the intake efficiency of the human head for particles carried in moving air (ICRP, 1994), or the aspiration efficiency of the human head for aerosols over representative ranges of aerosol size and external wind conditions

(Vincent *et al.*, 1990). Its numerical definition is the ratio of the number concentrations of particles with a particular d_{ac} inspired through the nose or mouth to the concentration present in the inspired volume of ambient air (ICRP, 1994).

Extrathoracic region fractional deposition

The extrathoracic region is assumed to consist of two physical compartments. Fractional deposition in the anterior nasal passage (ET₁ region) is determined solely by air inhaled through the nose. The total volumetric flow rate through the nasal region must be adjusted for the fraction of air being inhaled through the mouth. Therefore, deposition in the ET₁ region of mouth breathers is significantly reduced, due to the low fraction of air being inhaled through the nose. Fractional deposition in the posterior nasal passage, pharynx and larynx (ET₂ region) also depends on the individual's breathing pattern (nose or mouth breather). For mouth breathers, ET₂ deposition is increased, due to the larger volumetric flow rate and increased exposure to particulates inspired through the mouth. Total volumetric flow rate ventilating the lungs (V) is a function of BR and is the sum of the volumetric flow rates through the inhalation pathways.

Particle deposition occurs through aerodynamic and thermodynamic processes. Aerodynamic deposition occurs through impaction and sedimentation, which are dependent on aerodynamic particle size, total volumetric flow rate and the diameter of the trachea. Thermodynamic deposition occurs through Brownian diffusion and depends on the diffusion coefficient, along with total volumetric flow rate and trachea diameter (d_0). The diffusion coefficient is a function of the particle's effective volume diameter and a slip correction factor. The slip correction factor accounts for particle slip caused by the relative velocity of gas molecules at the particle surface (ICRP, 1994). Deposition of particulates within all regions occurs throughout the breathing cycle including inhalation and exhalation.

Thoracic region fractional deposition

The thoracic portion of the respiratory tract is assumed to be composed of three compartments: bronchial (BB), bronchiolar (bb) and alveolar–interstitial (AI). The BB region begins with the trachea and includes the bronchi to the eighth airway generation. The bb region is the second portion of the air conducting system in the lungs, and consists of the bronchioles from airway generations 9 to 15. The AI region is comprised of respiratory bronchioles and alveolar ducts from airway generations 16 to 26. The number of airway generations may vary in individuals, and other anatomic models may use a different number of airway generations and distribution. Uncertainty in the branching angles at the bifurcations between generations is a source of variability in the estimation of fractional

deposition in the respiratory tract. Fractional deposition in these regions is influenced by individual breathing patterns and the level of physiological exercise during inhalation. The total volumetric flow reaching the thoracic region is assumed to be the same for nose and mouth breathers.

Deposition in the thoracic region is the sum of aerodynamic and thermodynamic deposition of particulate material. Aerodynamic deposition depends on aerodynamic particle size, total volumetric flow rate, anatomical dead space, tidal volume, functional residual capacity (FRC) (combined residual and expiratory reserve volume or the amount of air remaining in the lungs after a tidal expiration) and diameter of the airways. Thermodynamic deposition depends on anatomical and physical characteristics, such as tidal volume, anatomical dead space, functional residual capacity and the transit time of air within each region. Thermodynamic particle size, which is derived from the diffusion coefficient, particle shape factor and the particle's mass density, influence thermodynamic deposition.

Parameter Distributions

Input parameter distributions and their characteristics are listed in Table 27.1 for all age and gender groups. The particle d_{ac} distribution was not varied when determining uncertainty in the model, because estimates of regional deposition fraction are numerically integrated over particle size distribution to determine the deposition of aerosols characterized by an activity median aerodynamic diameter. Regional particulate deposition is dependent on the particle size distribution of the inhaled aerosol, even though it is not varied for the probabilistic assessment.

Wind speed (U)

Ambient wind speed (U) of the air breathed affects particulate inhalability. Wind speeds typically encountered vary within the range of about 0–10 m·s⁻¹, in both indoor and outdoor environments (ICRP, 1994; Vincent *et al.*, 1990; Vincent, 1995). When determining inhalability for an occupational worker, wind speeds are within the range of 0–4 m·s⁻¹ for most occupational environments (Vincent *et al.*, 1990; Vincent, 1995). It is assumed herein that the distribution of wind speeds is uniform from 0 to 10 m·s⁻¹ with no preference for one speed over another (Vincent *et al.*, 1990; Vincent, 1995).

Aerodynamic diameter (d_{ae})

Environmental exposures to the general public are assumed to be from 1 μm AMAD aerosols; therefore, particulate d_{ae} is assumed to be log-normally distributed with a geometric mean of 1 μm and a geometric standard deviation of

2.47 (ICRP, 1994). The aerodynamic particle diameter is the diameter observed due to irregularities in shape and is used in the determination of aerodynamic deposition. This parameter was not varied to determine estimates of uncertainty; it cannot be varied because it is a function of the aerosol distribution, over which the resulting deposition fractions are numerically integrated.

Particle shape factor (χ)

The particle shape factor (χ) is a dimensionless constant used to relate drag force on an irregular particle moving in air to the particle's equivalent volume diameter (ICRP, 1994). The shape factor and the mass density are used to determine the particle's thermodynamic diameter in the model but in practice, the thermodynamic diameter can be measured for small particles. The shape factor is assumed to have a triangular distribution ranging from 1.1 to 1.9 with a mode of 1.5 (ICRP, 1994). The ICRP 66 default for the χ is 1.5.

Particle mass density (ρ)

Particle mass density (ρ) is assumed to be triangularly distributed ranging from 1 to $10 \text{ g}\cdot\text{cm}^{-3}$ with a mode of $3 \text{ g}\cdot\text{cm}^{-3}$ (ICRP, 1994). The ICRP recommends a reference value of $3 \text{ g}\cdot\text{cm}^{-3}$, because it is a typical value for many natural materials. The assumed range includes particles such as polystyrene, Teflon, iron oxide and uranium oxide (ICRP, 1994).

Diameter of trachea (d_0), bronchiole (d_9), and terminal bronchiole (d_{16})

Scaling factors used in the ICRP 66 methodology for regional deposition of particulates depend on the diameter of airways within the respiratory tract. These factors are based on average airway diameters of the adult male and are used to estimate airway diameters at various generations as functions of gender and age. Aerodynamic and thermodynamic deposition of particulate material within the ET₁, ET₂ and BB regions is dependent on the diameter of the trachea. Thermodynamic deposition of particulate material within the bb region is a function of the diameter of the bronchiole (d_9) at airway generation 9, while aerodynamic and thermodynamic deposition of particulates within the AI region varies with the diameter of the terminal bronchiole.

Mean values of airway diameter (Table 27.1) can be found in the literature (Horsfield and Cumming, 1968; Phalen *et al.*, 1978; Phalen *et al.*, 1985; Weibel, 1963; Yeh and Schum, 1980), but reference values for their corresponding standard deviation are more difficult to determine. Airway diameter also correlates well with an individual's height (Phalen *et al.*, 1985). The variability

in height (USDHEW, 1970, 1973, 1979), and hence the airway diameter, is shown to be normally distributed, and the relative standard deviation in height has been applied as a measure of the variability in airway generation diameter. Height and airway diameter data are both age- and gender-specific.

Breathing rate (BR)

BR assumptions by age and level of exercise are taken from the ICRP Committee 2 report by the Lung Dynamics Task Group (ICRP, 1960). These data are also used in ICRP 66 as the basis for BR estimates. ICRP 2 data were used to derive a distribution of annual BRs using a simple Monte Carlo technique to simulate assumed activity levels (Hamby, 1993). BR distributions are determined by two different methods for adults and children. Adult males and females, 15-year-old males and females, and 10-year-old children are handled in a similar manner. The methodology is similar for 5-year-old children, 1-year-old children and 3-month-old infants, but distinct from the method employed for more mature age groups.

Two BR distributions have been assumed for each of the more mature age and gender classifications – one for light-to-moderate exercise and another for heavy exercise. To introduce the least amount of bias into the deposition uncertainty calculation, and yet provide some weight to the expected ventilation rates, the distributions describing BRs are assumed to be triangular. The light-to-moderate distribution has a mode equal to the light activity BR default value, and is bounded by the resting BR and twice the light BR (ICRP, 1994). The distribution describing heavy exercise ranges from twice the light BR, to the maximum sustained BR of twice the heavy BR, with a mode equal to the heavy BR default value (ICRP, 1994).

BRs for adult males and females, 15-year-old males and females and 10-year-old children were determined by assuming that the individual is engaged in light-to-moderate activity for $163 \text{ h}\cdot\text{wk}^{-1}$ and heavy exercise for $5 \text{ h}\cdot\text{wk}^{-1}$. The distributions were sampled uniformly based on the amount of time an individual engages in each type of activity. The distribution best describing BR was found to be normally distributed for all age and gender groups (Table 27.1). The Nuclear Regulatory Commission BR default value is $0.91 \text{ m}^3\cdot\text{h}^{-1}$ (USNRC, 1977), demonstrating reasonable agreement.

Data in ICRP 2 were used to derive the distribution of annual BRs for young children and infants using a simple Monte Carlo technique to simulate assumed activity levels (Hamby, 1993). BR is assumed to have a triangular distribution with a mode equal to the light activity BR, and is bounded by the resting BR and twice the light BR (Hamby, 1993). The resulting BR distributions are found to be normally distributed, and default values are listed in Harvey (2003). These data were used as the basis for BR

in our determination of particle deposition in infants and children.

BRs at differing levels of activity were used to determine four input parameters for the ICRP 66 lung model. These parameters included tidal volume (V_T), V and the fractions of air breathed through the nose (F_n) and mouth (F_m). Functions relating these four parameters to BR were generated for various levels of exercise. BRs were limited to $0.2 \text{ m}^3 \cdot \text{h}^{-1}$ in adults, 15- and 10-year-olds to prevent sampling of unusually low BRs, and total volumetric flow rate was limited to $2000 \text{ ml} \cdot \text{s}^{-1}$. V_T was bounded by 100 ml and 2500 ml in adults, 15-year-old adolescents and 10-year-old children. Minimum BR was limited to $0.1 \text{ m}^3 \cdot \text{h}^{-1}$ in younger children and infants. V_T was limited to a lower level of 75 and 100 ml for 1- and 5-year-old groups, respectively, regardless of breathing pattern. In nose and mouth breathers, total volumetric flow rate was limited to $100 \text{ ml} \cdot \text{s}^{-1}$ for 1-year-olds. Monte Carlo techniques were then used to determine estimates of particulate deposition uncertainty.

Anatomical dead space (V_D)

Anatomical dead space (V_D) refers to the volume of air in the lung that does not undergo gas exchange. Total V_D values can be found in Table 27.1. Regional V_D within the respiratory tract is taken into account in this particulate model, and is based on the total V_D of individuals in each age and gender group. V_D is assumed to be normally distributed for all age and gender groups (ICRP, 1994; Roy *et al.*, 1991; Hart *et al.*, 1963; Cook *et al.*, 1955).

Values estimating the variability for V_D in 1-year-old children and 3-month-old infants were not available in the open literature; therefore, an alternative method was used to estimate their variability. V_D correlates better with an individual's height than any other anatomical parameter (Zapletal *et al.*, 1987; Phalen *et al.*, 1985). The variability in height (Stoudt *et al.*, 1960), and hence anatomical dead space, is assumed to be normally distributed, and the percent standard deviation in height has been applied as a measure of the age-specific variability in anatomical dead space.

Functional residual capacity (FRC)

The FRC is the combined residual and expiratory reserve volume, or the amount of air remaining in the lungs after a tidal expiration (Marieb, 1998). An individual's FRC varies by gender and age. For all age and gender groups, FRC is assumed to be normally distributed. FRC input parameter values can be found in Table 27.1 (Harvey, 2003; ICRP, 1994; Roy *et al.*, 1991; Quanjer, 1983; Gaultier *et al.*, 1979; Helliessen *et al.*, 1958). FRC values vary significantly with age and gender of the individual.

Uncertainty of Regional Deposition Fraction

Regional deposition fractions of a $1 \mu\text{m}$ AMAD aerosol, their distributions and characteristics have been determined as functions of age, gender and breathing pattern (Table 27.2 and Harvey, 2003). All are shown to follow similar trends (Harvey, 2003; Harvey and Hamby, 2002; Harvey and Hamby, 2001). The frequency distributions of regional deposition fraction are described by a normal distribution for the BB and extrathoracic regions. The bb and AI regions are best described by a lognormal distribution.

Table 27.2 Deposition fractions (unitless) for lung regions by age, gender and breathing characteristics^a

Parameter	Mean ^b	Standard deviation ^c	ICRP 66 value ^d
Adult Male			
<i>Nose breather</i>			
ET ₁	0.17	0.035	0.18
ET ₂	0.22	0.045	0.23
BB	0.013	0.0023	0.013
bb	0.018	1.21	0.015
AI	0.10	1.23	0.099
ET _{2seq}	0.00011	0.000021	0.00012
BB ₁	0.0069	0.0011	0.0068
BB ₂	0.0068	0.0012	0.0062
BB _{seq}	0.000096	0.000015	0.000091
bb ₁	0.0079	1.33	0.0074
bb ₂	0.0079	1.30	0.0072
bb _{seq}	0.00011	1.31	0.00011
AI ₁	0.031	1.20	0.03
AI ₂	0.061	1.19	0.059
AI ₃	0.010	1.22	0.0099
<i>Mouth breather</i>			
ET ₁	0.12	0.015	–
ET ₂	0.16	0.024	–
BB	0.025	0.0069	–
bb	0.021	1.20	–
AI	0.13	1.15	–
ET _{2seq}	0.000081	0.000013	–
BB ₁	0.012	0.0036	–
BB ₂	0.012	0.0042	–
BB _{seq}	0.00018	0.000045	–
bb ₁	0.011	1.27	–
bb ₂	0.0096	1.40	–
bb _{seq}	0.00015	1.20	–
AI ₁	0.040	1.15	–
AI ₂	0.079	1.15	–
AI ₃	0.013	1.13	–

^aET₁, ET₂ and BB regions are described by a normal distribution; AI and bb distributions are described by a log-normal distribution.

^bMean for normal distributions and geometric mean for log-normal distributions.

^cStandard deviation for normal distributions and geometric standard deviation for log-normal distributions.

^dICRP 66 deposition fractions are for members of the public that breathe $1 \mu\text{m}$ AMAD particles through the nose and are engaged in light exercises (values for mouth breathers are unavailable).

Uncertainty of compartmentalized deposition fraction

Deposition of particulates in the respiratory tract occurs in five different regions. These regions are further subdivided into compartments based on clearance, and deposition within the compartments is modeled accordingly. An introduction to clearance is necessitated in order to describe the particulate deposition model as developed. Once particulate material is deposited, particulates undergo clearance to other parts of the respiratory tract, lymph nodes, gastrointestinal tract and the bloodstream. Based on the method and rate of clearance, the respiratory tract is further subdivided from the five primary regions of deposition into 12 compartments (ICRP, 1994). The ET₂ region is divided into two regions – ET₂ and ET_{2seq}. Particulate material in the ET₂ region is cleared to the GI tract, and material in the ET_{2seq} compartment is sequestered in tissues and cleared to the lymphatic system.

The BB region is divided into the BB₁, BB₂, and BB_{seq} compartments. The designation of BB₁ and BB₂ refers to the rate of surface transport of particulate material deposited in the BB region. BB₁ is particulate material that is cleared by fast surface transport, and slow surface transport of particulates occurs in the BB₂ compartment. Particulate material that is sequestered in the BB region and moved to the lymph nodes is designated by the BB_{seq} compartment. Particulates that penetrate further into the respiratory tract may be deposited in the bb region. The bb region is further subdivided into three compartments: bb₁, bb₂, and bb_{seq}. The bb₁ compartment is cleared via fast surface transport, and the bb₂ compartment is cleared via slow surface transport. Particulate material sequestered in the bb region and cleared to the lymphatics is represented by the bb_{seq} compartment.

The AI region is cleared by three different rates of surface transport. The AI₁ compartment is cleared by the most rapid rate of surface transport, and the AI₂ by an intermediate rate. Particulates in the AI₃ compartment are cleared by two different methods. The first is slow surface transport, and the second is clearance to the thoracic lymph nodes.

Compartmental deposition fractions of a 1 μm AMAD aerosol, their distributions and characteristics have been determined as functions of age, gender and breathing pattern (Table 27.2 and Harvey, 2003) and are shown to follow similar trends (Harvey, 2003).

Comparison to ICRP estimates of deposition fraction

The ICRP 66 – recommended values shown in Table 27.2 – do compare well with those developed herein. The ICRP 66 values are for deposition of 1 μm AMAD particles, specifically for members of the public engaged in light

exercise. The deposition fractions determined in this assessment are for members of the public exposed to particles characterized by 1 μm AMAD aerosol and characteristic BR distributions. Deposition fraction estimates are within 4% of the ICRP fractional deposition estimates, for all regions of the respiratory tract.

Factors influencing estimates of particulate deposition

The larger the aerodynamic particle size, the greater the deposition of that material within the ET₁ region, as well as in the entire extrathoracic region. Particles with large aerodynamic diameters will have increasing difficulty in penetrating the respiratory tract, due to reduction in airway size as the particle moves deeper into the lung. The BB region of the respiratory tract has a reduced amount of deposition, due to its small surface area, and rapid transit time of air conduction to subsequent regions.

Deposition begins to increase again in the bb region, because as airway diameters decrease more particles become deposited. The deposition fractions of nasal augmenters demonstrate this, although mouth breathers do not, because a significant fraction of particulate material bypasses the ET₁ region; therefore, more particulate material is deposited in the BB region of mouth breathers versus that of nasal augmenters (Table 27.2). The maximum amount of thoracic deposition occurs in the deeper regions of the lung, due to the ability of small particulates to interact with the large surface area of the respiratory bronchioles and alveoli. These trends are consistent for all age groups, regardless of the breathing pattern and gender.

Particulate deposition's influence on radiation dose to the respiratory tract

The greater the fractional deposition in the extrathoracic region, the larger the dose estimate to this region of the respiratory tract. Increased deposition in ET₁, however, leads to a greater amount of clearance through physical removal, thus tending to decrease the total dose to the respiratory tract. Extrathoracic clearance is due to the movement of cilia on the mucosal surface and the upward flow of mucus. Particles can then be removed by swallowing, coughing and sneezing; therefore, increased extrathoracic deposition leads to a lower committed dose equivalent to the lung. However, increased amounts of particulate material enter the transfer compartment (blood), due to the effects of swallowing. Conversely, greater deposition in the thoracic regions increases the committed dose equivalent to the lung before subsequent clearance to the GI tract and blood. These are general observations, and specific information based on radionuclide, particulate material and chemical form are of great importance for estimating an individual's inhalation dose.

Trends demonstrated via particulate deposition

Distributions of fractional deposition are similar for all individuals, regardless of age, gender and breathing pattern. For example, particle deposition in each region of the respiratory tract of adult male nose and mouth breathers is shown in [Table 27.2](#). For 1 μm AMAD aerosols, the greatest regional deposition occurs in the extrathoracic regions of the respiratory tract and the largest thoracic fractional deposition occurs in the AI region, or deepest portion of the lung. When particles are large, deposition in the AI region is significantly reduced, because large particles are deposited in the extrathoracic region of the respiratory tract. Individuals that breathe primarily through the nose have greater deposition in the extrathoracic regions of the respiratory tract and lesser deposition in the thoracic regions, as compared to mouth breathers.

Parameter Sensitivity Analysis

Sensitivity analyses were conducted to determine which parameters influence deposition fraction in each region of the respiratory tract ([Harvey and Hamby, 2002](#); [Harvey and Hamby, 2001](#)). Parameter sensitivity analysis was performed by the rank transformation method ([Hamby, 1994](#); [Hamby, 1995](#)) based on breathing pattern, age and gender. Rank correlation coefficients were calculated ([Table 27.3](#) and [Harvey, 2003](#)), the value of which demonstrates the relative degree of importance that an input parameter has on output variability. The rank correlation coefficient may be positive or negative, demonstrating whether the model output is increasing or decreasing, respectively, with the magnitude of a given input parameter. Parameter sensitivity is dependent on the input parameter distribution and its characteristics, along with the mathematical structure of the model. BR, diameter of the trachea and particle mass density have the greatest influence on deposition in all regions of the lung. The model's sensitivity was tested with and without numerical integration. The numerical integration process does not appear to play a significant role in model sensitivity to input variables.

Sensitivity analysis for adults

The estimates of deposition in the respiratory tract are generally most sensitive to the input parameters of d_0 , ρ and BR. BR is always the most sensitive parameter in the model for adults. As shown by the rank correlation coefficients ([Table 27.3](#) and [Harvey, 2003](#)), BR has a more significant influence on regional deposition than other parameters in the extrathoracic and BB regions. The influence of BR on regional deposition in the bb and AI regions is also more important than other parameters, but to a lesser degree. BR is positively correlated in the BB and

extrathoracic regions, but negatively correlated in the bb and AI regions.

Extrathoracic regional deposition is also significantly influenced by the diameter of the trachea, being negatively correlated with extrathoracic deposition of particulate material. Particle mass density plays an important role in particulate deposition in the bb and AI regions, where it is positively correlated with deposition in the deep lung regions. Adults, regardless of gender and breathing pattern, demonstrate a similar relationship with respect to which parameters are most sensitive. The only exception is the second most important parameter in the BB region that varies by gender and breathing pattern.

Sensitivity analysis for 15-year-old adolescents

BR is generally the most sensitive parameter in the model, but there are two exceptions with regard to adolescents. In the BB region of 15-year-old female nose breathers and 15-year-old female mouth breathers, particle mass density is more sensitive than BR. Fifteen-year-old adolescents, regardless of gender and breathing pattern, demonstrate a similar relationship with respect to which parameters are most sensitive. The only exception is the second most important parameter: in the AI region of 15-year-old female mouth breathers where diameter of airway generation 16 (d_{16}) follows BR.

Sensitivity analysis for 5- and 10-year-old children

BR is also an important parameter in particulate deposition estimates of 5- and 10-year-old children, but unlike the older age groups it is not always the most important parameter. Five-year-old children considered to be mouth breathers are more sensitive to particle mass density and trachea diameter than to BR. In younger age groups, the importance of BR on fractional deposition decreases, while other parameters increase in significance. BR has a more significant influence on regional deposition than other parameters in the extrathoracic and BB regions, with the exception of 5-year-old mouth breathers. Five-year-old mouth breathers are more influenced by trachea diameter than BR. They are also an exception with regard to the influence of BR on deposition in the bb and AI regions. BR is important to deposition in these regions, but not at the same magnitude as in other ages and breathing patterns.

Anatomical dead space is an important parameter with respect to deposition of particulate material in the AI region of 5-year-old children. Ten-year-old children, regardless of breathing pattern, demonstrate a similar relationship with respect to which parameters are most

Table 27.3 Parameter sensitivity for particulate deposition in adults using rank correlation coefficients

Parameter	ET_1	ET_2	BB	bb	AI
Male nose breather					
ρ	-0.09	-0.08	-	0.57	0.49
FRC	-0.09	-0.09	-	-0.08	-
d_0	-0.16	-0.17	-	-0.05	0.05
d_9	-	-	-	-0.18	-
d_{16}	-	-	0.07	-0.10	-0.15
U	-0.09	-0.12	-0.14	0.27	0.18
V_d	-	-	-	0.16	-0.14
χ	0.08	0.08	0.11	-0.13	-0.16
BR	0.86	0.86	0.97	-0.76	-0.80
Male mouth breather					
ρ	-	-	-	0.58	0.45
FRC	-	-	-0.10	-	0.22
d_0	-0.42	-0.42	-0.10	0.36	0.20
d_9	-0.06	-0.06	-0.10	-0.11	0.07
d_{16}	-0.16	-0.16	-0.18	0.21	0.05
U	0.15	0.14	0.05	0.06	0.08
V_d	-0.09	-0.08	-0.06	0.08	-0.24
χ	-	-	-0.07	-	-0.08
BR	0.84	0.84	0.97	-0.67	-0.73
Female nose breather					
ρ	-	-	0.11	0.50	0.37
FRC	-0.10	-0.10	-0.12	0.06	0.17
d_0	-0.33	-0.32	-0.23	0.09	0.19
d_9	-0.06	-0.06	-	-	0.09
d_{16}	-0.11	-0.11	-0.10	0.09	-
U	-	-	-	-0.06	-
V_d	0.20	0.20	0.24	-	-0.34
χ	-0.07	-0.08	-0.12	-0.13	-
BR	0.94	0.94	0.96	-0.80	-0.86
Female mouth breather					
ρ	-	-	-	0.59	0.41
FRC	0.09	0.09	0.07	-0.10	-0.05
d_0	-0.49	-0.48	-0.22	0.34	0.26
d_9	-	-	-0.07	-0.09	-
d_{16}	-	-	-	0.07	-0.11
U	-	-	-	0.05	0.09
V_d	-	-0.05	-	0.15	-0.23
χ	-	-	-	-0.13	-0.16
BR	0.90	0.90	0.98	-0.71	-0.81

Note: Absolute values less than 0.05 have been excluded to increase clarity and the two most sensitive parameters are shown in bold.

sensitive. The parameters most influential to particulate deposition in older age groups are also important to the 5-year-old child, but anatomical dead space and particle shape factor have been shown to be more significant in this age group.

Sensitivity analysis for 3-month-old infants and 1-year-old children

Although the estimates of deposition in the respiratory tract are generally most sensitive to the input parameters of trachea diameter (d_0), particle mass density (ρ), and

BR, for infants and very young children anatomical dead space (V_d) is also an important parameter. As shown by the rank correlation coefficients (Harvey, 2003), the parameters that have a significant influence on regional deposition have greater variation in infants than more mature age groups. The influence of BR on regional deposition in the bb and AI regions is usually less important than other parameters, which is in direct contrast to the results for more mature age groups. BR is positively correlated in the BB and extrathoracic regions, but negatively correlated in the bb and AI regions with the exception of the 3-month-old mouth breather.

Anatomical dead space is an important parameter with respect to deposition of particulate material in the AI region of 1-year-old children and 3-month-old infants. The parameters most influential to particulate deposition in older age groups are also important to children and infants, but anatomical dead space and windspeed have been shown to be more significant in the youngest age groups. The most significant parameters for particulate deposition estimates are more variable in 1-year-old children and 3-month-old infants than in older age groups, and their rank correlation coefficients demonstrate greater variability in magnitude.

Comparison of Results with Other Investigators

Other investigators have experimentally or theoretically estimated particulate deposition in the respiratory tract using various experimental techniques (ICRP, 1994; Miller *et al.*, 1988; Stahlhofen *et al.*, 1983; Yu and Diu, 1982; Yeh and Schum, 1980; Hansen and Ampaya, 1975; Heyder *et al.*, 1975; Olson *et al.*, 1970; Weibel, 1963). Fractional deposition estimates will be compared by breathing pattern for adult males and females.

Regional deposition estimates were compared by partitioning the respiratory tract into three distinct regions: extrathoracic, bronchial and pulmonary. The extrathoracic region represents the total deposition of particulate material in the ET₁ and ET₂ regions of the respiratory tract. The BB region consists of the BB and bb regions of the respiratory tract, and deposition includes the sum of deposition in these regions. The AI region corresponds to the deeper regions of the lung or the pulmonary region, and depositions in the AI and pulmonary region are assumed to be equivalent. Investigative studies were conducted with different values for anatomical and physiological parameters; therefore, differences in the deposition fraction estimates can be expected.

Regional deposition comparison to experimental results of other investigators

Regional deposition estimates for 1 µm AMAD particles and particles with a specific aerodynamic diameter of 1 µm (Miller *et al.*, 1988; Yu and Diu, 1982) are shown in Table 27.4. Stahlhofen *et al.* (1983) determined regional deposition fractions for mouth breathers inhaling 2 µm aerodynamic diameter particles under various experimental conditions (Table 27.4). They either varied the flow rate of air while

Table 27.4 Adult deposition fractions (unitless) of 1 µm aerodynamic particles

Investigator	Breathing pattern ^{a,b}	ET	Bronchial	Pulmonary
Harvey ^c	NB (AM)	0.39	0.031	0.10
	MB (AM)	0.28	0.046	0.13
	NB (AF)	0.39	0.039	0.098
	MB (AF)	0.30	0.046	0.13
Stahlhofen <i>et al.</i> , 1983 ^d	P = 2, Q = 750	0.00	0.02	0.15
	P = 2, Q = 250	0.00	0.02	0.12
	P = 1, Q = 250	0.00	0.00	0.10
	P = 2, Q = 250	0.00	0.00	0.22
	P = 4, Q = 250	0.00	0.00	0.50
Miller <i>et al.</i> , 1988 ^e	NB	–	0.020	0.23
	MB	–	0.025	0.24
ICRP 66 ^f	NB (AM)	0.41	0.028	0.099
	NB (AF)	0.41	0.028	0.099
Yu and Diu, 1982 ^g	Weibel	0.30	0.035	0.11
	Olson	0.30	0.030	0.11
	Hansen and Ampaya	0.30	0.025	0.10
	Yeh and Schum	0.30	0.030	0.08

^aNB for nose breathers and MB for mouth breathers.

^bAM for adult male and AF for adult female.

^cDeposition fractions are for members of the public that breathe 1 µm AMAD particles.

^dP for period of inspiration time and Q for flow rate with units of s and cm³·s⁻¹, respectively. Data are for 2 µm particles (monodisperse spheres).

^eData for ET region were not measured.

^fICRP 66 deposition fractions are for members of the public that breathe 1 µm AMAD particles through the nose and are engaged in light exercises (values for mouth breathers are unavailable).

^gComputational estimates using anatomical models devised by other investigators.

keeping the period of inspiration time fixed, or kept the flow rate of air constant while varying the inspiration time period. Results of Stahlhofen *et al.* (1983) compare reasonably well with the probabilistic estimates determined in this study; however, this interpretation may vary based on results determined under various experimental conditions.

The extrathoracic deposition fraction estimates developed in this study do not compare well with the results from Stahlhofen *et al.* (1983), who observed no regional deposition of particulates in the extrathoracic region. Particles of these sizes, 1 and 2 μm aerodynamic diameters, are expected to be deposited in the extrathoracic regions; therefore, the discrepancy is of limited concern. This trend is consistent for individuals, regardless of the breathing pattern and gender; therefore, extrathoracic deposition estimates are contrasted with theoretical data (Harvey, 2003).

BB deposition estimates in this study compare reasonably well with the results achieved by Stahlhofen *et al.* (1983) for mouth breathers with a fixed inspiration period of 2 s and varying air flow rates (Harvey, 2003). Their experiments, where flow rates are fixed at $250 \text{ cm}^3 \cdot \text{s}^{-1}$ and the inspiration time period was varied, demonstrated the absence of particulate deposition in the BB region (Table 27.4). This data could not be used for comparison to results from other investigators. The results of Stahlhofen *et al.* (1983) are outside the one standard deviation range of the probabilistic estimate for adult male and female mouth breathers, although the results are within two standard deviations (Harvey, 2003).

The pulmonary deposition estimates in mouth-breathing individuals determined here compare well with the results of Stahlhofen *et al.* (1983) for inspiration periods of 1 and 2 s (Table 27.4). Their results show increasing amounts of alveolar deposition as the inspiration period is increased to a period of 4 s (Table 27.4). At longer inspiration time periods, the results determined by Stahlhofen *et al.* (1983) and those developed herein become more significantly different. Increased deposition of particulates occurs due to the increased time for deposition during longer inspiration periods, thus resulting in differences between the Stahlhofen data and our probabilistic deposition estimates. Pulmonary deposition fractions determined by Stahlhofen *et al.* (1983) fall within the 95% confidence interval of the probabilistic deposition estimate with the exception of the 4 s inspiration period (Harvey, 2003).

The Stahlhofen data are for mouth breathers only; therefore, nose breather deposition estimates are compared to experimental and theoretical results from other investigators. Most regional deposition results, when Stahlhofen *et al.* (1983) found particulate deposition, are within a factor of 2 and compare reasonably well with the deposition estimates determined here (Table 27.4 and Harvey, 2003).

Miller *et al.* (1988) have experimentally determined regional deposition of particulates in nose and mouth breathers for 1 μm aerodynamic diameter particles (Table 27.4). They did not determine particulate deposition in the extrathoracic region of the respiratory tract. Their BB deposition results compare reasonably well with the particulate deposition estimates herein. The results of Miller *et al.* (1988) for BB deposition are slightly reduced, as compared to the particulate deposition estimates determined in this assessment (Table 27.4 and Harvey, 2003). Their BB deposition results fall within the 68% confidence interval of the probabilistic deposition estimate, with the exception of adult female mouth breathers (Harvey, 2003). The pulmonary deposition results of Miller *et al.* (1988) were significantly greater as compared to the results achieved here (Table 27.4 and Harvey, 2003). The pulmonary deposition fraction determined by Miller *et al.* (1988) falls outside the 95% confidence interval of the pulmonary probabilistic deposition estimate, regardless of the gender and breathing pattern (Harvey, 2003). All results are within a factor of 2.

Regional deposition comparison to theoretical results of other investigators

Theoretical results have been determined by the ICRP 66 Committee (1994) and by Yu and Diu (1982). The ICRP 66 results are very similar to that determined herein (Table 27.4). Yu and Diu (1982) used four different anatomical models to determine regional deposition in nose breathers. The anatomical models employed were those of Weibel (1963), Olson *et al.* (1970), Hansen and Ampaya (1975) and Yeh and Schum (1980). All regional deposition estimates were within a factor of 2, thus demonstrating reasonable agreement (Table 27.4).

Extrathoracic deposition in adult nose breathers was slightly less than that determined by Yu and Diu (1982). Figure 27.1 graphically depicts the theoretical results

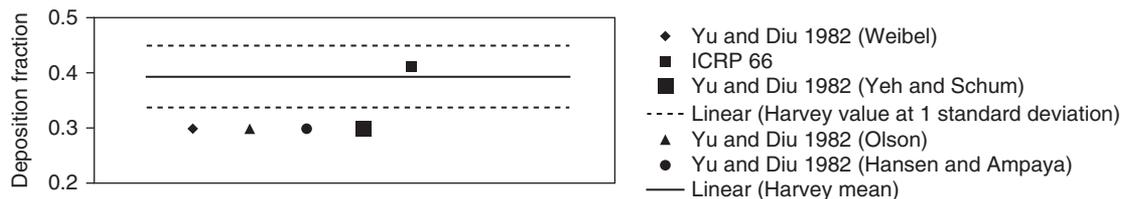


Figure 27.1 Extrathoracic deposition for adult male nose breathers.

determined by other investigators and the probabilistic estimate determined in this work for adult male nose breathers. Theoretical estimates of extrathoracic deposition in adult nose breathers by Yu and Diu (1982) fall outside the 68% confidence interval of the present study's probabilistic deposition estimate for extrathoracic deposition (Figure 27.1 and Harvey, 2003). ICRP 66 estimates of extrathoracic deposition, however, are within the 68% confidence interval. The absence of theoretical data for mouth breathers necessitated the use of nose breather data from Yu and Diu (1982) for comparison to the probabilistic deposition estimates in the extrathoracic region of mouth breathers. Investigators' theoretical estimates of extrathoracic deposition fall within one standard deviation of the mean determined for extrathoracic probabilistic deposition estimates in this work (Harvey, 2003).

Theoretical and experimental BB deposition estimates determined by other investigators fall within one standard deviation of the mean in this study's probabilistic estimate of BB deposition for adult nose breathers (Figure 27.2 and Harvey, 2003). For example, Figure 27.2 shows this work's results as compared to those of other investigators for adult male nose breathers.

Theoretical pulmonary deposition estimates for adult nose breathers are similar, and the investigators' results fell inside the one standard deviation range of the probabilistic estimate determined herein (Figure 27.3). Theoretical results by multiple investigators demonstrate good agreement for

regional deposition estimates in adult nose breathers (Figure 27.3 and Harvey, 2003). Theoretical deposition estimates for adult mouth breathers were unavailable.

Total lung deposition comparison to experimental results of other investigators

Probabilistic deposition estimates for the total lung in this assessment have been compared to the experimental results determined by other investigators (Table 27.5). Experimental conditions vary among investigators; therefore, some differences are expected in the comparison of experimental results and the probabilistic estimates determined herein.

For example, results determined for adult female nose breathers are shown in Figure 27.4. Even though this group uses $2\mu\text{m}$ particles, the results of Stahlhofen *et al.* (1983) show good agreement with the probabilistic estimates determined herein for mouth breathers (Figure 27.4 and Harvey, 2003). The results of Stahlhofen *et al.* (1983) are within one standard deviation of this study's mean.

Miller *et al.* (1988) show reasonable agreement with this study's probabilistic estimate for adults, regardless of breathing pattern and gender (Table 27.5). Results are within a factor of 2 in all cases. The total lung deposition estimates of Miller *et al.* (1988) in nose breathers do not fall inside the 68% confidence interval of probabilistic deposition for the total lung as determined in this work; however, their deposition fraction results are within two

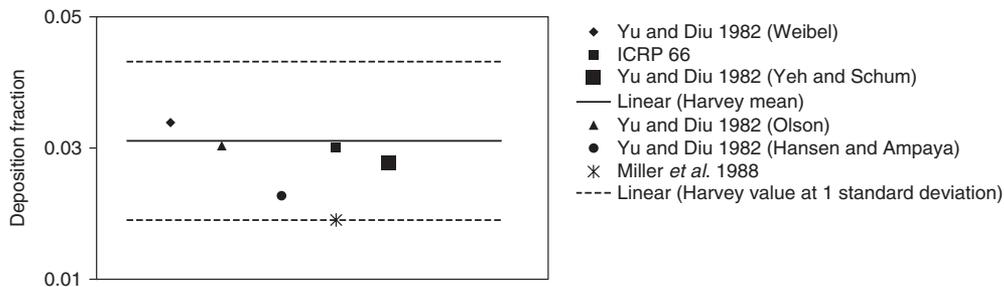


Figure 27.2 Bronchial deposition for adult male nose breathers.

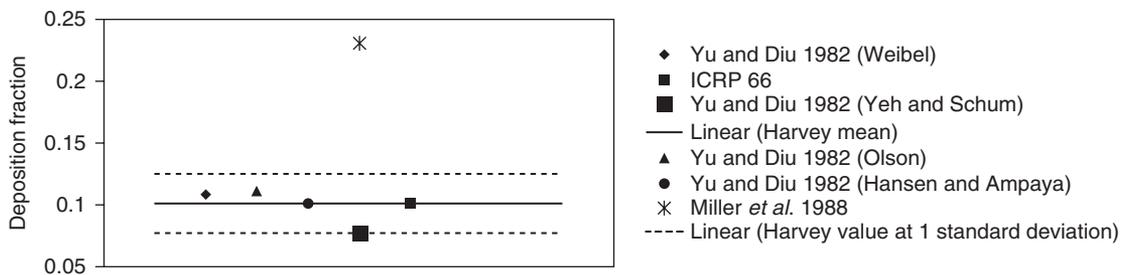


Figure 27.3 Alveolar deposition for adult male nose breathers.

Table 27.5 Total lung deposition fractions (unitless) in adults

Investigator	Breathing pattern ^{a,b}	Particle size (μm)	Deposition fraction	
Harvey ^c	NB (AM)	1.0	0.13	
	MB (AM)	1.0	0.18	
	NB (AF)	1.0	0.14	
	MB (AF)	1.0	0.18	
Stahlhofen <i>et al.</i> , 1983 ^d	P = 2, Q = 750	2.0	0.17	
	P = 2, Q = 250	2.0	0.14	
	P = 1, Q = 250	2.0	0.10	
	P = 2, Q = 250	2.0	0.22	
	P = 4, Q = 250	2.0	0.50	
Miller <i>et al.</i> , 1988	NB	1.0	0.25	
	MB	1.0	0.27	
Heyder <i>et al.</i> , 1975 ^e	$V_t = 250$	NB	1.0	0.15
	$V_t = 500$	NB	1.0	0.25
	$V_t = 1000$	NB	1.0	0.36
	$V_t = 1500$	NB	1.0	0.46
	$V_t = 250$	MB	1.0	0.07
	$V_t = 500$	MB	1.0	0.14
	$V_t = 1000$	MB	1.0	0.24
	$V_t = 1500$	MB	1.0	0.35
ICRP 66 ^f	NB (AM)	1.0	0.13	
	NB (AF)	1.0	0.13	
Yu and Diu, 1982 ^g	Weibel	NB	1.0	0.15
	Olson	NB	1.0	0.13
	Hansen and Ampaya	NB	1.0	0.13
	Yeh and Schum	NB	1.0	0.11

^aNB for nose breathers and MB for mouth breathers.
^bAM for adult male and AF for adult female.
^cDeposition fractions are for members of the public that breathe 1 μm AMAD particles.
^dP for period of inspiration time and Q for flow rate with units of s and $\text{cm}^3 \cdot \text{s}^{-1}$, respectively.
^e V_t for tidal volume with units of cm^3 .
^fICRP 66 deposition fractions are for members of the public that breathe 1 μm AMAD particles through the nose and are engaged in light exercise (values for mouth breathers are unavailable).
^gComputational estimates using anatomical models devised by other investigators.

standard deviations of the probabilistic mean (Figure 27.4 and Harvey, 2003). The results of Miller *et al.* (1988) for mouth breathing individuals are within one standard deviation of the probabilistic mean.

Total lung deposition estimates were determined by Heyder *et al.* (1975) for nose and mouth breathers at various tidal volumes of air (Table 27.5). Their deposition estimates for nose breathers at tidal volumes of 250 and 500 cm^3 demonstrate reasonable agreement with probabilistic deposition estimates for adult nose breathers; the results are within a factor of 2. At increased tidal volumes of 1000 and 1500 cm^3 , however, their results do not compare well with this assessment's probabilistic estimates. This occurs due to the significantly reduced volumetric flow rates of air that they used, as compared to those employed by our theoretical estimates. As volumetric flow rate is increased, a reduction in particle deposition occurs in the lung region, due to the decreased residence time in the respiratory tract.

The deposition estimates that Heyder *et al.* (1975) determined were at various tidal volumes for mouth breathers. Their deposition estimates for tidal volumes between 500 and 1500 cm^3 are within a factor of 2 of this study's mean for probabilistic deposition in the total lung of mouth breathers. Our deposition fraction estimates are within a factor of 3 of the results determined by Heyder *et al.* (1975) for a V_T of 250 cm^3 .

Total lung deposition comparison to theoretical results of other investigators

Theoretical results have been determined for total lung deposition in adults based on breathing pattern. Theoretical results for mouth breathers were not found in the literature; therefore, this comparison is limited to nose breathing individuals. The estimates determined in this study have been compared to the results of the ICRP 66 (1994) and the results of Yu and Diu (1982). These theoretical estimates are shown in Table 27.5.

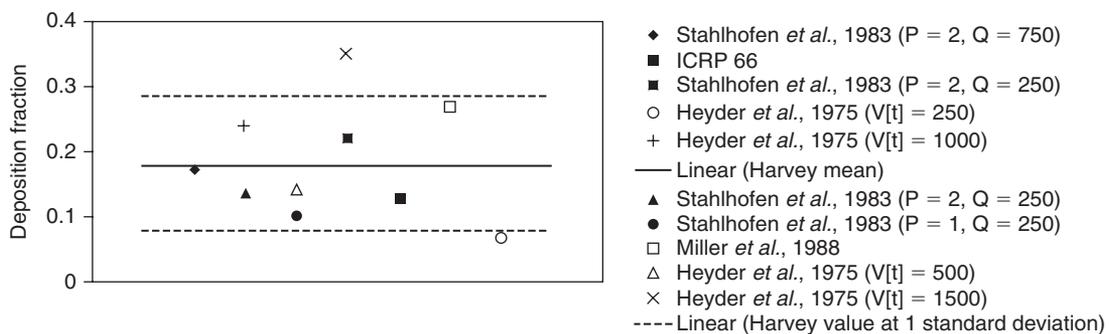


Figure 27.4 Total lung deposition for adult female mouth breathers.

The theoretical results of the ICRP and the present study show good agreement. The mean determined in this study and the ICRP estimate are the same for adult male and female nose breathers, the ICRP results being within one standard deviation of the probabilistic mean (Harvey, 2003).

The probabilistic estimates and their means compare well with the theoretical results of Yu and Diu (1982). The probabilistic mean of this work and the deposition estimate, using the models of Olson *et al.* (1970) and Hansen and Ampaya (1975), have the same value for adult male nose breathers. The results of Yu and Diu (1982), using the Weibel (1963) model and the Yeh and Schum (1980) model, are very similar to the present study's mean for adult male nose breathers. The mean determined herein for adult female nose breathers is very similar to the results of Yu and Diu (1982). All of the theoretical estimates for total lung deposition are within one standard deviation of the mean for adult female nose breathers determined in this study. Although theoretical estimates for particulate deposition in mouth breathers were unavailable, a comparison of this work's mouth breathing probabilistic estimates and the ICRP results for adult nose breathers was performed (Figure 27.4 and Harvey, 2003). The ICRP 66 theoretical results for nose breathing adults are within one standard deviation of the means determined herein for mouth breathing adults.

Conclusions

Better estimates of fractional deposition will improve our ability to estimate the committed dose equivalent to the lung and subsequent movement of particles to the bloodstream. Modeling of the regional deposition of particulate material within the lungs has inherent uncertainty, which depends on the input parameters, breathing patterns and gender. The resulting distribution and characteristics of each region contribute to the total uncertainty of particulate deposition, and thus the overall uncertainty of inhalation dose estimates. The particulate deposition estimates for the extrathoracic and BB regions are best described by a normal distribution. In contrast, estimates of particulate deposition in the bb and AI regions of the lung are best characterized by a log-normal distribution.

Generally, fractional deposition in the lung, as modeled in ICRP 66 (1994), is directly proportional to particle mass density and BR, and inversely proportional to trachea diameter. Other parameters play a relatively minor role in modeling regional deposition within the respiratory tract for adults, adolescents and 10-year-old children. The parameters of anatomical dead space and windspeed are more important to deposition in infants and children. Research into these more sensitive parameters and their distributions may lead to reduction in the uncertainty of

the deposition model of ICRP 66. Dose estimate uncertainties also can be reduced when data on particle size and density are gathered at the time of exposure.

Particulate deposition estimates determined herein compare reasonably well with experimental and theoretical results of other investigators. There are some expected differences in particulate deposition estimates, due to experimental conditions and specific investigator values for individual parameters.

Summary Points

- Radioiodine may be released to the environment as a particulate; therefore, particle deposition and dose assessment are necessary for members of the public.
- Extrathoracic and bronchial depositions follow a normal distribution.
- Bronchiolar and alveolar deposition are best fit by a log-normal distribution.
- Breathing rate, trachea diameter and particle mass density have the greatest influence on particulate deposition for 1 μm AMAD particles.
- The parameters used for dose assessment have inherent variability, which results in uncertainty for output variables that may be characterized by various known distributions.

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The Relationship between Thiocyanate and Iodine

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Abstract

Large amounts of thiocyanate are generated in people with a high intake of cyanide from tobacco smoking, from cyanide in food, or from industrial pollution of the environment with cyanide. Thiocyanate may also be directly consumed with certain foods. Thiocyanate is a competitive inhibitor of the sodium iodide symporter (NIS) at thiocyanate levels normally found in blood. Thereby, it worsens iodine deficiency by inhibition of thyroidal iodide accumulation and by inhibition of iodide transport into breast milk for infant nutrition. Cessation of smoking, reduction of industrial pollution and improved diet will reduce the role of thiocyanate in thyroid disease. In individuals exposed to high levels of thiocyanate, adverse effects may be prevented by an increase in iodine intake.

Abbreviations

NIS Sodium iodide symporter

Introduction

Thiocyanate [SCN^-], the anion of thiocyanic acid, is an important waste product from the chemical industry, it is present in various food items, it may be added to dairy milk to promote bacteriostatic processes, and it is synthesized in the human liver as part of detoxification of cyanide. A special case in medicine has been generation of large amounts of thiocyanate leading to hypothyroidism after sodium nitroprusside infusion for hypertension (Nourok *et al.*, 1964). After oral ingestion thiocyanate is readily absorbed. It is partly bound to albumin in blood, and mainly eliminated by excretion in urine. The half life of thiocyanate in humans is in the order of 1–2 weeks (Scherer, 2006), but varies between individuals. Thiocyanate may have a number of

biological roles (Weuffen *et al.*, 1990). Among these, interaction with iodide transport and thyroid hormone synthesis is the most important. As the overall effect of thiocyanate is to hamper utilization of iodide, the main effect of thiocyanate is to worsen iodine deficiency. By this mechanism thiocyanate is one of the most important environmental compounds influencing the occurrence of thyroid disease.

Thiocyanate and NIS

The most well-characterized effect of thiocyanate on iodine utilization is competitive inhibition of the sodium iodide symporter (NIS), although inhibition of iodide organification may also take place (Greer *et al.*, 1966). The inhibitory effect of thiocyanate on thyroidal iodide uptake (Wolff, 1964) and on iodide transport in the mammary gland (Brown-Grant, 1957), as well as elsewhere (Brown-Grant, 1961), was first described many years ago. More recently, human NIS has been cloned and characterized in detail (Levy *et al.*, 1997; Spitzweg *et al.*, 1998). *In vitro* studies illustrating the competition between iodide and various compounds, including thiocyanate, for transport by NIS have been performed by a number of investigators (Van Sande *et al.*, 2003; Tonacchera *et al.*, 2004). As shown in **Figure 28.1**, thiocyanate is one of the most powerful inhibitors of iodide transport.

NIS is found in various organs (Spitzweg *et al.*, 1998) and has important biological roles in the thyroid, the lactating mammary gland (Tazebay *et al.*, 2000), and probably the placenta (Logothetopoulos and Scott, 1956; Manley *et al.*, 2005). The serum concentration of thiocyanate in smokers and after oral ingestion of thiocyanate or cyanide-containing foods reaches a level around $100 \mu\text{mol/l}$. At this level NIS transport of iodide is clearly affected, even if part of the thiocyanate is bound to albumin (**Figure 28.1**).

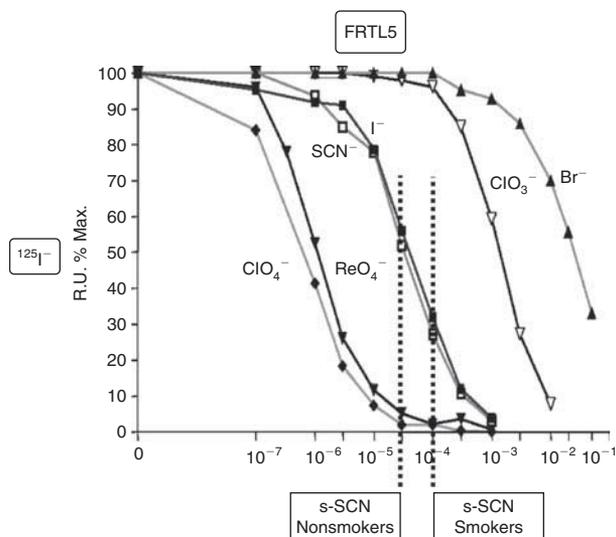


Figure 28.1 Anion selectivity by the sodium iodide symporter. Inhibition of the transport of $^{125}\text{I}^-$, by competing anions (NaI, NaBr, NaClO_4 , NaClO_3 , NaSCN, NaReO_4) in FRTL5 cells and COS NIS-6 cells *in vitro*. The competitor and tracer anions were added together, and the $^{125}\text{I}^-$ uptake was measured after 1 h. Reproduced from Van Sande *et al.*, (2003) with permission.

Thiocyanate and Tobacco Smoking

Tobacco smoke contains considerable amounts of cyanide, which is generated in the burning zone of the tobacco from proteins and nitrate at high temperatures. The amount of cyanide generated from a single cigarette may differ widely depending on the smoking conditions (Scherer, 2006). The cyanide is highly toxic but is rapidly detoxified in the liver of the smoker to thiocyanate by a sulfation process.

Serum concentrations of thiocyanate are two to three times higher in smokers than in nonsmokers. In populations not exposed to excessive amounts of thiocyanate from diet or industrial environmental pollution, thiocyanate concentrations in serum can be used to identify and quantify tobacco smoking (Scherer, 2006; Butts *et al.*, 1974). Because of the variability in cyanide generation from smoking a cigarette, the correlation between serum (or urine) concentration of thiocyanate and the number of cigarettes smoked per day is rather low (Foss and Lund-Larsen, 1986).

Other sources of thiocyanate may interfere with the use of this compound as a marker of smoking, and more specific markers such as cotinine in serum have now replaced thiocyanate for evaluation of smoking (Luck and Nau, 1984; Pichini *et al.*, 2000).

In a Belgian study the levels of thiocyanate in serum from heavy smokers were not much different from the serum concentrations found in people in Central Africa with a high intake of cyanide from only partly detoxified cassava (Delange *et al.*, 1980). Thus, heavy smoking may be as important in the generation of thiocyanate as

is severe dietary intake of cyanide, and serum levels of thiocyanate in smokers overlap the levels found in people exposed to severe industrial pollution.

Interaction between Smoking and Iodine Intake

Overall, tobacco smoking increases the risks for Graves' disease, and especially for Graves' orbitopathy (Wiersinga and Bartalena, 2002) and goiter (Knudsen *et al.*, 2002a). On the other hand, smoking may decrease the risk for thyroid cancer (Mack *et al.*, 2003) and for subclinical hypothyroidism (Knudsen *et al.*, 2002b; Belin *et al.*, 2004). Only the change in risk for goiter (and possibly subclinical hypothyroidism) seems to be mediated by thiocyanate (Laurberg *et al.*, 2007).

The important interaction between smoking and low iodine intake was well-illustrated in the DanThyr program (Laurberg *et al.*, 2006). This study included randomly selected people within predefined age and sex groups from two Danish subpopulations (Aalborg and Copenhagen), with a small difference in iodine intake level because of differences in groundwater iodine content. Median spot urinary iodine concentrations in people not taking iodine supplements were 45 $\mu\text{g}/\text{l}$ in Aalborg and 61 $\mu\text{g}/\text{l}$ in Copenhagen. All participants were investigated by thyroid ultrasonography using validated procedures (Knudsen *et al.*, 1999). As illustrated in Figure 28.2, both serum thyroglobulin (as a marker of iodine deficiency; Knudsen *et al.*, 2001) and thyroid volume increased with smoking. The increase differed between areas, and was more pronounced in the area with the lowest iodine intake (Figure 28.2), supporting the theory that smoking had impaired iodine utilization.

An even more significant interaction was found when previous pregnancy was taken into account. Pregnancy and lactation result in an increase in the need for iodine (Glinoe, 2001). Thus, previous pregnancy may be a risk factor for having goiter later in life. Figure 28.3 shows the effect of smoking on thyroid volume in female participants of the DanThyr cohort. The effect of smoking was much more pronounced in parous women compared to nulliparous. Previous pregnancy was not associated with an increase in thyroid volume in nonsmokers.

An effect of smoking on the prevalence of goiter has been found in many studies (Knudsen *et al.*, 2002a), and all evidence suggests that this effect is caused by thiocyanate inhibition of the utilization of iodide.

Thiocyanate and Iodine in Milk

The lactating mammary gland concentrates iodide from blood into milk, and thereby delivers necessary substrate for neonatal and infant thyroid hormone production. NIS in the mammary gland is identical to thyroidal

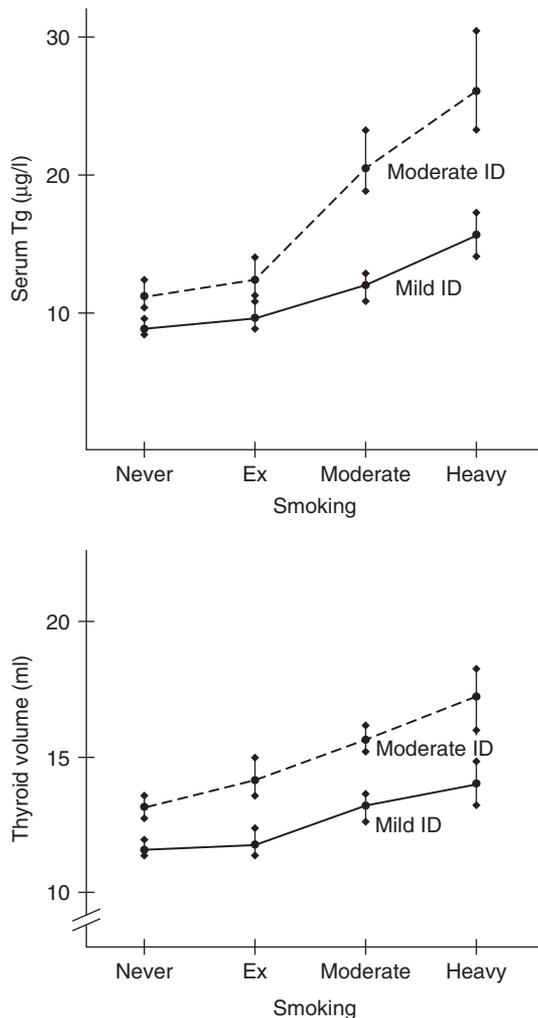


Figure 28.2 Interaction between smoking and low iodine intake. The association between tobacco smoking and serum thyroglobulin (upper panel) and between smoking and thyroid volume (lower panel) in two areas with different iodine intake. Data from the DanThyr population study including 4649 subjects. Significant associations with tobacco smoking were found for both thyroglobulin and thyroid volume in both areas. The association was significantly stronger in the area with the most pronounced iodine deficiency. Reproduced from Knudsen *et al.*, (2002a) with permission.

NIS (Tazebay *et al.*, 2000). However, the characteristic autoregulation by iodide of thyroidal NIS is absent, or at least much less developed, in the mammary gland. This difference in the effect of iodide is probably caused by thyroid autoregulation being mediated by organic iodide-containing substances only synthesized in the thyroid. Thus, breast milk iodide concentration varies much in parallel with iodine intake (Laurberg *et al.*, 2002). Inhibition of iodide transport into milk by thiocyanate was demonstrated in the lactating rabbit by Brown-Grant (1956), with a fall in ^{131}I molar ratio of milk/plasma from about 20 to unity after injection of thiocyanate into blood.

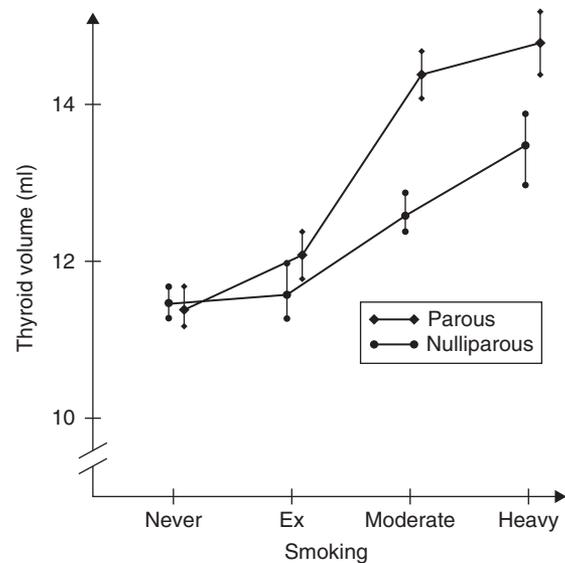


Figure 28.3 Interaction between previous pregnancy, smoking and low iodine intake. The association between tobacco smoking, parity and thyroid volume in the DanThyr population study, including 3712 women. A significant statistical interaction was found for tobacco smoking on the association between parity and thyroid volume. Reproduced from Knudsen *et al.*, (2002a) with permission.

Many plants, especially of the *Cruciferae* family, contain substances that may generate thiocyanate and they also contain a number of other goitrogens. Rape (*Brassica napus*) is an illustrative example of thiocyanate generation in plants of potential importance for human iodide nutrition. Rape is a 60–100 cm tall plant with yellow flowers, widely grown in many countries because the seeds contain 30–40% of oil. In addition, rapeseed contains high levels of protein, and the press-cakes generated after harvesting of the oil may be used for feeding domestic animals as a substitute for soybean proteins.

However, the seeds also contain sulfur-containing phytochemicals called glucosinolates. During processing of the seeds, the glucosinolates come into contact with the hydrolytic enzyme myrosinase, which is normally present in the seeds in a separate compartment. Hydrolysis of glucosinolates generates a variety of products, some of which may have goitrogenic effects. One of these products is thiocyanate (Bones and Rossiter, 2006).

A series of studies of domestic animals have demonstrated how thiocyanate-rich rapeseed feed may interfere with iodine utilization in animals (Laurberg *et al.*, 2002). The considerable effect of rapeseed feed on excretion of iodine in cow's milk is illustrated in Figure 28.4.

In sows given rapeseed, it has been shown that the low iodine transfer via sows milk to piglets caused by rapeseed inhibition of iodide transport into the milk leads to hypothyroidism and developmental defects in the piglets, and that this is preventable by iodine administration (Schöne *et al.*,

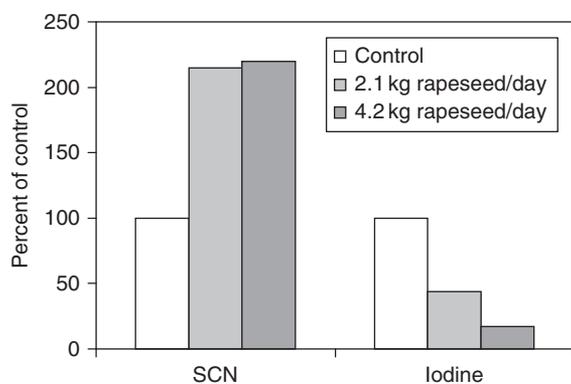


Figure 28.4 Thiocyanate and iodine in dairy milk. Milk content of thiocyanate and iodine during feeding with high glucosinolate rapeseed meal. Eighteen high-yielding dairy cows were randomly assigned to control (without rapeseed) and two levels of rapeseed intake for 3 weeks. Thiocyanate in milk was significantly higher and iodine significantly lower during rapeseed feeding ($P < 0.001$). Data from Hermansen *et al.*, (1995).

1997). Similar inhibition of mammary iodide transport may be induced by direct administration of thiocyanate.

No studies of humans have demonstrated inhibition of iodide transport into breast milk of mothers from thiocyanate in diet. In many countries the most important determinant of thiocyanate levels in blood is tobacco smoking, as discussed above, and smoking is associated with similar increases in thiocyanate in blood as may be found from diets with high cyanide content. We therefore investigated the effect of maternal smoking on iodine transfer to the breast-fed neonate.

Maternal Smoking and Iodine Nutrition of the Breast-Fed Child

Maternal smoking during pregnancy is now firmly advised against in Denmark as elsewhere, but this is a relatively new phenomenon. Twenty years ago there was less focus on the side-effects of smoking during pregnancy, and this was rather common in Denmark. As part of a characterization of iodine nutrition in Denmark we had previously (1987–1988) sampled serum from healthy pregnant women shortly after admission for labor and from cord blood. On day 5 after delivery we had collected spot urine from the mother and child and a spot breast milk sample. Results of measurements of iodine in urine and milk and thyroid function tests had been published (Nøhr *et al.*, 1993; Nøhr and Laurberg, 2000), but extra serum and urine samples had been kept frozen. In the late eighties there were few restrictions on smoking in hospitals, and mothers in general continued their usual smoking habits in the maternity ward. We hardly focused on smoking at the time of the initial study; hence smoking was not discussed with the participants of the study, and we had no data. However, from

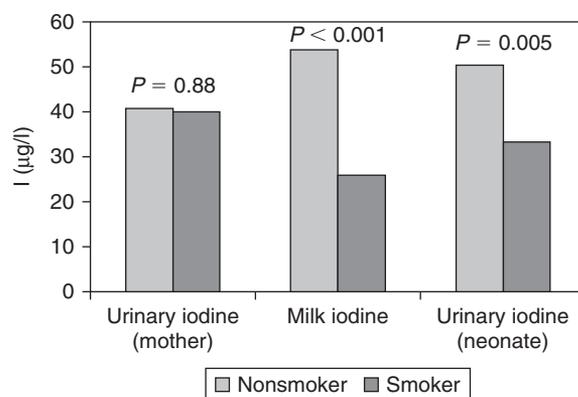


Figure 28.5 Smoking and iodine in breast milk. Comparison of urinary and breast-milk iodine contents in smoking and nonsmoking mothers. Morning samples of urine and breast milk from mothers and urine from breast-fed infants were collected on day 5 after delivery. For calculation of geometric mean values and statistical comparisons logarithmically transformed data were used. Iodine 1 µg/l corresponds to 7.88 nmol/l. Reproduced from Laurberg *et al.*, (2004) with permission.

the frozen samples patients could be clearly characterized as smokers or nonsmokers from measurements of cotinine in serum and urine (Laurberg *et al.*, 2004).

Figure 28.5 shows the iodine concentrations in urine and milk from nonsmoking and smoking mothers, and in urine from their neonates. Whereas urinary iodine concentrations were not different between groups of mothers, the iodine content of breast milk and of neonatal urine was reduced to around 50% if the mother was a smoker. This effect of smoking varied with the cotinine concentrations in mothers, and with the levels of thiocyanate in serum from the mothers and in cord serum (Laurberg *et al.*, 2004).

Urinary iodide excretion varies with plasma inorganic iodide concentration and it is not changed by inhibition of NIS (Brown-Grant, 1961). Iodide transport into breast milk also seems to vary with plasma iodide concentration, and NIS in the mammary gland is not autoregulated by iodide as is the thyroidal NIS. Thus, inhibition of mammary NIS can be evaluated by calculating the ratio between iodine in milk and iodine in urine in an individual. As the time sequence of iodide appearance may differ between urine and breast milk, the ratio can only be used in groups of women. This ratio is shown for the nonsmoking and the smoking mothers in Figure 28.6. The milk/urine iodine ratio was around 50% in the smoking mothers compared with the nonsmokers. As might be expected, the ratio between iodine in urine from the neonate/mother was also much lower in the smokers.

Interestingly, the ratio between iodine in neonate urine and breast milk was higher if the mother was a smoker (Figure 28.6). This probably indicates that iodide utilization by the neonatal thyroid was impaired if the mother was a smoker, even if the supply of iodine via breast milk was reduced.

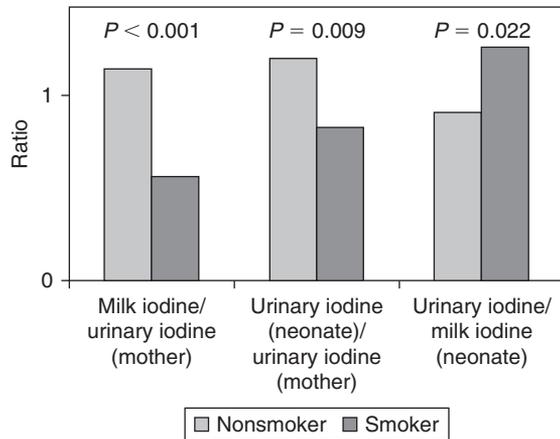


Figure 28.6 Maternal smoking and neonatal iodine nutrition. Breast-milk iodine and infant's urinary iodine content expressed as fraction of the mother's urinary iodine concentration. Both are measures of iodine transfer from mother to child during breast-feeding. The infant's urinary iodine content is expressed as a fraction of milk iodine content and is an inverse measure of iodine retention in the infant. Reproduced from Laurberg *et al.*, (2004) with permission.

Thiocyanate crosses the placenta, and serum levels were similar in mothers' serum and in serum from cord blood (Laurberg *et al.*, 2004). Thiocyanate has a long half life, and thiocyanate received from the smoking mother in fetal life may have induced a partial block of thyroidal NIS in the neonate. The levels of thiocyanate in breast milk are rather low, but higher in smokers, and passive neonatal smoking from mothers who smoke might also lead to inhibition of thyroidal iodide accumulation in the neonate.

The results shown in Figures 28.5 and 28.6 suggest that thiocyanate in smokers reduces NIS activity in the organs where this is operating by around 50%. In the thyroid such a reduction will be counteracted by iodide autoregulation to keep thyroid iodide uptake sufficient for hormone synthesis. However, up-regulation of thyroid activity may increase the risk of goiter. As illustrated in Figure 28.2, moderate-to-heavy smoking among people living in Copenhagen (median urinary iodine concentration 61 $\mu\text{g}/\text{l}$) was associated with serum Tg levels and thyroid volume corresponding to those found in nonsmokers living in Aalborg (median urinary iodine 45 $\mu\text{g}/\text{l}$). Thus, smoking and mild iodine deficiency, in combination, corresponded to being a nonsmoker living with moderate iodine deficiency.

Thiocyanate from Food: The Experience from Central Africa

Thiocyanate may be contained in food or generated in the liver after ingestion of cyanide-containing food. The most well-documented examples of the devastating effects of

a combination of iodine deficiency and high thiocyanate levels have been obtained in Central Africa. In Congo, myxoedematous cretinism was common in certain areas with low iodine intake. Characteristically, people living in these areas also had a high intake of cyanide from insufficiently detoxified cassava, and they had high levels of thiocyanate in the blood (Vanderpas *et al.*, 1984; Delange *et al.*, 1972; Bourdoux *et al.*, 1978). The developmental defects associated with myxoedematous cretinism seem to be caused by insufficient thyroid hormone supply in late fetal life, and during infancy and childhood, similar to sporadic congenital hypothyroidism (Boyages and Halpern, 1993).

Characteristically, the thyroid undergoes gradual involution in patients with myxoedematous cretinism, as opposed to the endemic goiter found in other members of the society (Delange and Ermans, 1976). Undoubtedly, high thiocyanate generation in people in this area of Congo worsened the consequences of iodine deficiency, both because of inhibition of thyroidal and mammary gland NIS. The possible role of inhibition of placental NIS (Logothetopoulos and Scott, 1956; Manley *et al.*, 2005) has not been elucidated. The cause of the gradual thyroid involution in patients with myxoedematous cretinism has not been firmly established. The thyroid insufficiency may be reversible during the first years of life (Vanderpas *et al.*, 1986). It has been suggested that selenium deficiency and high thiocyanate levels (Contempré *et al.*, 2004) may, in combination with iodine deficiency, lead to gradual thyroid necrosis and fibrosis, as discussed elsewhere in this book. It is conceivable that thiocyanate may, by several mechanisms, play a role in the early postnatal development of mental deficiency in patients with myxoedematous cretinism in Central Africa. Recently, data obtained in Congo in 1980 on iodine content of breast milk in mothers with various degrees of thiocyanate exposure and iodine deficiency have been re-evaluated (Vanderpas, 2007). The results confirmed the inhibitory effect of thiocyanate on iodine excretion in milk in iodine-deficient mothers.

The excretion of thiocyanate into milk is limited. However, thiocyanate may occasionally be added to dairy milk to activate the lactoperoxidase peroxide system, and thereby retard bacterial growth in raw milk. In India, ingestion of such milk led to impairment of thyroid function (Banerjee *et al.*, 1997a), whereas a short-term study from Sweden did not find any effect (Dahlberg *et al.*, 1984).

Another source of excess thiocyanate that may interfere with thyroid function that has been described in India (Banerjee *et al.*, 1997b) and elsewhere (Brauer *et al.*, 2006) is environmental pollution from industry. Cyanide is involved in, or generated as part of, many industrial processes. Thiocyanate may be synthesized from cyanide already at the factory, as part of the detoxification of industrial waste (Ebbs, 2004), or in the liver of cyanide exposed individuals. The effects of thiocyanate from industrial environmental pollution are similar to those described

above in heavy tobacco smokers and in people with high cyanide intake from food.

Conclusion

Thiocyanate is widespread in the environment and may play an important role in the development of disease by worsening the level of iodine deficiency.

Summary Points

- Thiocyanate in blood may originate from tobacco smoking, from industrial pollution of environment or from ingestion of certain foods.
- Thiocyanate is a competitive inhibitor of the sodium iodide transporter responsible for the accumulation of iodide in the thyroid gland and in breast milk.
- Exposure to thiocyanate corresponds to a decrease in iodine intake.
- In areas of low iodine intake, thiocyanate exposure increases the risk of developmental and other iodine deficiency disorders.

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Environmental Perchlorate and the Thyroid

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Abstract

Perchlorate is a competitive inhibitor of the sodium–iodide symporter (NIS) and has been used in pharmacological doses to treat hyperthyroidism, especially iodine-induced hyperthyroidism. Perchlorate appears to be ubiquitous in the environment, and has been detected in trace amounts in the urine in almost all subjects evaluated both in the United States and Europe. In prospective clinical studies and environmental studies, there is no convincing evidence that environmental perchlorate adversely affects thyroid function.

Abbreviations

HPLC	High-performance liquid chromatography
NIS	Sodium–iodide symporter
TSH	Thyrotropin/thyroid stimulating hormone
T4	Thyroxine
T3	Triiodothyronine

Perchlorate is a potent competitive inhibitor of the sodium–iodide symporter (NIS), a protein on the thyroid follicular cell membrane responsible for the active transport of iodine from the blood into the thyroid follicular lumen, and in the lactating breast providing the nursing infant with milk sufficient in iodine (De Groef *et al.*, 2006). Since iodine is essential for thyroid hormone synthesis, decreases in intrathyroidal iodine could potentially decrease the synthesis of the thyroid hormone, resulting in goiter and hypothyroidism, as occurs in regions of the developing world where iodine deficiency is pronounced. In areas where iodine deficiency is marginal, including regions of Europe, compensatory diffuse and nodular goiters are not uncommon.

This ability of perchlorate to decrease iodine uptake by the thyroid made it suitable to be used as an antithyroid drug in the therapy of hyperthyroidism. Indeed, perchlorate was

used successfully in the therapy of hyperthyroidism in the 1960s and 1970s, but caused aplastic anemia and death in a few reported cases treated with large doses (600–1600 mg/d) and its use was discontinued (Barzilai and Sheinfeld, 1966). However, more recent studies have demonstrated efficacy, with no severe side effects in the therapy of Graves' disease and iodine-induced hyperthyroidism, in doses up to 600 mg daily (Wenzel and Lente, 1984; Martino *et al.*, 1986).

Perchlorate salts are potent oxidizers and are used in solid propellants for rockets and missiles, fireworks, road flares, matches, and auto air bag inflation systems. They are also found in Chilean fertilizers, a wide variety of foods including vegetables and cows milk, prenatal vitamins, and as a result of natural processes (Dasgupta *et al.*, 2005). Thus, it appears likely that perchlorate is a naturally occurring anion and is probably ubiquitous.

Since 1997, methods have been developed to enable the detection of perchlorate in low levels, first by high-performance liquid chromatography (HPLC) and, more recently, by ion chromatography–mass spectrometry, with a detection level of 0.025 µg/l in urine (Valentin-Blasini *et al.*, 2005). Concern has been raised in the United States that drinking water contains perchlorate, especially in regions where perchlorate was manufactured, resulting in contamination of ground water with perchlorate which is extremely stable and not biodegradable. Perchlorate has been detected in tap water in concentrations ranging from 1 to 200 µg/l, especially in California and the Southwestern US. These findings, over the past several years, have raised concerns that low levels of consumed perchlorate could pose a public health risk and cause thyroid disease. These concerns have resulted in many recent reviews based upon theoretical considerations of these potential adverse effects (De Groef *et al.*, 2006; Ginsberg *et al.*, 2007; Hershman, 2005).

Several clinical studies have not reported any adverse effects of perchlorate on thyroid function in newborns, pregnant women, and nonpregnant adults (Li *et al.*, 2000; Tellez *et al.*, 2005; Amitai *et al.*, 2007). The one study that

found higher serum thyrotropin/thyroid-stimulating hormone (TSH) values in newborns whose mothers drank tap water containing 6 µg perchlorate/l during pregnancy (Brechner *et al.*, 2000), was subsequently reported to be due to different demographics and altitudes in the two nearby towns studied in the original report (Lamm, 2003).

We and others have reported a series of studies in ammonium perchlorate production workers who are exposed intermittently to perchlorate, resulting in urine perchlorate values of up to 40 mg daily, and in normal volunteers given 3–35 mg perchlorate daily for 2 weeks or 0.5 and 3 mg daily for 6 months (Lawrence *et al.*, 2000, 2001; Greer *et al.*, 2002; Gibbs *et al.*, 1998; Braverman *et al.*, 2005, 2006). Thyroid function studies, including serum TSH, thyroxine (T₄), free T₄, and total triiodothyronine (T₃), were not affected by perchlorate exposure in the plant or perchlorate administration to normal volunteers, despite a decrease in the thyroid uptake of ¹²³I at the higher exposures. Despite a mean exposure of 3 years to high levels of perchlorate in the production workers, no abnormalities of the thyroid evaluated by ultrasound were detected compared to a nonexposed local population (Braverman *et al.*, 2005).

Since it has been suggested that the fetus and infant might be more susceptible to the adverse effects of perchlorate on neonatal thyroid function, the perchlorate content of breast milk and infant formulae was assessed. We found that perchlorate was detected in all 49 breast milk samples (median, 9.1 µg/l) and in 17 infant formulae (median, 1.5 µg/l). There was no correlation between breast milk perchlorate and breast milk iodine content, even in those 27 samples with perchlorate concentrations greater than 10 µg/l (Pearce *et al.*, 2007 b). This is in contrast to the data reported by Kirk *et al.* (2005) in 6 breast milk samples in which there was a negative correlation between perchlorate and iodine content. Perchlorate has also been detected in cows' milk in the US (5.9 ± 1.8 µg/l) (Kirk *et al.*, 2005) and in Japan (9.4 ± 2.7 µg/l) (Dyke *et al.*, 2007).

Recent studies from the US reported that all urine samples obtained from 2820 US residents in the National Health and Nutrition Examination Survey (2001–2002) contained perchlorate with a median value of 3.6 µg/l (Blount *et al.*, 2006a, b), and that in women, but not in men, with a urinary iodine <100 µg/l, there was an inverse correlation of urine perchlorate with serum T₄ values and a direct correlation with serum TSH values (Blount *et al.*, 2006a, b). However, we have recently assessed urine perchlorate concentrations in 398 first-trimester pregnant women, residing in Wales and Italy, with a median value of 2.7 µg/l and low urine iodine values <100 µg/l, and found no correlation between urine perchlorate and serum free T₄ and TSH concentrations (Pearce *et al.*, 2007a). Further studies are underway to obtain more information on this obviously important public health issue.

It is obvious that drinking water is not the only source of perchlorate in the US, and probably worldwide, and that perchlorate appears to be ubiquitous in the environment. Thus, it is difficult to reconcile the presence of perchlorate in drinking water alone as a potential health hazard, and it appears unlikely that the potential adverse effects of perchlorate on the thyroid pose a risk to the general population.

Summary Points

- Perchlorate is a competitive inhibitor of NIS and is ubiquitous in the environment.
- In large doses, it has been used as an antithyroid agent by decreasing the entrance of iodide into the thyroid. NIS is also present in the lactating breast.
- In many clinical and environmental studies of subjects exposed to perchlorate, there is no convincing evidence that the low quantities found in the environment adversely affect thyroid function.

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Iodate and Perchlorate in Bottled Water: Methods for Discovery and Impact on Humans

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Abstract

Iodate and perchlorate (Figure 30.1) are oxyhalide anions that are present in water and originate from natural and anthropogenic sources. Iodate is rapidly formed when water-containing iodide is disinfected with ozone. Perchlorate is a common contaminant that is formed during the manufacture and use of munitions and fireworks. More recently, perchlorate has been shown to form and accumulate in sodium hypochlorite solutions used for water disinfection. Therefore, iodate and perchlorate have the propensity to contaminate bottled water, from both contamination of source water and from the use of disinfectants during the treatment process. Modern analytical equipment has made the routine analysis of these oxyhalides possible with nanogram per liter sensitivity. Iodate is essential for thyroid function, while perchlorate is known to inhibit the thyroid's ability to take up iodide. Some regulatory agencies have started to address the adverse health impacts of perchlorate in drinking water. Currently, less is known regarding the relative source contribution of perchlorate from sources beyond tap water. With global increases in bottled water consumption, additional efforts are needed to determine relative human exposure due to iodate and perchlorate in bottled waters. The present study reviews the literature regarding iodate and perchlorate in bottled waters, and presents a survey of locally available bottle water for these oxyhalides.

Abbreviations

CDHS	California Department of Health Services
DBP	Disinfection by-product
DWEL	Drinking water equivalent level
EPA	Environmental Protection Agency
IC	Ion chromatography
LC	Liquid chromatography

MDL	Method detection limit
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NRC	National Research Council
PCR	Postcolumn reaction
UCMR	Unregulated contaminant monitoring rule
UV	Ultraviolet

The oxidation of halides results in the formation of highly soluble and potentially “toxic” species, including perchlorate, iodate and bromate. The presence of these chemicals in drinking water supplies has become an important issue for municipal water supplies, as well as for the bottled water industry. These species are not only present in many source waters, but also can be formed or introduced during water treatment.

Iodate

Iodate is generally considered to be an important component of the human diet, as it is rapidly reduced to iodide in the body and iodide is essential for thyroid function (Burgi *et al.*, 2001). However, high levels of iodate (> 600 mg/day) have been shown to cause damage to the retina, resulting in ocular toxicity (Burgi *et al.*, 2001).

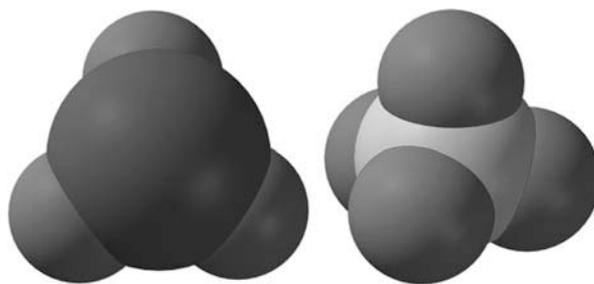


Figure 30.1 Molecular representation of iodate and perchlorate.

Iodate has been reported as present in drinking water at various levels (Nyman *et al.*, 1996; Weinberg and Yamada, 1998). Although the levels are low, it has been speculated that the presence of iodate and, by implication, iodide can have far-reaching consequences on water quality through the formation of iodine-substituted organic by-products (Plewa *et al.*, 2004; Weinberg and Yamada, 1998). For example, Cancho *et al.* (2000) reported the detection of iodinated trihalomethanes in treated waters. Iodinated disinfection by-products (DBPs) are believed to be more toxic than their chlorine and bromide analogs (Plewa *et al.*, 2004).

Perchlorate

Perchlorate was first discovered as an impurity in nitrates mined from the Atacama Desert in Chile (Dafert, 1908). Chilean nitrates have been utilized commercially since 1840 (National Organic Standards Board Technical Advisory Panel Review, 2002). It has been postulated that perchlorate has accumulated in desert soils because of atmospheric deposition, where insufficient rainfall negates the transport of this salt (Ericksen, 1983). More recent reports have supported this notion, based on isotopic patterns capable of discerning naturally occurring perchlorate from industrially utilized and synthesized perchlorate (Bao and Gu, 2004). Recently, additional studies have reported the natural occurrence of perchlorate in soils, and subsequently in water (Crump *et al.*, 2000; Orris *et al.*, 2003; Urbansky

et al., 2001). Perchlorate production in the United States was recorded as soon as the early 1900s (The Libraries, 2004).

Despite the early discovery of perchlorate impurities in Chilean nitrate, perchlorate was not widely utilized until the 1940s and 1950s, when its strong oxidative properties were exploited in the production of explosives and propellants (Davis, 1940; Hampel and Leppla, 1947; Medard, 1950; Summerfield *et al.*, 1960). Perchlorate synthesis using electrolytic conversion was promoted, allowing mass production of the oxyhalide (Hampel and Leppla, 1947). Since World War II, millions of pounds of perchlorate per year have been synthesized in the United States (Urbansky, 2000a). Only since the mid-1990s has perchlorate production waned; it is now manufactured at only one US facility in Cedar City, Utah.

Perchlorate Analysis: Premodern Era

Methods for the detection of perchlorate have developed over the past century (Table 30.1). The nitrates obtained from salt deposits in the Chilean Desert for use as fertilizers, contained undesirable perchlorate impurities. These impurities led to the development of the first analytical methods for perchlorate detection in soils and resulting aqueous solutions. However, these early methods for detecting perchlorate were crude and insensitive. Some of the earliest measurement techniques were reported in a publication from 1908 (Dafert, 1908). These methods

Table 30.1 Methods for the detection of perchlorate developed over the past century

Year	Method	Detection limit ($\mu\text{g/l}$)	Confounding factors	Reference
1908	Colorimetric	>100000	Estimated detection limit from data provided	Dafert (1908)
1912	Gravimetric	>100000	Estimated detection limit from data provided	Lamb and Marden (1912)
1926	Colorimetric	>100000	Estimated detection limit from data provided	Junck (1926)
1930	Gravimetric	>100000	Estimated detection limit from data provided	Willard and Thompson (1930)
1958	Colorimetric	>100000	Estimated detection limit from data provided	Feigl and Goldstein (1958)
1959	Colorimetric	>100000	Estimated detection limit from data provided	Nabar and Ramachandran (1959)
1968	Colorimetric	>100000	Estimated detection limit from data provided	Hayes (1968)
1968	Atomic Absorption	≈ 700	Requires complexation with copper	Collison and Boltz (1968)
1979	Colorimetric	>20000	Estimated detection limit from data provided	Shahine and Khamis (1979)
1980	Raman Spectroscopy	>1000	Less commonly used instrumentation	Miller and Macklin (1980)
1985	Colorimetric	$\approx 20\text{--}50$	Extremely high false-positive/poor QA/QC	Margolis (1986)
1993	Capillary Electrophoresis	$\approx 2\text{--}10$	Custom built detector/insufficient QA/QC	Avdalovic <i>et al.</i> , (1993)

(Continued)

Table 30.1 (Continued)

Year	Method	Detection limit ($\mu\text{g/l}$)	Confounding factors	Reference
1994	Capillary Electrophoresis	5	Custom-built detector/weak QA/QC	Nann and Pretsch (1994)
1995	Raman Spectroscopy	1000	Less commonly used instrumentation	Kowalchuk <i>et al.</i> , (1995)
1996	Ion-selective Electrode	≈ 50	High degree of interference from other ions	Neuhold <i>et al.</i> , (1996)
1996	IC-MS	>1000	Lack of sensitivity and commercial hardware	Corr and Anacleto (1996)
1997	Ion-selective Electrode	>50	High degree of interference from other ions	Siswanta <i>et al.</i> , (1997)
1997	IC Conductivity	$\approx 25,000$	Good separation, poor selectivity/sensitivity	Biesaga <i>et al.</i> , (1997)
1997	IC Conductivity	4	Good sensitivity, limited selectivity	CDHS (1997)
1998	Flow Injection Spectroscopy	3000	Requires preextraction	Ensafi and Rezaei (1998)
1999	IC Conductivity	4	First US EPA method, limited selectivity	(Hautman, <i>et al.</i>) (1999)
2000	IC-MS	0.27–0.30	Required extraction/preconcentration	Magnuson <i>et al.</i> , (2000b)
2004	IC-MS	0.05	Costly MS/MS required	Winkler <i>et al.</i> , (2004a)
2005	LC-MS	0.05	Costly MS/MS required	Snyder <i>et al.</i> , (2005)

Abbreviations: IC, ion chromatography; LC, liquid chromatography; MS, mass spectroscopy; MS/MS, tandem spectrometer.

liberated the anions in an aqueous solution, followed by colorimetric titration with various reagents. A publication from 1926 compared the various colorimetric methods available (Junck, 1926). These early colorimetric methods had detection limits of approximately 0.1%, or roughly grams per liter concentrations. As early as 1912, gravimetric methods for perchlorate were employed. Perchlorate was reduced to chloride, and further reacted with silver nitrate to form a silver chloride precipitate, that could then be weighed and correlated to the perchlorate concentration (Lamb and Marden, 1912; Willard and Thompson, 1930). Gravimetric methods are limited by the accuracy and sensitivity of the weighing devices; therefore, gravimetric methods for perchlorate detection likely had sensitivities in the range of grams or milligrams per liter. Colorimetric methods utilizing dyes and chemical reactions continued to develop through the 1970s and remained the most common analytical procedures for perchlorate measurement, despite poor detection limits of milligram to gram per liter (Feigl and Goldstein, 1958; Hayes, 1968; Nabar and Ramachandran, 1959; Shahine and Khamis, 1979).

Perchlorate Analysis: Modern Era

Although these analytical methods seem insensitive compared to modern techniques, trace analysis was not required to detect the relatively high levels of perchlorate contained in Chilean caliche. Furthermore, early toxicological reports did not indicate that perchlorate was relevant to human health at milligram per kilogram and higher

doses (Eichler, 1929; Kahane, 1936). However, a publication by Wyngaarden *et al.* (1952) indicated that perchlorate could displace iodide from the thyroid, which led to the subsequent use of perchlorate as a pharmaceutical for overactive thyroid and stimulated efforts to develop more sensitive analytical methods for the measurement of perchlorate in body fluids. In 1968, a more sensitive method was reported, in which aqueous perchlorate was complexed with copper followed by the measurement with atomic absorption spectroscopy (Collison and Boltz, 1968). This was the first analytical method reported to be capable of submilligram per liter perchlorate measurements (detection limit approximately 700 $\mu\text{g/l}$). This method was later revised to analyze perchlorate in biological fluids (Weiss and Stanbury, 1972). Raman spectroscopy was applied to the analysis of perchlorate in 1980; however, the technique was quite insensitive (milligram per liter detection limits) (Miller and Macklin, 1980; Urbansky, 2000b). In 1995, a report showed that preconcentration followed by Raman spectroscopy resulted in a detection limit of 1000 $\mu\text{g/l}$ (Kowalchuk *et al.*, 1995). Despite the publication of methods with detection capabilities as low as 700 $\mu\text{g/l}$, these spectroscopic methods did not become widely used. These methods were tedious, and subject to potential interference when applied to environmental matrices.

Two publications from the mid-1990s indicated that perchlorate could be analyzed at microgram per liter concentrations using capillary electrophoresis separation (Avdalovic *et al.*, 1993; Nann and Pretsch, 1994). The initial paper describes a custom-built suppressed conductivity detector that was capable of “minimum detection limits

in the range of $(2-10) \times 10^{-8}$ M ($<10 \mu\text{g/l}$) for common inorganic anions" (Avdalovic *et al.*, 1993). However, the report provides only one chromatogram showing perchlorate in a stock solution at $990 \mu\text{g/l}$. No formal method detection limit (MDL) study was performed using the perchlorate anion. This custom-built system was not commercially available and has not received support as a viable analytical method for perchlorate detection. A second report using capillary electrophoresis was published the following year; however, this method employed an ion-selective microelectrode for perchlorate detection (Nann and Pretsch, 1994). While this method boasts a limit of detection of $5 \mu\text{g/l}$ for perchlorate, the method required a self-constructed anion-selective microelectrode. This electrode was created by hand, under a microscope, following a tedious procedure (Nann and Pretsch, 1994). Furthermore, the analytical detection limit was simply estimated from the signal-to-noise ratio of perchlorate spiked into a "background buffer." The report concedes that "...proper peak identification becomes difficult..." due to the low signal-to-noise ratio. While these capillary electrophoresis methods offered excellent sensitivity, they utilized custom, "hand-constructed," detectors that were not commercially available. Likewise, formal quality assurance and quality control procedures were not applied to accurately determine sensitivity, reproducibility, and precision of perchlorate measurement.

Perchlorate Analysis: Recent Advances

In 1996 and 1997, ion-selective electrodes were developed that were able to detect perchlorate at concentrations of $50 \mu\text{g/l}$ and higher (Neuhold *et al.*, 1996; Siswanta *et al.*, 1997). While these probes offered good sensitivity, they lacked accurate selectivity. Unfortunately, these ion-selective probes suffered from interferences such as nitrate, nitrite, chlorate, bromate, arsenate, arsenite and so on, thus rendering them inappropriate for most water matrices (Urbansky, 2000b). The use of capillary electrophoresis and ion chromatography (IC) coupled to a mass spectrometer for the analysis of perchlorate was first reported in 1996; however, this early attempt yielded minimal sensitivity at milligram per liter levels (Corr and Anacleto, 1996). IC with conductivity detection became the analytical method of most frequent application in the late-1990s (Biesaga *et al.*, 1997; Eaton *et al.*, 1998; Jackson *et al.*, 1999; Okamoto *et al.*, 1999; Urbansky, 1998, 2000a, b; Wirt *et al.*, 1998). Initial IC conductivity methods generally had detection limits higher than $1000 \mu\text{g/l}$ (Biesaga *et al.*, 1997; Maurino and Minero, 1997; Okamoto *et al.*, 1999; Urbansky, 1998; Urbansky *et al.*, 1999). Thus, as of early 1997, no standard methods using common analytical equipment existed by which perchlorate could be accurately quantified at less than approximately $1000 \mu\text{g/l}$.

A major breakthrough occurred in 1997, when the California Department of Health Services (CDHS) published an analytical method with a reporting limit of $4 \mu\text{g/l}$ (1997). This method was successfully applied to natural waters with conductivity up to $1000 \mu\text{mhos/cm}$. The CDHS method set forth the necessary performance criteria for proper quality assurance, including blanks and matrix spike/recovery tests. However, this method utilizes conductivity detection, which has limited selectivity. In 1999, the US Environmental Protection Agency (EPA) released method 314.0, "Determination of perchlorate in drinking water using ion-chromatography," which has become the standard for perchlorate analysis (Hautman, *et al.*, 1999). EPA method 314.0 was mandated for use in the unregulated contaminant monitoring rule (UCMR), under which drinking water samples from across the United States were analyzed for perchlorate and other chemicals. Since 1999, a variety of analytical methods have become available for perchlorate analysis; however, EPA method 314.0 remains the standard method for the analysis of perchlorate in water. Recently, developments in mass spectrometry (MS) have led to extremely sensitive (nanogram per liter) and selective detection methods for perchlorate (Mathew *et al.*, 2005; Handy *et al.*, 2000; Koester *et al.*, 2000; Magnuson *et al.*, 2000a, b, Richardson, 2002; Rickman, 2003a; Roehl *et al.*, 2002; Winkler *et al.*, 2004a; Zwiener and Frimmel, 2004). The US EPA is currently evaluating two LC-MS methods for the analysis of perchlorate, methods 330.0 and 331.0. Despite their ultratrace sensitivity and mass selectivity, LC-MS methods are not as widely used as IC conductivity methods, due to the greater cost and complexity of LC-MS analyses. As analytical techniques continue to improve, it is becoming apparent that perchlorate is a ubiquitous contaminant at trace levels.

Interestingly, some of the largest perchlorate-contaminated sites in the United States were not found until the advent of the CDHS IC method that allowed the detection of perchlorate at low microgram per liter concentrations. Initial trials using the CDHS method resulted in the detection of perchlorate in tap water in Southern California in 1997. Eventually, this contamination was traced to the Colorado River, and ultimately to former perchlorate manufacturing facilities in Henderson, Nevada (Okamoto *et al.*, 1999; Roefer *et al.*, 2000; Urbansky, 2000a). Recently developed MS procedures allow perchlorate quantitation to submicrogram per liter levels (Mathew, *et al.*, 2005; Rickman, 2003a; Snyder *et al.*, 2005; Winkler *et al.*, 2004a; Zwiener and Frimmel, 2005). Using an LC-MS method, Los Alamos National Laboratory has recently reported that all US drinking waters tested were positive for perchlorate (Rickman, 2003a). Another recent study utilizing LC-MS detected perchlorate in nearly 50% of 21 commercially available bottled waters (Snyder *et al.*, 2005). This same study showed that water treatment technologies can generate perchlorate and add this anion to the finished drinking water. Without question, the advent

of more sensitive analytical techniques has led to detection of perchlorate in a plethora of sources and locations where it would not have been detected just 10 years ago.

Perchlorate and Iodate in Water

Contamination of water with perchlorate has become a major environmental and health concern in recent years, as toxicological associations of perchlorate to abnormal endocrine functions have emerged. In mammals, it has been shown that perchlorate is a competitive inhibitor of thyroidal iodide uptake (Greer *et al.*, 2002; Lamm *et al.*, 1999; Wolff, 1998; York *et al.*, 2001); hence drinking water perchlorate contamination has been associated with abnormal newborn thyroid function in humans (Brechner *et al.*, 2000). Based on human clinical trials by Greer *et al.* (2002), the National Research Council's (NRC) technical review on perchlorate suggests a reference doses with a drinking water equivalent (DWEL) of 24.5 µg/L (National Research Council, 2005). The co-occurrence of iodate/iodide with perchlorate is of great importance. In iodide-deficient populations, the impact of perchlorate on the thyroid will be significantly more profound. Therefore, modern analytical methodologies capable of providing simultaneous iodate and perchlorate identification and quantification are critical (Snyder *et al.*, 2005).

Although many papers have focused on the detection of iodate and perchlorate in water (Charles and Pepin, 1998; Handy *et al.*, 2000; Kitamaki and Takeuchi, 2004; Krynskiy *et al.*, 2004; Li and George, 2005; Winkler *et al.*, 2004b), few have reported their occurrence in bottled water. This chapter presents a review of the occurrence of iodate and perchlorate in bottled water.

Iodate

To the best of the author's knowledge, iodate was first reported in bottled water by Weinberg and Yamada (1998), who developed a novel method for detecting

iodate in water. Iodate was separated from other anions using IC and then reacted, postcolumn, with HBr and HNO₂ to form the tribromide ion (Br₃⁻). Tribromide was then detected by a ultraviolet (UV) spectrophotometer at 267 nm. Using this method, iodate was detected in nonmineral bottled water at 0.5 µg/l (Table 30.2). Bichsel and von Gunten (1999) improved upon this method by replacing the toxic nitrite solution with one of neutral iodide. Iodate reacts with iodide to form I₃⁻, which was detected in this method using UV at 288 nm. Under acidic conditions, the reaction proceeds at a much faster rate. Therefore, the membrane exchange suppressor was placed before the postcolumn reaction (PCR) coil to provide the necessarily low pH. Iodate was determined in 16 mineral waters, and was found in all but 5. Concentrations of iodate detected in the mineral waters ranged from 0.4 to 99 µg/l. Interestingly, some waters contained high concentrations of iodate but little iodide, indicating that the waters had been strongly oxidized, perhaps to eliminate manganese and iron (Bichsel and von Gunten, 1999).

Snyder *et al.* (2005) analyzed iodate, along with perchlorate, using reverse-phase liquid chromatography (LC) coupled with tandem mass spectrometry detection (LC-MS/MS). Figure 30.2 presents the chromatogram obtained for the separation of these two anions. Twenty-one bottled waters were surveyed, and perchlorate concentrations ranged from >0.1 to 25 µg/l with 15 containing detectable levels of iodate. Four of these 15 were >10 µg/l and two of those used ozonation as treatment technology (the other two did not divulge the treatment technology used). Finally, Zhu *et al.* (2006) detected iodate in bottled water using IC-PCR with UV detection at 450 nm. This method used a solution of 0.5 g/l *o*-dianisidine·2HCl, 4.5 g/l KBr, 25% methanol, and 5.6% nitric acid as a PCR. Nine of the 14 mineral waters tested contained iodate, with an average concentration of 38 µg/l and a standard deviation of 92 µg/l. Of the 20 nonmineral bottled waters,

Table 30.2 Summary of iodate occurrence in bottled water

Detection method	Reporting limit (µg/l)	Bottled water type	Number of bottled waters analyzed	Range of concentrations (µg/l)	Reference
IC-UV after PCR with acidic bromide	0.1	Nonmineral	1	0.50	Weinberg and Yamada (1998)
IC-UV after sequential PCR with iodide and acid	0.3	Mineral	16	< 0.3–99	Bichsel and Von Gunten (1999)
LC-MS/MS	0.1	Unknown	4	< 0.1–0.14	Snyder <i>et al.</i> , (2005)
		Nonmineral	14	< 0.1–18	
		Mineral	3	1.8–25	
IC-UV after PCR with <i>o</i> -dianisidine·2HCl	0.72	Nonmineral	4	3.8 ± 3.5 (SD)	Zhu <i>et al.</i> , (2006)
		Mineral	9	38.5 ± 92.5 (SD)	

Abbreviations: UV, ultra violet; PCR, postcolumn reaction.

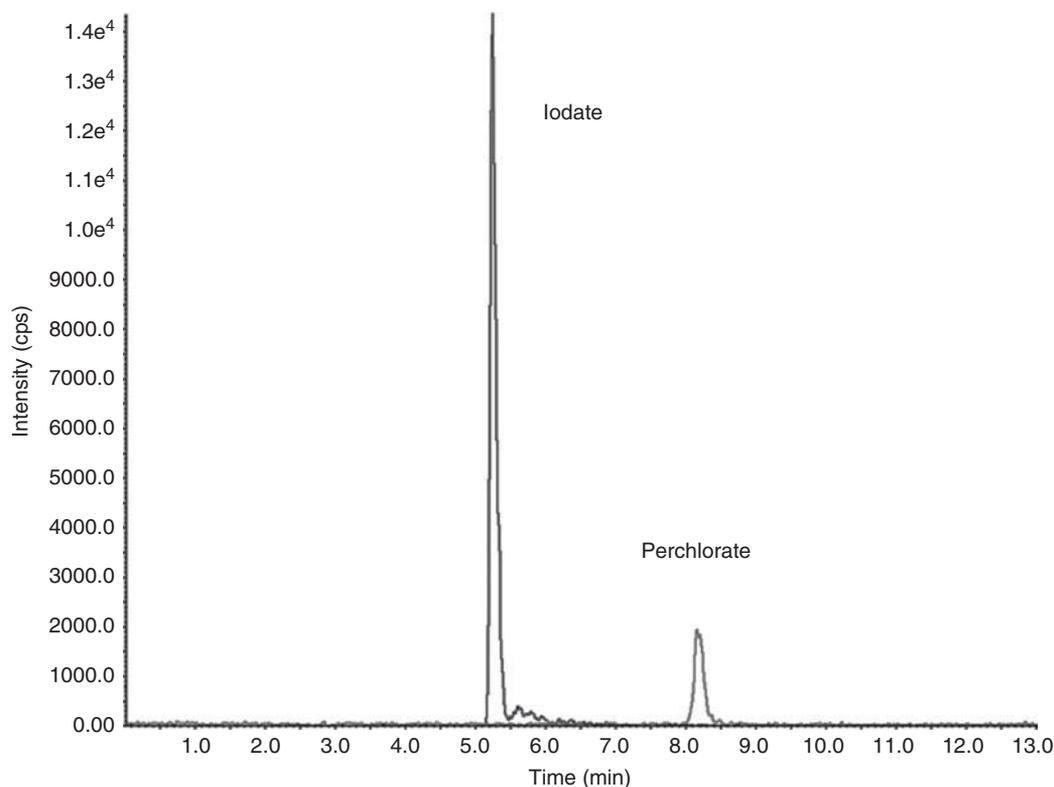


Figure 30.2 Chromatogram showing iodate and perchlorate in a bottled water sample using method by Snyder *et al.*, (2005).

Table 30.3 Summary of perchlorate occurrence in bottled water

Detection method	Reporting limit ($\mu\text{g/l}$)	Bottled water type	Number of bottled waters analyzed	Range of concentrations ($\mu\text{g/l}$)	Reference
Extraction followed by IC conductivity and IC-MS	5.0	Nonmineral	12	< 5.0	Urbansky <i>et al.</i> , (2000)
		Mineral	3	< 5.0	
LC-MS/MS	0.05	Unknown	4	< 0.05	Snyder <i>et al.</i> , (2005)
		Nonmineral	14	< 0.05–0.74	
		Mineral	3	< 0.05–0.34	
IC-MS/MS	0.005	Unknown	10	< 0.05–5.1	El Aribi <i>et al.</i> , (2006)

4 contained iodate with an average concentration of 3.8 $\mu\text{g/l}$ and a standard deviation of 3.5 $\mu\text{g/l}$.

Perchlorate

The first study of perchlorate in bottled water was performed by Urbansky *et al.* (2000), using IC coupled with both conductivity and MS detection. Sixteen bottled waters were studied in this report however, perchlorate was not detected above the reporting limit of 5 $\mu\text{g/l}$ (Table 30.3). Snyder and coworkers tested 21 bottled waters for perchlorate and detected it in only 10, the highest at 0.74 $\mu\text{g/l}$ using LC-MS/MS (Snyder *et al.*, 2005). El Aribi *et al.* (2006)

used IC-MS/MS with an oxygen-18 labeled perchlorate internal standard to analyze perchlorate in 10 bottled water samples. Perchlorate was detected in seven of those samples, and concentrations ranged from 0.067 to 5.1 $\mu\text{g/l}$.

This review demonstrates the tremendous advantages of modern analytical technology for the detection of iodate and perchlorate in aqueous samples, in addition to providing a brief overview on these two species. The measurement of iodate and perchlorate simultaneously, and at nanogram per liter sensitivity, provides great advantage to risk assessors who must balance exposure of these oxyhalides and also helps in the evaluation of the differences between potable water and bottled water.

The data presented here for bottled water will contribute to the greater knowledge on iodate and perchlorate by providing details on relative source contributions of iodate and perchlorate from bottled waters. Bottled water would generally not contribute significant amounts of iodate and perchlorate; however, in some cases iodate and perchlorate concentrations as large as 99 and 5.1 µg/l have been reported.

Summary Points

- Perchlorate and iodate occur in natural waters from natural and anthropogenic sources.
- Iodate is essential for thyroid function and can be formed from iodide during the ozonation of drinking water.
- Perchlorate is commonly found in natural waters and is known to inhibit the ability of the thyroid to take up iodide.
- Perchlorate and iodate detection in water has improved significantly in recent years, due to improvements in analytical methodology.
- Perchlorate and iodate have been detected in bottled water, but not in significant amounts.

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Dioxins, PCBs and Iodine: Effects on Synthesis and Metabolism of the Iodine Containing Thyroid Hormones

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Abstract

Dioxins and dioxin-like PCBs induce a broad spectrum of toxic responses, such as loss of body mass, hepatotoxicity, immunotoxicity, epidermal changes, embryotoxicity and carcinogenicity. Moreover, they act as endocrine disruptors in the reproductive system and also interfere in thyroid hormone metabolism. The changes in plasma thyroid hormone concentrations, mostly the depression of circulating T₄ concentrations, due to exposure to dioxins and PCBs can be the consequence of several “modes of action.” First, a direct effect on the thyroid gland, both on thyroid gland morphology and on iodine transporters in the thyroid gland can lead to a decreased synthesis of thyroid hormones. Secondly, an altered metabolism of thyroid hormones, such as an increased biliary excretion of T₄, can decrease thyroid hormone concentrations. Thirdly, binding of PCBs to the plasma thyroid hormone transport proteins can result in a displacement of the natural ligand of T₄. And, finally, interference of PCBs with binding of thyroid hormones on their receptors has been described. In contrast to other environmental chemicals, such as perchlorates and nitrates, it appears that dioxins/PCBs do not compete directly with iodine uptake at the thyroid gland, but exert their effect through changes in NIS expression.

Abbreviations

AhR	Aryl hydrocarbon receptor
ARNT	Aryl hydrocarbon nuclear translocator
CatB	Cathepsin B
CRH	Corticotrophin-releasing hormone
CRMP	Collapsing response mediator proteins
CYP	Cytochrome p450
IRD	Inner ring deiodination
NIS	Sodium iodide symporter
ORD	Outer ring deiodination

PAPS	3'-Phosphoadenosine 5'-phosphosulfate
PCB	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo- <i>p</i> -dioxins
PCDFs	Polychlorinated dibenzofurans
SS	Somatostatin
T ₃	3,5,3'-Triiodothyronine
T ₄	3,5,3',5'-Tetraiodothyronine
TBG	Thyroxine-binding globulin
TCDD	2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin
TH	Thyroid hormones
TPO	Thyroid peroxidase
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
TTR	Transthyretin
UDP	Uridine diphosphate
XREs	Xenobiotic response elements

Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are chemically classified as halogenated (polychlorinated) aromatic hydrocarbons (PHAH). Dioxins are formed as a by-product of chemical processes, whereas PCBs are synthesized by direct chlorination of biphenyls. Due to their lipophilic character, they are concentrated in the food chain and both humans and wildlife are exposed to them. The major source of human exposure is through the diet, as these substances are concentrated in fatty tissues of beef, poultry, pork and fish, and through cigarette smoking. Moreover, maternal milk contains considerable amounts of PCBs and dioxins.

The basic chemical structure of PHAHs consists of two fused aromatic rings made up of hydrogen and carbons (Figure 31.1). Substitution of hydrogen atoms by chlorine

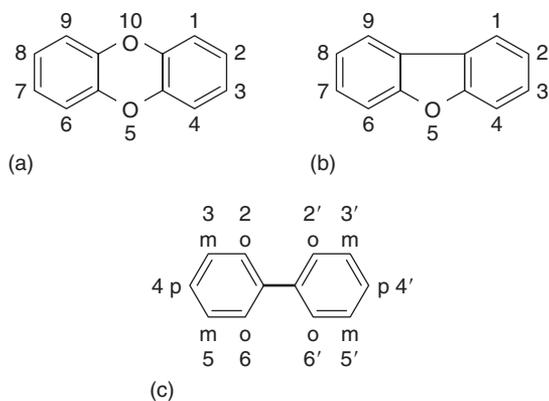


Figure 31.1 Molecular structures of (a) polychlorinated dibenzo-*p*-dioxins (PCDDs); (b) polychlorinated dibenzofurans (PCDFs); and (c) polychlorinated biphenyls (PCBs). Numbers indicate positions on the basic structure of the molecules where chlorine substitutions can take place. *m*, *meta*; *o*, *ortho*; *p*, *para* position.

atoms on the rings produces one of many chlorinated congeners. There are 75 PCDD, 135 PCDF and 209 PCB congeners, of which about 30 are considered to give dioxin-like toxicity. The most toxic PCDDs and PCDFs are those chlorinated at the 2,3,7 and 8 positions; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is considered to be the most toxic dioxin, and is used as a reference molecule. The most toxic PCBs are those with four or more chlorines with just one or no substitution in the ortho position. These PCBs (e.g., PCB77) are referred to as “coplanar” enabling them to bind to the aryl hydrocarbon receptor (AhR), also called the dioxin receptor (Safe, 1994). The noncoplanar or *ortho*-substituted PCBs (e.g., PCB153, PCB180) do not bind to the AhR.

Dioxins and dioxin-like PCBs induce a broad spectrum of toxic responses, such as loss of body mass, hepatotoxicity, immunotoxicity, epidermal changes, embryotoxicity and carcinogenicity. In addition, they can act as endocrine disruptors, thereby mimicking or interfering with the action of endogenous hormones and other molecules of the endocrine system. Besides their interference with the reproductive system, there are indications of their role in thyroid hormone (TH) metabolism and function. The toxic and biological effects of these PHAHs depend on several factors, such as doses, route of administration, species, age, strain and sex of the animals (Safe, 1986).

General Mechanisms of Action of Dioxins and Dioxin-Like PCBs

A large body of evidence suggests that the biological and toxic responses associated with dioxins and dioxin-like PCBs are not the results of direct insult of the toxin. However, many of their toxic effects have been shown to be mediated through a specific protein known as the aryl

hydrocarbon receptor (AhR). After entry of the toxin into the cell and binding to the AhR, the ligand-activated AhR translocates from the cytoplasm to the nucleus where it binds its dimerization partner aryl hydrocarbon nuclear translocator (ARNT). This AhR/ARNT heterodimer complex binds to its cognate DNA sequences, termed xenobiotic response elements (XREs), and activates the expression of AhR target genes, such as the well-known cytochrome *p*450 (CYP) genes (*CYP1A1* and *CYP1B1*; Hankinson, 1995). The activation of the CYP enzyme family is followed by a cascade of events essentially aimed at the formation of more hydrophilic metabolites to facilitate subsequent elimination of the toxic compounds (Bandeira, 2001). Several of the enzymes activated in this pathway also influence the metabolism of steroids and TH. This may change the availability of these hormones in tissues, and hence hormone-dependent gene expression. Several groups of proteins appear to be responsive to AhR agonists; the best known are the drug-metabolizing enzymes (glucuronyl transferase, glutathione-transferase, etc.) via the induction of CYP enzymes, growth regulatory proteins, and NADPH-generating enzymes. Moreover, it seems that even some action via steroid/thyroid receptors (Okey *et al.*, 1995) or cross-talk among AhR and nuclear receptors cannot be excluded (Mathews and Gustafsson, 2006). In humans, genetic variations within the AhR pathway have been identified that may determine individual responsiveness to dioxin and dioxin-like PCBs (Landi *et al.*, 2005).

Certain PCBs and chlorinated dibenzo-*p*-dioxin congeners are structurally similar (two phenol rings and halogen substitutions) to the active TH (McKinney and Waller, 1994). Laterally-substituted chlorinated aromatic compounds, such as the *meta* and *para* PCBs, particularly when hydroxylated, are ideally suited to serve as binding ligands for 3,5,3',5'-tetraiodothyronine (T₄) binding proteins (McKinney and Waller, 1994). It is therefore not surprising that these dioxins and dioxin-like PCBs can interfere with TH availability (reviewed by Brouwer *et al.*, 1998) acting as agonist or antagonist to the naturally occurring hormone. Theoretically they thus have the potential of binding to TH binding proteins or to the TH receptor.

Effects of PCBs on Circulating Iodine Containing Thyroid Hormones

Changes in thyroid hormone concentrations

During the past years, much attention has been given to the effect of endocrine disruptors, such as PCBs and dioxins, on TH and their involvement in brain and neural development. Disruption in TH homeostasis leads to developmental deficits and neurological abnormalities (McNabb and

King, 1993; Porterfield, 1994; Sher *et al.*, 1998; Zoeller, 2001; Bernal, 2002; Boas *et al.*, 2006; Darras, 2007). It has to be mentioned that while dioxin-like PCBs are considered most dangerous in terms of general toxicity (including effects on circulating TH concentrations), *ortho*-substituted PCBs are more frequently brought into relation with neurotoxicity. The main explanation for this divergence is that these *ortho*-substituted PCBs are more potent in disrupting Ca^{2+} homeostasis and Ca^{2+} signaling pathways in the nervous system, consequently leading to changes in, e.g., neurotransmitter concentrations. For a review on the specific neurotoxicological effects of these PCBs, we refer to Darras (2007). Brain and neural development of the fetus can thus be influenced by PCBs indirectly, by affecting maternal thyroid homeostasis (effects of altered TH levels), but also directly as a consequence of specific neurotoxicological effects of some PCBs.

Alterations in thyroid homeostasis by organochlorine compounds have been documented for many species, including humans. There are several epidemiological studies describing changes in thyroid function in adults and infants, but these data on circulating TH and thyroid-stimulating hormone (TSH) levels are not always consistent, which may be related to methodology, mixed exposures, sample size, and so on. For extensive overview of epidemiological data in adults and newborns we refer to the recent reviews of Hagmar (2003), Langer (2005) and Giacomini *et al.* (2006). However, as also shown in experimental animal models, individual congeners as well as PCB mixtures mostly depress the plasma T_4 levels, while there is no clear effect on circulating 3,5,3'-triiodothyronine (T_3) concentrations (Barter and Klaassen, 1994; Bastomsky *et al.*, 1976; Brouwer *et al.*, 1998; Kato *et al.*, 2003; Morse *et al.*, 1993; Van Birgelen *et al.*, 1994). Studies in wildlife birds, as well as laboratory experiments on avian species, reveal ambiguous effects of PCBs and dioxins on plasma T_4 and T_3 levels (reviewed by Scanes and McNabb, 2003; Janz and Bellward, 1996; Bruggeman *et al.*, 2003).

The changes in plasma TH concentrations due to exposure to dioxins and dioxin-like PCBs can be the consequence of several "modes of action." First, a direct effect on the thyroid gland can lead to a decreased synthesis of TH. Secondly, an altered metabolism of TH, such as an increased biliary excretion of T_4 , can decrease TH concentrations. Thirdly, binding of PCBs to the plasma TH transport proteins can result in a displacement of the natural ligand of T_4 . And, finally, interference of PCBs with binding of TH on their receptors has been described. However, when interpreting data and generalizing working mechanisms, it has to be taken into account that there is a divergence of results originating from studies using different types of congener/mixture, animal species, animal ages and times of exposure. The divergence in these parameters can explain the apparent discrepancies between data from literature.

Mechanisms of action of PCBs and dioxin-like PCBs

Direct Effects on the Thyroid Gland Most mammals have a bilobed thyroid gland with the lobes joined by an isthmus. The structural and functional unit of the vertebrate thyroid is a spherical structure, the follicle, consisting of a single layer of epithelial cells, surrounding the lumen. This lumen is an extracellular space filled with a proteinaceous fluid (colloid) that consists primarily of thyroglobulin, a large protein prohormone carrying the hormones T_4 , T_3 , other iodoproteins and albumin. The relative activity of the gland is reflected in changes in the appearance of the follicles by alterations in epithelial cell height, in cellular organelle density, and in the relative size of the colloid-filled lumen. An essential enzyme in the production of TH is the thyroid peroxidase (TPO), which oxidizes the captured iodide in the follicle. These oxidation products are used for the iodination of the tyrosine residues of thyroglobulin. Furthermore, the oxidative coupling of tyrosines to form thyronines is catalyzed by TPO. The TH biosynthesis is dependent on the level of expression and the correct action of TPO, but also involves the TSH receptor, the Na^+/I^- symporter (NIS), both on the membrane, as well as thyroglobulin. TSH originates from the pituitary gland and is regulated by stimulating factors (thyrotropin-releasing hormone, TRH; corticotrophin-releasing hormone, CRH) and an inhibiting factor, somatostatin (SS). TSH causes the thyroid gland to produce and release TH, predominantly T_4 . Low circulating TH concentrations result in an increased TSH stimulation of the thyroid gland. When T_4 reaches target cells, it is converted through deiodinase enzymes to T_3 , the biologically active form (Figure 31.2).

Collins *et al.* (1977) published the first report on the direct effects of PCBs on thyroid morphology; a striking hypertrophy and hyperplasia of thyroid follicular cells was accompanied by a significant decrease in serum T_4 . Later reports also describe morphological evidence for direct effects of individual PCB and dioxin congeners and mixtures on thyroid follicles, thereby inducing an increase in

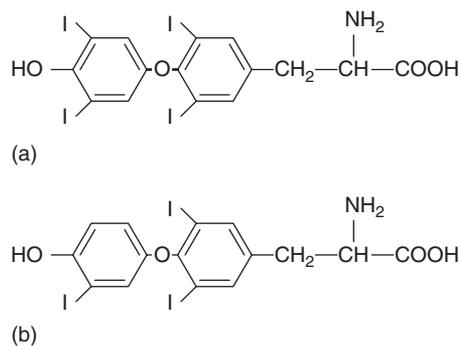


Figure 31.2 Molecular structures of (a) 3,5,3',5'-tetraiodothyronine (T_4); and (b) 3,5,3'-triiodothyronine (T_3).

the volume of the thyroid by hypertrophy and hyperplasia of the follicular cells (Collins and Capen, 1980; Saeed and Hansen, 1997; Sewall *et al.*, 1995; Nishimura *et al.*, 2002). A recent study of Langer *et al.* (2007a, b) showed, by using ultrasound, that the thyroid volume was increased in humans exposed to high PCB levels, pesticides and dioxins, apparently by consuming contaminated fish in specific areas of Slovakia (Langer *et al.*, 2007a). Since urinary iodine concentrations did not differ between groups, the possibility of any interference of iodine deficiency was excluded (Langer *et al.*, 2007b). These authors hypothesize that the stimulation of thyroid growth could be due to estrogen-like effects of some of these dioxins and dioxin-like compounds, since estrogen receptors are reported to be present on the thyroid (Manole *et al.*, 2001; Shiraishi *et al.*, 2003).

Other disturbances at the level of the thyroid are a decreased follicular colloid area, accumulation of large colloid droplets in follicular cells, and disturbances of enzymes directly involved in TH synthesis and release. The latter was shown *in vitro* by using primary porcine thyrocytes in which TCDD and PCB126 downregulated NIS and cathepsin B (CatB) (lysosomal cysteine protease involved in proteolysis of thyroglobulins) gene expression, which is thought to be mediated by the AhR. This downregulation of NIS would result in restricted iodine uptake by the thyroid gland, causing the cellular hypertrophy of thyrocytes, while the decrease of CatB could lead to an attenuated proteolytic cleavage of thyroglobulins and thus their accumulation in the thyroid follicles (Pocar

et al., 2006) (Figure 31.3). It has to be noted that, whereas PCBs seem to act directly on NIS gene expression, other environmental chemicals, such as perchlorate and nitrate, act as competitive inhibitors for iodine uptake by the thyroid gland through the NIS (reviewed by De Groef *et al.*, 2006). Another group of chemicals, phthalates, also affect NIS, enhancing both mRNA and activity (Breous *et al.*, 2005). Langer *et al.* (2005) showed that another effect of long-term exposure to organochlorinated pollutants was the occurrence of TPO antibodies in human blood.

The observed histological changes are often associated with normal or high serum TSH levels and low serum T₄ levels (Koopman-Esseboom *et al.*, 1994). The results of Desaulniers *et al.* (1999) also showed a decrease in both T₃ and T₄ in adult rats treated with a dioxin-like PCB (PCB126) but no increase in TSH concentration, suggesting a primary thyroidal disorder and a limited role of TSH in this type of hypothyroidism, induced by dioxin-like PCBs. A similar conclusion was formulated by Byrne *et al.* (1987) saying that PCB-induced decreases in serum TH would be the result of direct damage to the thyroid gland, rather than any enhanced hepatic catabolism or change in TSH. However, signaling at the level of the TSH receptor was inhibited by the PCB mixture Aroclor 1254 (Santini *et al.*, 2003).

Exposure to the most toxic dioxin, TCDD, resulted in an increase in TSH-positive cells in the anterior pituitary (Nishimura *et al.*, 2002), which is consistent with a compensatory production of T₄ in the thyroid gland. This group (Nishimura *et al.*, 2005) also reported a sex

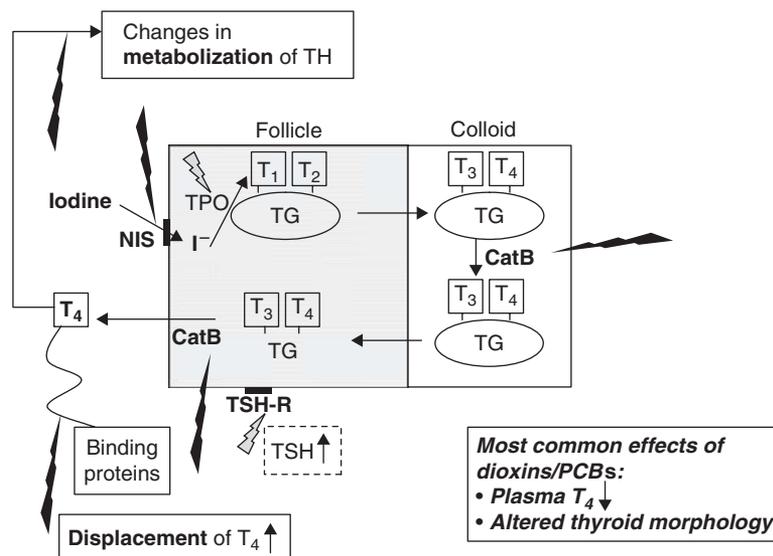


Figure 31.3 Possible intervention sites of dioxin-like compounds at the level of the thyroid gland and in other processes such as thyroid binding to their binding proteins and metabolism. Adapted from Pocar *et al.*, (2006). Full bold line boxes indicate the most consistent effects of dioxin-like compounds, whereas dotted boxes indicate conflicting results in literature. Black ⚡, intervention of dioxin-like compounds in specific pathways; consistent effect through literature; gray ⚡, conflicting results in literature. TG, thyroglobulin; T₄, thyroxine; T₃, triiodothyronine; TSH-R, thyroid-stimulating hormone receptor; NIS, sodium iodide symporter; TPO, thyroid peroxidase; CatB, cathepsin B.

difference in response to TCDD in rats, with male rats being more susceptible to TCDD than female rats in terms of TSH response.

Not only in adults, but also in offspring of mothers exposed to dioxins, morphological alterations of the thyroid gland are reported. Administration of a single dose of TCDD to rats on gestational day 15 resulted in hyperplasia of thyroid follicular cells and functional disruption of the thyroid in offspring, due to exposure via milk but not by placental transfer (Nishimura *et al.*, 2003, 2005). In addition, circulating T_4 levels at weaning were lower and serum TSH was increased in pups with postnatal TCDD exposure due to intake of mother's milk; this suppression in serum T_4 was highly correlated with induction of the *UGT1* gene (uridine diphosphate, UDP-glucuronosyltransferase-1) in liver. The results of Beck V. (2006) showed a downregulation of TPO gene expression in chicken embryo thyroids during late stages of embryonic development, which was accompanied with lower plasma TH concentrations after PCB77 treatment.

Effects on Thyroid Hormone Transport: Interaction with Binding Proteins Dioxin and PCB competition with TH may displace TH from their binding proteins, thus increasing their elimination rates. This would contribute to the hypothyroidism-like hormonal alterations described in toxicity studies, characterized by low T_4 and elevated TSH concentrations (not always when PCB treated). Cheek *et al.* (1999) suggested that disruption of TH transport could be an important mechanism by which organochlorine compounds alter thyroid homeostasis, due to the high affinity of OH-PCBs for thyroid-binding proteins. Many of the hydroxylated congeners of PCBs have a high affinity for transthyretin (TTR; binds most of circulating T_4 and a small proportion of T_3) (Cheek *et al.*, 1999). Kato *et al.* (2004) attributed the decreased T_4 levels in rats after exposure to a PCB mixture (Kanochlor 500) partially to the binding of two major OH-PCB metabolites to TTR. Brouwer *et al.* (1990) also observed both *in vivo* and *in vitro* inhibition of T_4 binding to TTR by OH-metabolites of PCB77. Also in mice, maternal exposure to PCB77 resulted in decreased fetal T_4 levels due to similar effects on TTR binding (Darnerud *et al.*, 1996). TTR is also thought to be important for T_4 delivery across the blood-brain barrier; competition between OH-PCBs and this binding protein could contribute to the observed hypothyroidism at the level of the brain.

Only a small number of the PCB-OHs bind to thyroxine-binding globulin (TBG), the major transport protein in many mammals, including man, binding a large proportion of T_3 (Lingappa and Mellon, 1997).

Effects on Thyroid Hormone Metabolism TH can be metabolized in four different ways: by deiodination, by conjugation (glucuronidation and sulfation), by cleavage

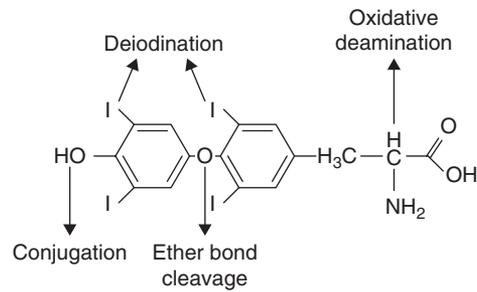


Figure 31.4 Metabolic pathways for thyroxine (T_4).

of the ether bond between the two aromatic rings, and by oxidative deamination or decarboxylation of the alanine side chain (reviewed by Visser T. J., 1990) (Figure 31.4). Only the conjugation process is reversible. Deiodination and conjugation are the major pathways for the degradation and excretion processes of TH and will be discussed more in detail.

Deiodination In order to initiate TH action, T_4 originating from the thyroid gland must be activated in tissues by outer ring deiodination (ORD) to form T_3 . To balance the activation pathway, both T_4 and T_3 are irreversibly inactivated by monodeiodination of the tyrosyl ring of the iodothyronines, called inner ring deiodination (IRD). Mammals and birds have three types of deiodinases: type I deiodinase (D1) with ORD and IRD activity, type II (D2) with only ORD activity, and type III with only IRD activity Visser T. J. (1990).

A single dose of TCDD was reported to decrease type I deiodinase activity in a dose-dependent manner in adult rats (Raasmaja *et al.*, 1996). Hydroxylated PCB metabolites also interfere with T_4 metabolism by inhibition of deiodinase activity, which prevents the formation of T_3 (Adams *et al.*, 1990).

Adaptive changes in deiodinase activity have been reported in certain regions of the brain of fetal rats exposed *in utero* to the PCB mixture Aroclor in order to compensate the local depression of T_4 levels (Morse *et al.*, 1996). In chicken embryos, *in ovo* exposure to PCB77 temporarily reduced TH levels (Roelens *et al.*, 2005) both in plasma and brain areas, which was combined with an adaptive increase in the activity of the type 2 deiodinase activity and decrease in activity of the T_3 inactivating type 3 deiodinase Beck V. (2006).

Conjugation: Glucuronidation and Sulfation Glucuronidation is a major metabolic reaction, and mainly takes place in the liver, for disposal of a variety of endogenous (such as TH) and exogenous substrates (such as PCBs). It involves the transfer of glucuronic acid from the cofactor UDP-glucuronic acid to functional groups, in particular hydroxyl groups, of the substrates. Lipophilic compounds are thus converted to water-soluble derivatives, which are then excreted in bile or urine. TH concentrations are decreased

by enhanced liver glucuronidation, which is the rate-limiting step in biliary excretion of T_4 and T_3 (Sewall *et al.*, 1995). PCB/TCDD exposure of perinatal and adult rats increased biliary excretion of T_4 by inducing the enzyme T_4 -uridine diphosphoglucuronyl-transferase (UDP-UGT), thereby increasing hepatic T_4 glucuronidation (Brouwer *et al.*, 1998; Bastomsky, 1977; Klaassen and Hood, 2001). As a result, T_4 clearance is facilitated from serum through liver metabolism, reducing the half life of T_4 in the blood. Besides this enzyme, other drug-metabolizing enzymes are also induced by TCDD, mediated via the AhR pathway (Safe, 1995; Sewall *et al.*, 1995; Schrenk, 1998).

The transfer of a sulfonate group from the donor compound 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to an acceptor compound (such as TH, steroids, ... but also xenobiotics) is catalyzed by a large family of enzymes called sulfotransferases, located in the cytoplasmic fraction of, e.g., liver cells. Unlike glucuronidation, sulfation does not facilitate the excretion of TH, but interferes with the deiodination process. Sulfated THs strongly facilitate the IRD activity of D1 while they inhibit the D2, D3 activity, and the ORD of D1 (Moreno *et al.*, 1994; Visser T. J., 1990).

Hydroxylated PCBs can influence T_4 metabolism by a strong inhibition of T_4 sulfation (Schuur *et al.*, 1998). As sulfation is a major regulation pathway of T_4 in the fetus, the interference of these metabolites with T_4 sulfation may have consequences for fetal development, and in particular for brain development (Brouwer *et al.*, 1998).

Interaction with Binding of Thyroid Hormones to Thyroid Receptors and Induction of Thyroid Hormone Responsive Genes

As a result of structural similarities between hydroxylated PCBs and TH, PCBs are capable of competing with TH for binding the TH receptor. Iwasaki *et al.* (2002) showed a strong suppression of the effect of T_3 on TH receptor and coactivator complex by PCBs in various cell lines. In addition, several PCBs, and especially their hydroxylated forms, were found to inhibit the binding of T_3 to its receptor (Jacobson and Jacobson, 1996; Kitamura *et al.* 2005). Recent data showed that dioxins may even inhibit postreceptor activity events induced by TH (Birnbaum and Tuomisto, 2000; Bogazzi *et al.*, 2003). The results of Yamada-Okabe *et al.* (2004) showed that TCDD positively and OH-PCB negatively affected cellular responses to T_3 in HeLa cells overexpressing TH receptors, by enhancing or suppressing the T_3 -mediated gene expression.

Quite a lot of literature exists on TH-dependent gene expression in brain, which can be influenced by PCBs. The results of Zoeller *et al.* (2000) demonstrated that Aroclor 1254 influenced TH-dependent genes, such as myelin basic protein and RC3/neurogranin, in rats. Also in birds, PCB77 appeared to directly interact with TH responsive genes in brain, such as collapsing response mediator proteins (CRMP) and NADH-ubiquinone oxidoreductase

(Roelens *et al.*, 2005 and Roelens, unpublished results). It remains however, to be established whether direct binding to TH receptors is the main mechanism responsible for the interaction of PCBs at the level of gene expression in fetal rat brain, since a wide array of PCBs and hydroxylated metabolites do not have the capability to bind to the TH receptors (Gauger *et al.*, 2004; You *et al.*, 2006).

Summary Points

- PCBs alter thyroid homeostasis, with the most consistent finding being the decrease in plasma T_4 concentrations.
- The most critical period for reduction of TH concentrations by PCBs and dioxins is during fetal and neonatal development.
- Mechanisms on how these pollutants interfere with TH status are numerous and complex, since they act at several levels in the thyroid system.
- New results point to a direct affect of PCBs on iodine uptake by the thyroid gland. PCBs cause a change in gene expression of the NIS, but do not compete directly with I^- at the NIS, as reported for other environmental chemicals such as perchlorates and nitrates.
- In conclusion, it appears that most effects caused by dioxins and PCBs on circulating TH concentrations are mainly due to mechanisms not related to direct effects on iodine uptake by the thyroid gland, although the latter cannot be excluded.

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Iodide Effects on the Thyroid: Biochemical, Physiological, Pharmacological, and Clinical Effects of Iodide in the Thyroid

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Abstract

Iodide, the main and limiting substrate in the synthesis of thyroid hormones by the thyroid gland, also exerts physiological and pharmacological signaling effects on this organ. *In vivo* studies in humans and animals have demonstrated that at low or physiological levels ($<0.4\mu\text{M}$), iodide uptake limits the synthesis of thyroid hormones and therefore their secretion. These serum thyroid hormones depress the secretion of the main physiological activator of the gland, pituitary thyroid stimulating hormone (TSH). Thus, iodine deficiency leads to decreased thyroid hormone levels and compensatory TSH secretion. At these levels, there is an inverse relationship between iodide supply and TSH secretion. This is a negative indirect control. At high concentrations ($0.4\text{--}10\mu\text{M}$), iodide directly inhibits several activating thyroid signaling pathways. Such effects require an intact follicular structure and the oxidative organification of a postulate intermediate X to XI. Two iodinated lipids reproduce these effects of iodide: iodolactone and 2-iodohexadecanal. Only the latter is found in the thyroid. The direct effects of iodide and XI account for an inhibition of iodide oxidation itself, of iodide uptake, and of thyroid hormone secretion and thyroid growth. Besides the XI effects, iodide at very high pharmacological or toxicological concentrations ($>10\mu\text{M}$) appears to directly inhibit thyroid blood flow and secretion. The latter effects are used in the preoperative treatment of patients suffering from Graves' disease with Lugol. The various experimental models used to elucidate the direct effects of iodide on the thyroid are discussed and analyzed. The chapter also describes the clinical consequences of the diverse iodide actions.

Abbreviations

2-IHDA 2-Iodohexadecanal
cAMP Cyclic 3',5'-adenosine monophosphate

DBcAMP	$\text{N}_2\text{-}0_6$ dibutyryl cAMP
DUOXes	Dual oxydases
Gq	Stimulatory G protein of phospholipase C
Gs	Stimulatory G protein of adenylyl cyclase
mRNA	Messenger RNA
NIS	Sodium iodide symporter
T3	3,5,3'-Triiodothyronine
T4	3,5,3',5'-Tetraiodothyronine or thyroxine
TPO	Thyroid peroxidase
TRH	Thyrotropin-releasing hormone
TSAb	Thyroid-stimulating antibody
TSH	Thyroid-stimulating hormone

Introduction

The thyroid gland is mainly regulated by two systems: the thyroid-stimulating hormone (TSH) control system ensuring the homeostasis of thyroid hormone serum levels, and control by iodide of the adaptation of thyroid function to the supply of this main substrate. In the first system, hypothalamic thyroid-releasing hormone (TRH) activates TSH synthesis and secretion by the pituitary thyrotrophs, TSH itself stimulating the function, differentiation and growth of the thyroid. The secreted thyroid hormones, thyroxine (T4) and 3,5,3'-triiodothyronine (T3), inhibit TRH and TSH secretion at the hypothalamic and pituitary level. This negative feedback, like a thermostat, maintains serum T4 and T3 at a steady level (Figure 32.1).

Iodide interferes with the TSH system as the limiting factor in thyroid hormone synthesis. In iodine deficiency, low thyroid hormone synthesis and secretion lead to increased TSH secretion and thyroid stimulation.

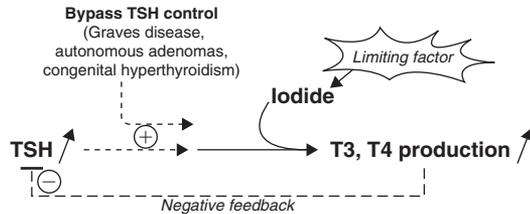
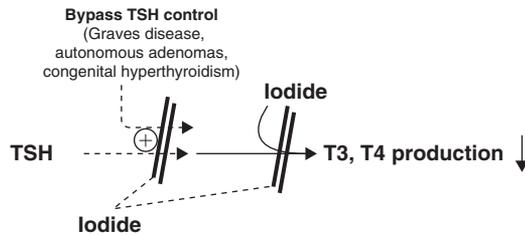
Low iodine diet: iodide as a substrate**High iodine intake: iodide as a signal/regulator**

Figure 32.1 Dual role of iodide on thyroid function. ---+---> Stimulation; ---|--- Inhibition; → chemical transformation.

However, besides this external control, iodide also exerts direct effects on the thyroid: the autoregulation of the thyroid (Halmi and Stuelke, 1956; Wolff, 1948, 1969).

The direct physiological and pharmacological controls of the thyroid by iodide are presented in this chapter.

Effects of Iodide on the Thyroid

There are two approaches to study the effects of iodide: *in vivo* and *in vitro* model systems. The *in vivo* effects are complex and difficult to analyze, but they represent the *in vivo* reality. The *in vitro* model systems lead themselves to a much more precise delineation of the effects and their mechanisms, but are only interesting insofar as they reproduce the *in vivo* situation.

Iodide as substrate

Under conditions of iodide deficiency, synthesis of thyroid hormones is impaired, which leads to decreased levels of T₄, and in severe deficiency, also of T₃, and to consequent increases of serum TSH and thyroid activation (Figure 32.1). With higher levels of dietary iodide, more thyroxine is formed and secreted, the thermostat of the negative thyroid hormone feedback operates, and thyroid activation decreases accordingly. This system operates when the negative feedback operates, but not when thyroid functioning no longer depends on TSH stimulation. This occurs in the case of Graves' disease in which unregulated levels of thyroid-stimulating antibody (TSAb) stimulate the thyroid, as well as in autonomous adenomas and congenital

hyperthyroidism (the "Leclère" disease) in which the TSH receptor is constitutively activated. In both cases, the negative feedback loop is interrupted at the level of the TSH receptor. Therefore, increasing the iodide supply will automatically increase thyroid hormone synthesis and secretion, and cause or increase thyrotoxicosis.

Iodide as signal

Administration of moderately elevated doses of iodide to rats, mice, or humans has immediate effects on thyroid function, independently of later possible effects through changes in thyroid hormone serum levels. These effects are:

1. an inhibition of iodide oxidation and binding to proteins, secondary to an inhibition of H₂O₂ generation by dual oxidases (DUOXes): the "Wolff–Chaikoff effect." This effect directly inhibits thyroid hormone synthesis (Wolff, 1948). This phenomenon was first described *in vivo* by Wolff and Chaikoff. Rats were injected with increasing doses of iodide, and the level of organification, as well as the thyroid and plasmatic distribution of iodide, was measured at different times. It was shown that the inhibitory effect of iodide on its own organification is acute (<2.5 h), and occurs above an administered critical dose (>1 μM) (Wolff, 1948).
2. a delay (after several hours or a day) in the inhibition of iodide transport secondary to a repression of sodium iodide symporter (NIS) expression: adaptation to the Wolff–Chaikoff effect. This effect decreases the loading of the thyroid with iodide, and hence the Wolff–Chaikoff effect itself. This effect, complemented by the negative feedback of thyroid hormone on the hypophysis and hypothalamus, accounts for the inverse relationship of radioiodide thyroid uptake and dietary iodine supply (Wolff, 1969).
3. an inhibition of thyroid hormone secretion (Degroot and Greer, 1956; Wartofsky *et al.*, 1970) and of thyroid blood flow (Rognoni *et al.*, 1984) at high doses of iodide.
4. an inhibition of thyroid growth (Pisarev and Itoiz, 1972) at high doses of iodide.

A rapid stimulation of H₂O₂ generation by relatively low concentrations of iodide (1–10 μM) has been demonstrated for some species *in vitro*. The effect leading to increased iodide oxidation with increased iodide concentrations increases the efficiency of the system over a wider range of iodide concentrations. Although it is difficult to demonstrate *in vivo*, its physiological interest, activating the system when substrate is available, is obvious. It is observed only sometimes in dog and human thyroid, but in dog thyroid it is enhanced by prior iodine deprivation (Corvilain *et al.*, 2000).

For delayed effects of iodide, it is difficult to separate the direct effects on the thyroid and the secondary action on thyroid hormone synthesis, secretion and serum levels, unless the experiments are performed in hypophysectomized animals or subjects, or as responses to administered TSH. An effect of iodide on a hypophysectomized animal has been shown for (1) the inhibition of TSH-induced thyroid hormone synthesis (Katakai and Yamada, 1966); (2) the trapping of iodide (Taurog *et al.*, 1958); (3) the inhibition of TSH-induced thyroid hormone secretion (Yamada and Lewis, 1968) or spontaneous secretion in autonomous adenomas (Green and Ingbar, 1962); and (4) the inhibition of TSH-induced thyroid growth (Bray, 1968). Of course, direct effects *in vitro* are also independent of the T₄–TSH loop, but their *in vivo* relevance should be proved; these effects are examined in Chapter Mechanism(s) of Action of Iodide, third part.

All these direct effects on the thyroid supplement the indirect effects through the negative feedback of thyroid hormones in ensuring an inverse relationship between iodine supply and thyroid stimulation.

The overall inhibitory effect of high doses of iodide on thyroid function tends to decrease over several weeks, which led to the abandonment of iodine treatment of thyrotoxicosis (Harden *et al.*, 1964), except for the temporary use of Lugol before surgery to reduce thyroid blood flow.

These effects of iodide have a great significance in medicine, as iodide excess induces hypothyroidism in several categories of patients: patients with Hashimoto's disease, postpartum thyroiditis, or previously treated with radioactive iodide or surgery, for Graves' disease, near-term fetuses, and so on. The absence of autoregulation of iodide transport in the fetus (Price and Sherwin, 1986; Sherwin, 1982) may explain amniocentesis and topical iodine-induced hypothyroidism of neonates (Chabrolle and Rossier, 1978; Rodesch *et al.*, 1976) as it does in some mice strains (Li and Carayanniotis, 2007). On the other hand, iodide deficiency leads to goiter by mechanisms that may in part be direct (Dumont *et al.*, 1995).

Models for the Study of Iodide Action

Clinical investigation

Various models have been used for the study of thyroid regulation. Of course, the main object of interest is the human being *in vivo*. The overall effects of iodide demonstrated by clinical investigation are as follows.

- The Wolff–Chaikoff effect is evidenced by the perchlorate discharge test, i.e., the release of radioiodide by the thyroid in response to perchlorate after administration of high levels of iodide. The fraction of the trapped iodide that is not organified is released by perchlorate.

- The adaptation of the Wolff–Chaikoff effect is evidenced by a decreased uptake of radioiodide or pertechnetate within hours of iodide administration.
- The iodide inhibition of thyroid hormone secretion is proven by the fall of serum thyroxine.
- The decreased thyroid blood flow, which was an object of controversy, has been confirmed (Arntzenius *et al.*, 1991) by Doppler measurement.

Animal models *in vivo* (rats, mice) reproduce the same results.

Thyroid slices and follicles

Thyroid slices of various animals (dog, pig, sheep) or humans are used as an *in vitro* model with the structure, composition and functional activity of the tissue *in vivo*. They are constituted of closed follicles and open follicles. They first exhibit all the functional properties of the tissue *in vivo*: active iodide uptake, iodide oxidation and binding to thyroglobulin, thyroglobulin synthesis, thyroglobulin endocytosis by macro- and micropinocytosis, secretion of thyroid hormone, response to TSH, and so on. The open follicles offer access to the normally inaccessible apical border of the cells. They are as functional as the closed follicles but of course, in the absence of a follicular lumen, do not concentrate iodide, organify iodide, or endocytose thyroglobulin any more. They allow direct measurements of H₂O₂ generation. Thyroid slices have provided the main experimental system for the study of the acute effects of iodide and their mechanisms. Follicles cultured in three dimensions have largely conserved the *in vivo* thyroid structure, and are therefore a suitable experimental model.

Primary cultures

Monolayer primary cultures of human or animal material have lost totally or in great part the follicular organization, the cell polarity, and thus the iodination and secretory processes. However, the cultures have largely retained their differentiated character and signal transduction organization. They are particularly useful to study, on a time scale of days, the effects of various regulations. Polarized cultures on filters exhibit iodide transport and thyroglobulin micropinocytosis and digestion (Croizet-Berger *et al.*, 2002; Ericson and Nilsson, 2000). In the case of iodide action however, such systems lack the capacity to synthesize organic iodine derivatives. Except at early stages of primary cultures (Rapoport *et al.*, 1975, 1977), the only observed effects of iodide have been observed at high concentration (e.g., 1 mM, which is 10000 times higher than normal serum levels). Although it might be argued that these high concentrations are necessary to compensate for the absence of iodide concentration and of colocalization of iodide, H₂O₂ and thyroid peroxidase (TPO), we consider

that, more probably, they might be considered artefacts and hence we do not use such results in this review.

Cell lines

Rat thyroid cell lines have been much in use. They have also lost all structural aspects and functions of the thyroid except transport. In spite of differences in signal transduction, they are useful to study the effects of hormones, oncogenes, specific proteins by siRNA technology, and so on, provided they are validated for the metabolism studied. PCCl3 cell line is considered as the choice material (Kimura *et al.*, 2001). Some iodide effects have been observed at very high iodide concentrations (10 mM), but as explained above they are not considered here. It is interesting to note that when reconstituted as follicles *in vivo* after transplantation, the same cells are 200 times more sensitive to iodide (Aeschimann *et al.*, 1994). In this review, we only consider the effects observed with suitable models at iodide concentrations comparable to those obtained *in vivo*.

Mechanism(s) of Action of Iodide

The XI model

In 1973, we showed that several effects of iodide on thyroid slices were inhibited by perchlorate which blocks iodide uptake, and by methimazole or propylthiouracil which inhibit thyroid peroxidase and iodide oxidation (Van Sande *et al.*, 1975, 1977; Van Sande and Dumont, 1973). This suggested that, to act, iodide had to penetrate the follicles and be oxidized at the apex of the cell where TPO is active. It supported the hypothesis, called the XI hypothesis, according to which the inhibitory effect of iodide is mediated by an iodinated molecule XI (Halmi and Stuelke, 1956; Van Sande *et al.*, 1975). The evidence for such a mechanism was the relief of an iodide effect by perchlorate and methimazole, and the absence of iodide effect in adenomatous tissue with an iodide organification defect (Demeester-Mirkin *et al.*, 1984). On the other hand, at high concentrations of iodide, enhanced H₂O₂ generation could by itself generate iodinated derivatives in the absence of thyroperoxidase. Such generation and its consequences would not be prevented by antithyroid drugs.

Such XI effects were studied in well-structured thyroid material, such as the whole thyroid or thyroid slices, but of course were not obtained in models in which, for lack of follicular structure, no concentration of iodide or iodide organification takes place, i.e., cells in primary cultures or cell lines.

A number of iodide effects satisfy the criteria of the XI model, i.e., the relief of the effect by drugs blocking

iodide uptake (e.g., NaClO₄) and organification (e.g., methimazole):

- the inhibition of H₂O₂ generation and iodide organification, and consequently the activation of glucose oxidation;
- the inhibition of cyclic 3',5'-adenosine monophosphate (cAMP) generation;
- the inhibition of the stimulated phospholipase C-IP₃-diacylglycerol cascade;
- the inhibition of TSH-induced secretion; and
- the inhibition of the transcription of NIS messenger RNA (mRNA).

All these effects, except perhaps the inhibition of H₂O₂ generation, imply an intracellular action. They are multiple, but very specific.

In dog thyroid slices, the inhibitory effect of iodide on cAMP response to TSH is relieved partially after 2 h and even more after 4 h in the presence of methimazole, which indicates that the half life of XI, or at least its effect, is about 2 h (Van Sande *et al.*, 1985). In the absence of methimazole, there is no relief after 4 h.

Effects of iodide not explainable by the XI model

As is well-known to thyroidologists, administration of Lugol to patients with Graves' disease during the 15 days before surgery is a recognized treatment to decrease thyroid blood flow and vascularization, even in methimazole-treated patients. Given the dosages of antithyroid drugs used, there is little possibility of an XI-mediated effect. Therefore, an alternative mechanism must be postulated.

Mechanism of some effects of iodide

Many effects of iodide on thyroid cell metabolism have been reported in various thyroid models of various species with various kinetics. To simplify the analysis, we shall consider the results mainly obtained on human thyroid material under conditions in which iodide oxidation and binding to organic molecules occurs. Moreover, we shall apply the Occam's razor rule i.e., consider the minimum number of mechanisms necessary to explain all findings. Our scheme, therefore, may not involve other mechanisms still to be demonstrated.

Iodide by an XI-type mechanism inhibits both cAMP and the phospholipase C cascades (Figure 32.2). The inhibition of TSH, prostaglandin E1 (PGE1), cholera toxin and forskolin-activated cAMP cascade bears on Gs-adenylyl cyclase couple and on cAMP generation (Cochaux *et al.*, 1987; Filetti and Rapoport, 1983;

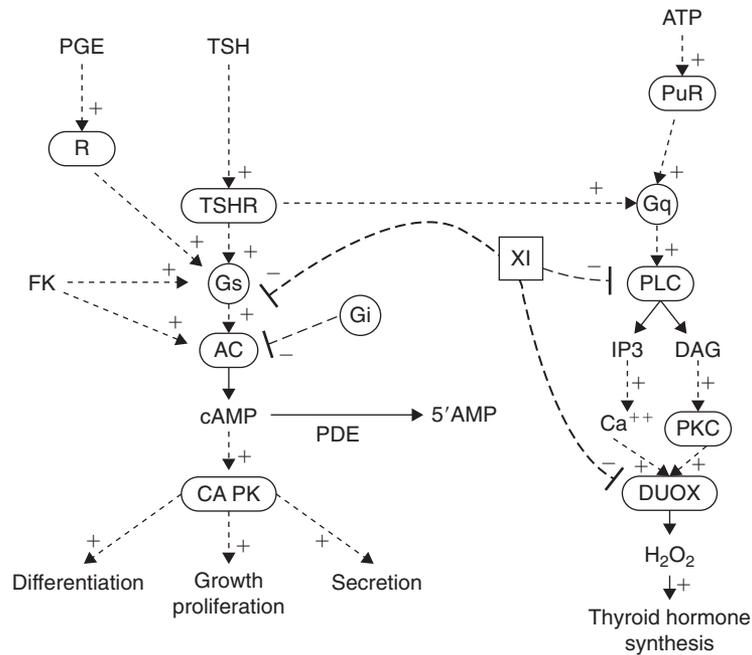


Figure 32.2 Inhibitory effects of XI on human thyroid signaling intracellular cascades. R, receptor; ATP, adenosine triphosphate nucleotide; PuR, purinergic receptor; Gs, stimulatory G protein of adenylyl cyclase; Gi, inhibitory G protein of adenylyl cyclase; Gq, stimulatory G protein of phospholipase C; AC, adenylyl cyclase; PLC, phospholipase C; IP₃, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C; DUOX, dual oxidase; PGE, prostaglandin E₁; TSHR, TSH receptor; cAMP, cyclic 3'-5'-adenosine monophosphate; PDE, cAMP phosphodiesterase; 5'AMP, adenosine monophosphonucleotide; cA PK, cAMP-dependent protein kinase; FK, forskolin; $\cdots \rightarrow +$ Stimulation; $\cdots \rightarrow -$ Inhibition; \rightarrow generation.

Heldin *et al.*, 1985; Rapoport *et al.*, 1975; Van Sande *et al.*, 1977). It persists in membranes after cell disruption. It does not bear on the disposal of cAMP (Van Sande *et al.*, 1977) in dog thyroid, but it does so in rat thyroid where it accelerates cAMP release (Mashita *et al.*, 1982). The fact that some effects of TSH, but not of N₂-O₆ dibutyryl cAMP (DBcAMP), i.e., an effector downstream of the TSH receptor-adenylyl cyclase system, are inhibited by iodide clearly suggests that iodide acts upstream of cAMP (Van Sande *et al.*, 1975). As cAMP in human thyroid stimulates thyroglobulin endocytosis and hydrolysis, and thus thyroid hormone secretion, this inhibition of cAMP synthesis accounts for the inhibition of hormone secretion by iodide, although another complementary effect downstream of cAMP has been demonstrated in rat (Yamamoto *et al.*, 1972) and mice (Bagchi *et al.*, 1985). The action on endocytosis may also account for the inhibition of TSH, cAMP-induced, stimulated calcium efflux, presumably accompanying thyroglobulin hydrolysis (Hashizume *et al.*, 1984). Similarly, cAMP in human thyroid mediates the growth-proliferation effect and the generation of IGF₁ (Hofbauer *et al.*, 1995) by TSH. Therefore, decreasing cAMP intracellular levels explains the growth-reducing effect of iodide. The same reasoning may be applied to the induction of NIS and

consequent iodide transport (Dohan *et al.*, 2003), and to the final maturation of DUOX2 in some systems (Morand *et al.*, 2003). The inhibition of iodide transport (the adaptation to the Wolff–Chaikoff effect), implying a decreased synthesis of the transporter NIS, is necessarily a delayed (several hours) effect of iodide. It does not explain shorter-term inhibitions of iodide transport that were reported in some systems (Socolow *et al.*, 1968).

As Ca⁺⁺ and diacylglycerol, presumably through their respective kinases, stimulate H₂O₂ generation and iodide oxidation in human thyroid (Corvilain *et al.*, 1994), the inhibition by an XI-type effect of the Gq–phospholipase C cascade accounts for the inhibition of iodide organification (the Wolff–Chaikoff effect) (Laurent *et al.*, 1989), and of prostaglandin synthesis (Boeynaems *et al.*, 1979). However, as direct effects of calcium ionophore and phorbol ester on H₂O₂ generation are also inhibited by iodide, a supplementary direct effect of XI on DUOX must be postulated (Figures 32.2 and 32.3). Those works are supported by other results which showed in bovine thyroid slices that the Wolff–Chaikoff effect can be overcome by adding exogenous H₂O₂, i.e. that H₂O₂ is the main limiting factor for organification in those conditions (Chiraseveenuprappund and Rosenberg, 1981). As H₂O₂ generation requires NADPH oxidation, it drives the activity

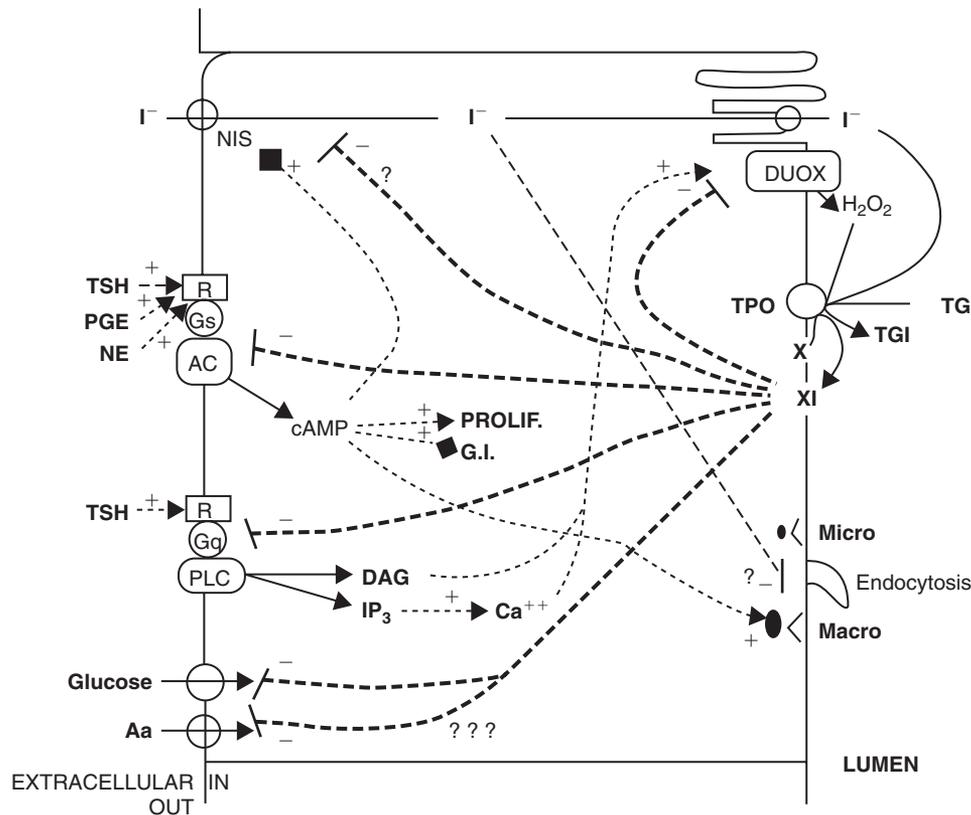


Figure 32.3 Inhibitory effects of iodide on human thyrocyte function. I^- , iodide; NIS, sodium iodide symporter; DUOX, dual oxidase; TPO, thyroperoxidase; TG, thyroglobulin; TGI, iodinated thyroglobulin; X, the substrate converted into the active inhibitory iodinated molecule XI; micro, micropinocytosis; macro, macropinocytosis; prolif, proliferation; G.I., gene induction; R, receptor; Gs, stimulatory G protein of adenylyl cyclase; AC, adenylyl cyclase; cAMP, cyclic 3'-5' adenosine monophosphate; PGE, prostaglandin E1; NE, nor epinephrine; Gq, stimulatory G protein of phospholipase C; DAG, diacylglycerol; IP₃, inositol 1,4,5-trisphosphate; Aa, amino acid; - - - ■ Induction; - - - -> Stimulation; - - - -| Inhibition; -> transport or chemical transformation.

of the pentose phosphate pathway and glucose oxidation. Its inhibition by iodide, therefore, also leads to the inhibition of the pentose phosphate pathway (Corvilain *et al.*, 1991). The stimulatory effect of iodide on H_2O_2 generation observed in some species does not involve a stimulation of cAMP or the phospholipase C cascade. It therefore, most probably reflects a direct XI effect (Corvilain *et al.*, 2000).

Other effects of iodide include the inhibition of glucose transport, presumably by reduction of the number of available glucose carriers in pig cells (Filetti *et al.*, 1986), and the inhibition of amino acid and uridine uptake (Kleiman de Pisarev *et al.*, 1978; Pisarev and Itoiz, 1972). The relationship of these effects to the previously described effects is not known.

All these effects, when obtained *in vitro*, satisfy the XI concept criteria, i.e., they are relieved by inhibitors of iodide transport and organification. However, a careful *in vivo* study of the relief of iodide inhibition by a fully active antithyroid drug treatment would be necessary to exclude supplementary, more direct, non-XI mechanisms in humans *in vivo*.

Inhibition of endothelial cells and angiogenesis must be indirect. As endothelial cells do not metabolize iodide, it is probable that the effects on thyroid endothelial cells result from paracrine information from thyrocytes. Indeed, iodide has been reported to inhibit VEGF expression and secretion by dog thyrocytes (Yamada *et al.*, 2006) and TGF has been reported as an intermediary in the action of XI (Cowin *et al.*, 1992; Yuasa *et al.*, 1992).

Candidates XI

A candidate for the XI role should be an iodinated compound synthesized in the thyroid gland and should, by itself, reproduce the effects of iodide. Several candidates have been proposed for XI.

- I_2 itself, which may inhibit TPO and thus iodination: however, such a specific mechanism would only explain in part the inhibition of iodination, but not of H_2O_2 production (Nunez and Pommier, 1982), i.e., part of the Wolff–Chaikoff effect.

- Iodine and oxygen radicals: such a short-lived mechanism would only explain part of the inhibition of iodination or the toxic effects of iodide (Denef *et al.*, 1996; Many *et al.*, 1992). It would presumably be short-lived and indiscriminate.
- Iodinated peptides (Lissitzky *et al.*, 1961): however, none has been found with suitable properties. Moreover, as iodination takes place at the luminal side of the apical membrane, a peptide iodinated there would have to access the inside of the cell.
- Thyroid hormones (Juvenal *et al.*, 1981): however, thyroid hormones are only synthesized within the thyroglobulin. They would have to be synthesized, then released from the thyroglobulin inside the cell, i.e., a stimulated process, and a rather long delay would then be necessary.
- General protein iodination: however, such intracellular protein iodination does not take place without the addition of huge amounts of H₂O₂ to the thyroid preparation. Moreover, the concentrations of methimazole necessary to relieve the XI effect are 10 times higher than those inhibiting protein iodination (Pereira *et al.*, 1990).
- Iodinated lipids: the only model retained in the literature was proposed by us and involves two iodinated lipids, i.e., iodolactones and iodoaldehydes (Figure 32.4). The presence of the first in thyroid is still controversial, whereas the second represents the most abundant iodinated lipid in the thyroid (Pereira *et al.*, 1990). Addition of the aldehyde 2-iodohexadecanal (2-IHDA) to thyroid slices and membranes reproduces two known XI-type effects of iodide: the inhibition of adenylyl cyclase (Panneels *et al.*, 1994b) and the inhibition of H₂O₂ generation (Ohayon *et al.*, 1994; Panneels *et al.*, 1994a). Iodolactone at high concentrations inhibits the proliferation of porcine thyroid cells induced by EGF (Dugrillon and Gartner, 1995).

Thus, there are good arguments to consider 2-IHDA as the mediator of iodide effects on cAMP accumulation and H₂O₂ generation and the Wolff–Chaikoff effects, but the question of the possible roles of iodolactone and 2-IHDA in other iodide effects remains open.

Iodolactones and iodoaldehydes as XI mediators

Iodolactones In 1980, Boeynaems and coworkers showed that lactoperoxidase was able to catalyze the iodination of some polyunsaturated fatty acids, resulting in the formation of a range of iodolactones (Boeynaems *et al.*, 1981b; Boeynaems *et al.*, 1981a; Boeynaems and Hubbard, 1980). In particular, arachidonic acid was

converted mainly into 6-iodo-5-hydroxyeicosatrienoic acid, δ -lactone (Boeynaems and Hubbard, 1980), and to a lesser extent a mixture of macrolides (15-iodo-14-hydroxyeicosatrienoic acid, ω -lactone, and 14-iodo-15-hydroxyeicosatrienoic acid, ω -lactone) (Boeynaems *et al.*, 1981a). Similarly, docosahexaenoic acid was converted into 5-iodo-4-hydroxydocosapentaenoic acid, γ -lactone (Boeynaems *et al.*, 1981b). Iodolactone formation is likely to result from the simultaneous interaction of the carboxylate moiety and a reactive form of iodine (I⁺, I^o) with a double bond, a reaction well-known by organic chemists. Rat thyroid lobes and isolated porcine thyroid follicles (Boeynaems and Hubbard, 1980; Dugrillon *et al.*, 1990; Dugrillon and Gartner, 1995) were able to convert exogenous free arachidonic acid into δ -iodolactone, when they were incubated in the presence of micromolar concentrations of iodide. It must be emphasized that no δ -iodolactone could be detected in these preparations, as well as in horse or dog thyroid slices (see below), unless exogenous arachidonic acid was added. This is consistent with the role of the carboxylate moiety in the iodolactonization reaction, and the fact that in tissues most of endogenous arachidonic acid is esterified in phospholipids. The δ -iodolactone was identified in a sample of thyroid tissue obtained during surgery from a patient with Graves' disease treated with high doses of iodide (Dugrillon *et al.*, 1994). This constitutes, so far, the only evidence that the δ -iodolactone is a genuine thyroid constituent and not an artifact resulting from unphysiological exposure to exogenous arachidonic acid.

Iodoaldehydes In 1990, Pereira *et al.* isolated the major iodolipid formed in horse thyroid slices incubated *in vitro* with radioiodide (Pereira *et al.*, 1990). It was identified as 2-iodohexadecanal (2-IHDA) (Figure 32.4) on the basis of proton nuclear magnetic resonance spectroscopy, mass spectrometry and coelution with authentic 2-IHDA, obtained by chemical synthesis, in reversed-phase, high-performance liquid chromatography and gas chromatography. In the same experiments, the formation of δ -iodolactone could not be detected, an observation consistent with the dependency of δ -iodolactone synthesis on exogenous arachidonic acid supplementation. 2-IHDA was also detected in the thyroid lobes of rats following intraperitoneal injection of a dose of iodide known to induce the Wolff–Chaikoff effect. In dog thyroid slices, both 2-IHDA and 2-iodooctadecanal (2-IODA) were detected, 2-IODA being the predominant species. Since 2-IHDA and 2-IODA are also formed during an incubation of bovine brain plasmalogens with lactoperoxidase, iodide and H₂O₂, their biosynthesis is likely to involve the addition of a reactive form of iodine (I⁺ or I^o) to the vinyl ether moiety of plasmalogens, followed by the rapid cleavage of that unstable intermediate (Figure 32.4). This hypothesis was supported by experiments demonstrating

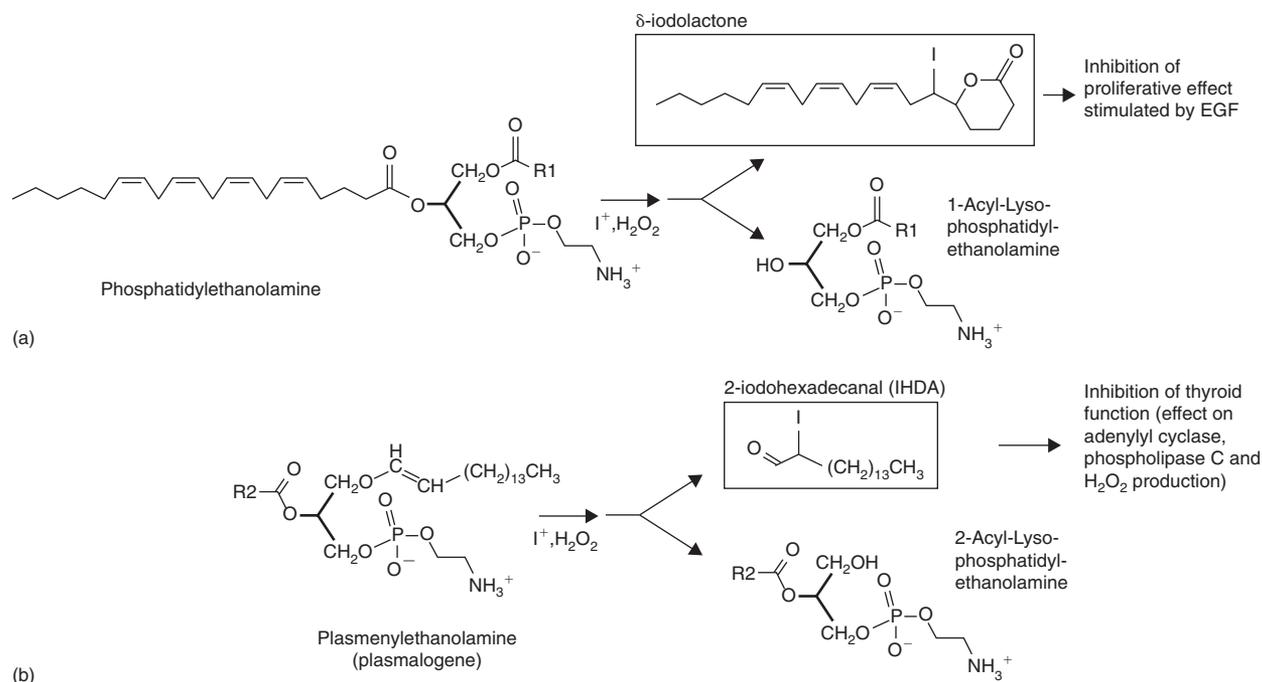


Figure 32.4 Biosynthesis of the two proposed mediators of iodide action in thyrocytes. R1, R2: acyl groups.

the formation of radioactive 2-IHDA following the addition of [3H]-hexadecanol, a plasmalogen precursor, to cultured dog thyroid cells. As a control, no 2-IHDA labeling could be obtained with [3H]-palmitate, which is incorporated into several classes of glycerophospholipids, but not into plasmalogens (Panneels *et al.*, 1996).

Role of Iodolactones The hypothesis that the δ -iodolactone derived from arachidonic acid or related compounds is involved in thyroid autoregulation by iodide has been investigated by the groups of Gartner and Pisarev. Pisarev and coworkers tested the effects of δ -iodolactone (6-iodo-5-hydroxyeicosatrienoic acid, δ -lactone), an ω -iodolactone (14-iodo-15-hydroxyeicosatrienoic acid, ω -lactone) and the free acid formed upon hydrolysis of ω -lactone (14-iodo-15-hydroxyeicosatrienoic acid). It must be emphasized that the formation of ω -iodolactone or the corresponding free acid has never been demonstrated in the thyroid, even when exogenous arachidonic acid is provided. In calf thyroid slices, the three compounds inhibited the incorporation of radioiodine into thyroid iodoproteins, as a result of decreased H_2O_2 availability (Chazenbalk *et al.*, 1988; Krawiec *et al.*, 1988). Although the free acid was the most potent of the three, a 100 μM concentration was needed to produce 50% inhibition. ω -iodolactone and the corresponding free acid also reduced the uptake of 2-deoxyglucose and aminoisobutyric acid in calf thyroid slices, with an almost equal potency (Krawiec *et al.*, 1991). However, when the mechanisms of action of

iodide and δ -iodolactone were compared, different results were obtained. While iodide exerted its action at the membrane and transcriptional levels (inhibiting the expression of some thyroid-specific genes), the action of δ -iodolactone was mainly at the membrane level (Krawiec *et al.*, 1991). Daily intraperitoneal injection of δ -iodolactone, ω -iodolactone, or its free acid derivative into rats reduced the increase in thyroid weight resulting from chronic stimulation by TSH induced by methimazole administration (Pisarev *et al.*, 1988): the three compounds had roughly equivalent effects. The inhibition of thyroid growth was not mediated by iodide released by deiodination of these compounds, because methimazole had no effect impaired organification. It was associated with a decrease in the thyroid cAMP content. *In vitro*, ω -iodolactone inhibited the proliferation of FRTL-5 cells at μM concentrations, and was more potent than δ -iodolactone (Pisarev *et al.*, 1992). As the proliferative effect of forskolin was also inhibited, the authors suggested that the inhibitory action of iodolactones might be linked to a reduction in cAMP or to a step beyond cAMP formation. This is not consistent with the results of Dugrillon and Gartner, who found that in pig thyroid follicles δ -iodolactone inhibited the proliferative effect of EGF (Dugrillon *et al.*, 1990) and the formation of inositol phosphates stimulated by EGF, but had no effect on TSH-induced accumulation of cAMP (Dugrillon *et al.*, 1990; Dugrillon and Gartner, 1995). However, a control effect of cAMP inhibition by KI in this system was not reported. Interestingly, δ -iodolactone was 50-fold

more potent than iodide itself, and inhibitory effects were detected in the 0.1–1 μM range. The inhibition was mimicked neither by the γ -iodolactone derived from docohexanoic acid, nor by an uniodinated δ -lactone.

In summary, the studies of Pisarev and coworkers must be interpreted with caution, since the concentrations used were in general quite high (most of the data were obtained at 100 μM) and there was little selectivity, with δ -iodolactone, ω -iodolactone, and its free acid form all being active. Furthermore, the formation of ω - and δ -iodolactone or of its free acid form in the thyroid gland is still controversial.

The data provided by Dugrillon and Gartner are more impressive: reasonable potency, evidence (though limited) of structural specificity and selectivity for the EGF signaling pathway. However, the evidence that δ -iodolactone is a mediator of iodide is restricted to one particular parameter, the inhibition of EGF proliferative action, and a conclusive demonstration that it can be formed in sufficient amounts from the endogenous pool of arachidonic acid remains to be provided.

Role of Iodoaldehydes In parallel to these studies, the biological actions of 2-iodoaldehydes have been studied in Brussels. The two components of the inhibitory action of iodide on H_2O_2 production (generation of intracellular signals and direct action on the H_2O_2 generating system) were mimicked by 2-IHDA. In porcine thyroid membranes, 2-IHDA strongly inhibited the NADPH oxidase involved in H_2O_2 generation (Ohayon *et al.*, 1994), now known to be DUOXes. The aldehyde function at C1 was absolutely necessary, but a halogen at C2 was not. However, inhibition by hexadecanal was fully reversible, whereas inhibition by 2-IHDA was irreversible. A partial inhibition of the thyroid peroxidase was also observed, while other enzymes, such as NADPH-cytochrome c reductase, were unaffected. In cultured dog thyroid cells, 2-IHDA decreased the production of H_2O_2 in response to muscarinic and bradykinin receptor activation, with no adverse effect on cell viability (Panneels *et al.*, 1994a). This decrease resulted from an inhibition of the phospholipase C cascade. Since these cells do not organify iodide, and since the inhibition was not suppressed by methimazole, it cannot result from the deiodination of 2-IHDA with recycling of iodine into another intermediate. The comparison of various analogs of 2-IHDA allowed the identification of two major structural features required for this biological activity: the aldehyde function at C1 (i.e., 2-iodohexadecanol was inactive) and a halogen at C2, with iodine conferring greater activity than bromine (chlorine and fluorine being completely inactive). These structural requirements are clearly different from those characterizing the direct action of 2-IHDA on the DUOXes, suggesting the involvement of distinct interactions and biochemical mechanisms.

In cultured dog thyroid cells, 2-IHDA mimicked the inhibitory effect of iodide on cAMP accumulation (Panneels *et al.*, 1994b). It also directly inhibited the adenylyl cyclase activity in human thyroid membranes (Panneels *et al.*, 1994b), whereas iodide has no effect on that system (because it is not oxidized by the thyroid peroxidase under the experimental conditions used). These actions of 2-IHDA share the following characteristics of the inhibition of cAMP formation by iodide in intact cells or in membranes prepared from thyroid tissue exposed to iodide (Cochaux *et al.*, 1987).

- The inhibition is stable and cannot be reversed by simple washings.
- It is due to a decreased V_{max} of the adenylyl cyclase, with no effect on the K_m for ATP- Mg^{2+} .
- The effects of all stimuli of adenylyl cyclase are inhibited (TSH, GTP- γ -S and forskolin).
- The inhibition is not mediated by Gi (Panneels *et al.*, 1994b).

The inhibitory action of 2-IHDA had some target specificity, since 2-IHDA had no effect on other enzymes involved in ATP metabolism (Mg^{2+} -ATPase, creatine kinase) or on the TSH receptor. A comparison with various analogs also revealed reaction specificity involving the crucial role of the same structural determinants for the inhibition of H_2O_2 production and adenylyl cyclase: the aldehyde function at C1 (alcohol function at C1 was inactive) and iodine at C2 (noniodinated aldehydes were less or not active; chlorine and fluorine derivatives were inactive). Taken together, these data strongly suggest that 2-IHDA might be the mediator of two important regulatory actions of iodide in the thyroid: the Wolff–Chaikoff effect and the inhibition of adenylyl cyclase (Figure 32.3).

Preliminary results suggest that 2-IHDA also has an antigoitrogenic action. The simultaneous injection of IHDA (10 μg) reduced the goiter induced by MMI (Juvenal, unpublished).

It is interesting to mention that the synthesis of iodolipids is not only restricted to the thyroid, but has also been reported in the mammary gland, which organifies iodide (Freinkel and Ingbar, 1956).

Clinical Significance of the Effects of Iodide

The range over which iodide concentrations vary *in vivo*, as well as the spectrum of iodide effects, is very large. Although no detailed study has been made of the concentration or dose-effect relationships of the various actions of iodide in one system *in vivo*, we can propose a qualitative explanatory scheme. With normal European diets, the extracellular iodide concentration would be around

2×10^{-8} to 5×10^{-8} M, with US-type diets being 5×10^{-8} to 5×10^{-7} M (Wolff, 2001). In these ranges, autoregulation by the relative expression of NIS, and also of TPO (Uyttersprot *et al.*, 1997), plays its role as shown by the negative correlation between radioiodine uptake and dietary iodine in these populations. This regulation probably reflects both the direct effects of iodide and the homeostatic regulation of the thyroid hormone–TSH negative feedback (Brabant *et al.*, 1992). Below this range, an activation of H_2O_2 generation by iodide would make sense. Higher levels of iodide ($> 0.4 \mu\text{M}$) are necessary to induce the Wolff–Chaikoff effect. The inhibition of thyroid secretion and blood flow takes place at a much higher iodine intake, i.e., above 6 mg/day up to 125 mg/day (1 ml of Lugol), leading to serum concentrations of 5×10^{-5} M. These are therapeutic or toxic levels of iodide. Of course, such levels may be achieved through the ingestion or administration of iodine-containing drugs (e.g., amiodarone) or radiology contrast agents (e.g., lipiodol). The thyroid is particularly sensitive to iodide-induced hypothyroidism in some strains of mice (Li and Carayanniotis, 2007) in the fetus and neonate (for lack of adaptation to the Wolff–Chaikoff effect) (Momotani *et al.*, 1992; Sherwin, 1982; Wolff, 2001), and probably for the same reason in thyroiditis (Vagenakis and Braverman, 1975).

Iodide at high concentrations induces thyroiditis in some individuals. The mechanism of this effect is unknown (Wolff, 2001), although it might be related to the described induction by iodide of genes involved in lymphocyte modulation in cultured human thyroid cells (Yamazaki *et al.*, 2003).

At high concentrations and in the absence or before the onset of autoregulation, iodide can lead to apoptosis and necrosis (Belshaw and Becker, 1973; Hindie *et al.*, 2001) and consequently thyroiditis (Bagchi *et al.*, 1985, 1995). When the gland is hyperstimulated, i.e., in Graves' disease, autonomous adenomas, or even goiters presumably containing autonomous areas (e.g., endemic goiters), iodide in high normal doses corresponding to serum 0.1–1 μM induces thyrotoxicosis (Stanbury *et al.*, 1998). This demonstrates a lack of autoregulation in these glands (Wolff, 2001). It represents dangerous side-effects at the initiation of iodine prophylaxis programs (Bulow *et al.*, 2006). It must be emphasized that all these toxic effects of iodide occur only in a minority of individuals, with the majority adapting very well (Bulow *et al.*, 2006; Theodoropoulou *et al.*, 2007).

Regarding goiter, in most cases no correlation was found between the size of the goiter and plasma TSH levels. In experimental goitrogenesis, there is evidence that thyroid weight increases significantly before an increase in TSH levels, and thus may be due to a lowered intrathyroidal iodine concentration (Berthier and Lemarchand-Beraud, 1978; Naeije *et al.*, 1978). Moreover, during goiter growth, thyroid DNA content is weakly correlated to TSH, but inversely related to thyroidal iodine (Stubner

et al., 1987). This would explain the coexistence of goiter with normal TSH levels (Pisarev *et al.*, 1970 1971).

There is a correlation between the histological type of thyroid cancer and the intake of iodine. The introduction of iodine prophylaxis increased the ratio of papillary to follicular carcinomas (Feldt-Rasmussen, 2001; Harach *et al.*, 2002). Of course, depending on the selective strength of the different iodine or XI effects in various species, or even in various genetic backgrounds or conditions in one species, the final results of the same iodine treatment may be different or even opposite. The same iodine supply may lead to hyperthyroidism (as in endemic goiter) or to hypothyroidism. This is well-illustrated by a study showing that the same iodide treatment induces goiters and hypothyroidism in one strain of mice, but not in another (Li and Carayanniotis, 2007).

Summary Points

- Iodide, the main substrate used in the synthesis of thyroid hormone, exerts a negative control on the activity and growth of the thyroid gland.
- Iodide is the limiting factor in the synthesis of thyroid hormones. More iodide allows the synthesis of more thyroid hormones, which inhibit the secretion of pituitary TSH, the main thyroid stimulant.
- Iodide also directly inhibits the activity and growth of the thyroid due to the generation of an organified inhibitor XI in the thyroid.
- Two iodolipids have been identified as candidates for XI: iodolactones which act on the thyroid but may not be synthesized in the gland, and 2-IHDA which is found in the thyroid and may account for some iodide effects including the Wolff–Chaikoff effect.
- The indirect effects of iodide through thyroid hormones are observed at low or physiological levels of iodide intake and serum concentrations. The direct effects are mostly observed at supraphysiological, therapeutic, or toxic levels.
- However, at very high doses, iodide may directly inhibit thyroid secretion and blood flow, effects that are used in the preoperative Lugol treatment of Graves' disease.

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Influences of Iodine on the Immunogenicity of Thyroglobulin

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Abstract

Among known autoantigens, thyroglobulin (Tg) is unique in its capacity to store iodine, an element that we receive through our daily diet. Evolutionary pressure has shaped Tg into a large scaffolding molecule, to allow enzyme-mediated organification of iodide and formation of thyroid hormones via intramolecular coupling of specific iodotyrosyl residues. Post-translational modification of Tg by iodine has inevitable immunological consequences: challenge of genetically susceptible hosts with highly iodinated Tg has been known to induce experimental autoimmune thyroiditis (EAT) with increased incidence and severity than EAT elicited by Tg with a normal iodine content. The molecular mechanisms underlying this process remain mostly unknown. To gain further insight, several studies have mapped distinct iodine-containing Tg peptides that are recognized by thyroiditogenic T-cells in EAT. This chapter summarizes the work in this area and discusses how the immune recognition of Tg can be influenced by its iodine content in genetically susceptible hosts.

Abbreviations

EAT	Experimental autoimmune thyroiditis
HI-Tg	Tg of high iodine content
HT	Hashimoto's thyroiditis
hTg	Human Tg
LI-Tg	Tg of low iodine content
MHC	Major histocompatibility complex
mTg	Mouse Tg
T4	Thyroxine
Tg	Thyroglobulin
Treg	Regulatory T cell

Introduction

The immunopathogenic role of thyroglobulin (Tg), well-known since 1956 from the pioneering studies of Rose and

Witebsky in rabbits (Rose and Witebsky, 1956), has been documented in Tg-induced experimental autoimmune thyroiditis (EAT), a T-cell-mediated disease considered to be a model for Hashimoto's thyroiditis (HT) in humans (Stafford and Rose, 2000). Recently, this has been highlighted by linkage and association studies implicating Tg as the first thyroid-specific susceptibility gene in human autoimmune thyroid disease (Ban *et al.*, 2003; Tomer *et al.*, 2002). Several studies have delineated defined peptide fragments within the 2749 aa sequence of Tg (Dunn and Dunn, 2000) which are recognized by thyroiditogenic T-cells in mice (Carayanniotis and Kong, 2000; Carayanniotis, 2003; Gentile *et al.*, 2004). The immunopathogenicity of these determinants can vary considerably, depending on the genetic make-up of the host strain and, in particular, the major histocompatibility complex (MHC) class II genes that encode susceptibility and resistance. Even for susceptible hosts, an immunodominant determinant that mirrors the thyroiditogenicity of the intact Tg molecule has not been found and, so far, all known pathogenic Tg determinants have been classified as subdominant or cryptic. Peptides containing subdominant determinants are recognized by Tg-primed T-cells *in vitro*, but peptide-primed T-cells are not consistently activated by Tg *in vitro*. Cryptic Tg determinants are immunopathogenic, but Tg-primed T-cells do not respond to them *in vitro* and, conversely, peptide-primed T-cells are not activated by Tg *in vitro*. This "cryptic self" (Carayanniotis, 2003) within Tg is not of minor importance, since all these peptides must be intrathyroidally expressed in order to be recognized by infiltrating, preactivated peptide-specific T-cells. Therefore, investigation of mechanisms that avert their generation or suppress their immunogenic behavior once they are formed deserves considerable attention. Iodination as a post-translational modification (Anderton, 2004; Doyle and Mamula, 2001) is unique to Tg, which has evolved to facilitate iodine storage, and ultimately its incorporation into thyroid hormones via intramolecular coupling of specific iodotyrosyls (Dunn *et al.*, 1983). Increasing ingestion of iodine has been linked to autoimmune thyroiditis (Rose

et al., 2002), and Tg may be a molecular link in this process. Pathogenic Tg peptides are, accordingly, divided into three categories: (a) noniodinated peptides, whose formation during Tg processing in antigen-presenting cells (APC) is either unrelated or only indirectly linked to the iodination status of Tg; (b) iodinated peptides encompassing hormonogenic sites; and (c) iodinated peptides containing iodotyrosyls. This chapter focuses mainly on the last two categories, and discusses molecular processes whereby iodine can promote the immunopathogenicity of Tg.

Categories of Iodinated Pathogenic T-Cell Determinants in Tg

Pathogenic Tg peptides containing primary hormonogenic sites

To examine the contribution of iodination to the immunogenicity of Tg peptides, a logical place to begin is the primary hormonogenic sites containing thyroxine (T4), particularly since *in vitro*-iodinated Tg was shown to induce more severe EAT than poorly-iodinated Tg (Champion *et al.*, 1987). These workers further showed that a 12mer peptide (2459–2560) containing T4 at aa position 2553, T4(2553), induced EAT, whereas a similar peptide containing only tyrosine was not pathogenic (Hutchings *et al.*, 1992). However, since a peptide containing a noniodinated thyronine (T0) was not compared in parallel, it was not firmed that the presence of four iodine atoms was the sole contributor to its pathogenicity. Two questions were thus addressed by one of us: (a) whether other conserved primary hormonogenic sites were likewise immunogenic, and (b) whether iodination was a requisite for T4(2553) to be pathogenic, and whether this observation extended to the other hormonogenic sites (Kong *et al.*, 1995).

Accordingly, in addition to T4(2553), the synthetic 12mer peptides (1–12) and (2559–2570) containing T4 or (T0) at aa position 5 or 2567, respectively, were examined in parallel for the capacity to stimulate Tg-primed T-cells, and their thyroiditogenicity was tested by direct immunization of *H2^k* strains (CBA/J and C57BR) and subsequent activation of either Tg- or peptide-primed T-cells for thyroiditis transfer into naive recipients (Kong *et al.*, 1995; Wan *et al.*, 1997). Furthermore, since these epitopes were conserved among mammalian species, cells from both human and mouse Tg-primed mice were included in the study. T4(2553) was classified as a subdominant, naturally-processed epitope: it activated both human and mouse Tg-specific T-cells for thyroiditis transfer and induced mild thyroiditis in 20–50% of mice only after vigorous immunization with mouse Tg (mTg) in complete Freund's adjuvant. T4(5) was more weakly stimulatory for either Tg-primed or T4(5)-primed T-cells. Both T4(5) and T4(2553) induced specific antibodies. In contrast, T4(2567) was devoid of immunogenicity; it

neither stimulated peptide-primed T-cells nor elicited peptide-specific antibodies.

We also synthesized peptides containing the fourth primary hormonogenic site at the C-terminus at aa 2746, derived from both human and mTg, as they are not conserved. Neither of them was immunopathogenic (Wan and Kong, unpublished data). Thus, the answer to the first question is that peptides with T4-containing hormonogenic sites other than T4(2553) are either not immunogenic or very weakly so. Comparing T0(2553) with T4(2553), where iodination was considered critical in pathogenicity (Hutchings *et al.*, 1992), both activated Tg-primed or peptide-primed T-cells for thyroiditis transfer. Furthermore, each reciprocally stimulated the other peptide-primed T-cells, indicating that T0(2553) was immunogenic in the absence of iodotyrosyl residues. Each also generated *in vitro* CD8⁺ cytotoxic T cells from Tg-primed T-cells and served as antigen on peptide-labeled target cells in a reciprocal manner (Wan *et al.*, 1998). Thus, the answer to the second question is that the antigenicity of conserved hormonogenic sites is intrinsic, dependent more on their amino acid composition than on thyronine substitution with iodine on four sites. Where the peptide is immunogenic, the presence of T4 increases the stimulatory activity of the peptide *in vitro* (Kong *et al.*, 1995). Interestingly, since T4(5) is the most active hormonogenic site made by the thyroid even at low iodine availability (Dunn *et al.*, 1987; Marriq *et al.*, 1984), its low antigenicity cannot be explained by its absence in the Tg preparation used for immunization. Since it is weakly stimulatory for Tg-specific T-cells *in vitro*, it appears to exist after natural processing (Kong *et al.*, 1995). Thus, even a T4-containing, naturally processed epitope may not be pathogenic. T4(2567), the third peptide from a primary hormonogenic site, appears not to be naturally processed nor to serve as a cryptic epitope.

These three conserved T4-containing peptides were also tested in *HLA-DRB1*0301* (DR3)-transgenic, murine class II-knockout mice, since DR3 molecules were permissive for both human and mouse Tg-induced EAT (Kong *et al.*, 1996), as well as for human TSHR plasmid DNA induction of thyroid-stimulating antibodies (Flynn *et al.*, 2004c) and TPO plasmid DNA induction of EAT (Flynn *et al.*, 2004a). Only T4(5) was mildly stimulatory for Tg-primed T-cells, indicating that the T4-containing peptides are not major Tg epitopes presented by DR3 molecules (Flynn and Kong, unpublished data). However, identification of naturally processed, pathogenic Tg epitopes in DR3⁺ mice was possible using computer-assisted prediction of putative DR3-binding peptides. Of the 39 15–23mer peptides selected for testing, four stimulated human Tg (hTg)-primed cells (Flynn *et al.*, 2004b). One of the four, a 15mer peptide hTg2079 (aa 2079–2093), that does not contain tyrosine and thus has no possibility of being iodinated, activated hTg-primed T-cells for thyroiditis transfer, and induced EAT with a 50–70% incidence of mild thyroiditis. Interestingly, the identical

sequence of this subdominant peptide was found independently, after sequencing peptides eluted from DR3-associated peptide complexes isolated from the thyroid tissue of a GD patient, confirming the natural ligand property of our peptide (Muixí *et al.*, 2008).

Pathogenic Tg peptides containing iodotyrosyls

Recently, studies using computerized algorithms have focused on the search for tyrosine-containing Tg T-cell epitopes with pathogenic potential (Li and Carayanniotis, 2006). Three A^k-binding peptides – I-p117 (aa 117–132), I-p304 (aa 304–318), and I-p1931 (aa 1931–1945) (Table 33.1) – have been found to activate autoreactive T-cells and cause EAT in CBA/J mice only in their iodinated (i.e., iodotyrosyl-containing) form, whereas the noniodinated analogs are not immunogenic (Figure 33.1). The bulky iodine atom (atomic radius of ~133 pm) facilitated either peptide binding to MHC or T-cell recognition of the peptide–MHC complex. Lymph node cells (LNC) activated by the iodinated analog did not cross-react with the noniodinated form of the peptide, suggesting that adoptively transferred peptide-specific effector T-cells (T_{eff}) mediating EAT must recognize the iodinated peptides *in situ*, under steady-state conditions.

A further work showed that iodotyrosyl formation at three other sites can exert variable effects, as it can enhance (p179, aa 179–194), suppress (p2540, aa 2540–2554), or not alter (p2529, aa 2529–2545) the immunogenic profile of peptides at the T-cell level (Li *et al.*, 2007). Such effects did not seem to influence the weak pathogenicity of these peptides, and subsequent investigations proceeded to examine how the addition of a single iodine atom to a single Tg T-cell epitope can affect T-cell recognition at the clonal level. A panel of T-cell hybridoma clones were generated against the 16mer peptide p179 or its iodinated analog I-p179, to examine the possible effects iodination of Y192 might have on T-cell recognition (Jiang *et al.*, 2007). p179 binds to both A^k and E^k molecules, and this binding was not significantly influenced by iodine while truncation analysis mapped the minimal T-cell epitope within the 11mer peptide (184–194). Iodination of Y192 had unpredictable effects: some clones were activated only when the iodine atom was present, others when the iodine atom was lacking, and yet other clones by both the p179 and I-p179 analogs, tolerating the presence of iodine. These results were interpreted to mean that the formation of a neoantigenic determinant by iodine can have unpredictable consequences at the polyclonal level, depending on the relative number and/or effector function of autoreactive T-cell clones that are switched on or off by this determinant.

Table 33.1 Immunopathogenic properties of iodine-containing Tg peptides in EAT

Mouse strain	H2 haplotype	MHC restriction	Relative position ^a	EAT induction		T-cell proliferation	Ab response	References
				Directly	Transfer			
CBA, SJL	k,s	ND	Tg (1–12) ^b	Weak	+, weak in s	+/-	ND	Kong <i>et al.</i> , (1995)
CBA	k	A ^k	mTg (117–32) ^c	Weak	+	+	+	Li and Carayanniotis (2006)
CBA	k	A ^k , E ^k	mTg (179–94) ^d	Weak	ND	+	+ ^e	Li <i>et al.</i> , (2007)
CBA	k	A ^k	mTg (304–18) ^c	–	+	+	+/-	Li and Carayanniotis (2006)
CBA	k	A ^k	mTg (1931–45) ^c	+	+	+	–	(Li and Carayanniotis (2006)
CBA	k	A ^k	mTg (2529–45) ^d	Weak	ND	+	+	Li <i>et al.</i> , (2007)
CBA	k	A ^k	mTg (2540–54) ^d	Weak	ND	+	+	Li <i>et al.</i> , (2007)
CBA	k	A ^k	Tg (2549–60) ^b	Weak	+ ^f	+	+	Hutchings <i>et al.</i> , (1992); Kong <i>et al.</i> , (1995); Wan <i>et al.</i> , (1997)

Note: ND: not determined.

^aAmino acid coordinates were assigned as previously (Carayanniotis, 2003) and do not include the leader sequence. For some peptides, these coordinates may differ slightly from those that appeared in the original publication. The peptides (1–12) and (2549–2560) are identical in mTg and hTg.

^bPeptides containing homonogenic sites.

^cPeptides immunopathogenic only in their iodinated form.

^dPeptides immunopathogenic in either the iodinated or noniodinated form.

^eAb responses elicited only by the iodinated analog.

^fWith transfer of LNC primed *in vivo* with either peptide or mTg and boosted *in vitro* with peptide.

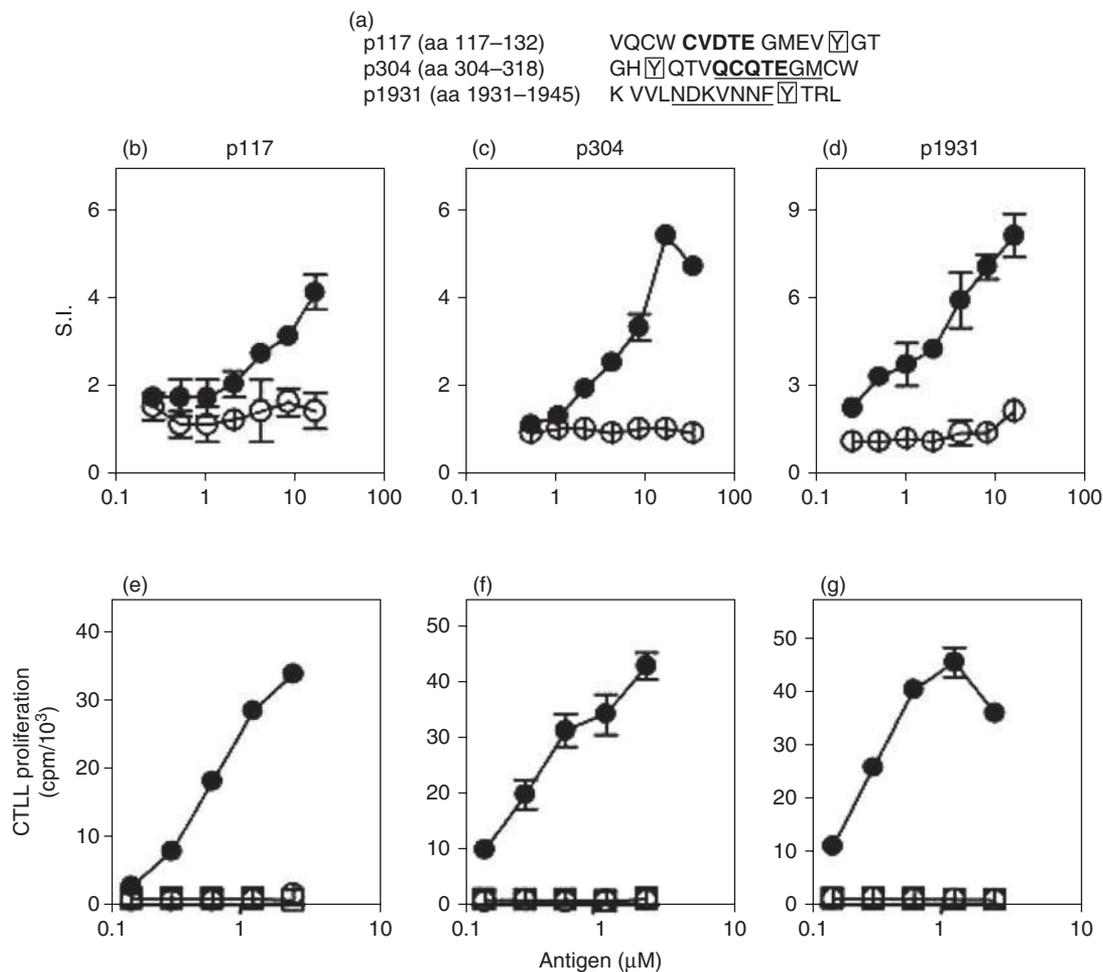


Figure 33.1 (a) aa coordinates and sequence of peptides used in the study. A^K-binding motifs are underlined (motif a) or in bold-face (motif B). Tyr residues with or without iodine are shown in boxes. (b,c,d) CBA/J mice (two mice per group) were primed with the noniodinated (○) or iodinated (●) form of the indicated peptides, and LNC responses were tested, 9 days later, against the respective peptide. (e,f,g) IL-2 secretion by the T-cell hybridoma clones 4A6, 10C1, and 1H7 cultured in the presence of DC and iodinated (●) or noniodinated (○) forms of p117, p304, and p1931, respectively, as well as intact Tg (□). Data are representative of two to four independent experiments. Background values ranged from 2000 to 5000cpm. Reprinted from [Li and Carayanniotis \(2006\)](#) with permission from The American Association of Immunologists.

Thus, the enhanced immunogenicity of an iodine-modified epitope may not lead to increased pathogenicity if peptide-reactive cells do not adopt a T_{eff} phenotype. Such a mechanism may also be operational against T4-containing peptides such as T4(5), (see 'Pathogenic Tg Peptides Containing Primary Hormonogenic Sites'), as there may have been evolutionary pressure to safeguard the host response against this important functional site.

Iodine and Peripheral T-Cell Reactivity to Tg

Twenty years ago, Roitt's group ([Champion *et al.*, 1987](#)) reported an enhancing effect of iodine on the immunogenicity of Tg at the T-cell level, and suggested that

iodothyronines were involved in this process. They found that mouse T-cell hybridomas were activated by hTg or mTg with a normal iodine content (3–4 T4 residues/molecule), but were unresponsive to hTg or mTg virtually devoid of iodine content (<0.05 T4 residues/molecule). Subsequently, these hybridomas were found to be specific for the T4(2553) peptide ([Champion *et al.*, 1991](#)). In the same year, [Sundick *et al.* \(1987\)](#) showed that normal K strain chickens challenged with chicken Tg of high iodine content (HI-Tg, 60–145 I atoms/dimeric molecule) produced Abs that reacted with HI-Tg, T3 and T4, whereas chickens challenged with Tg of low iodine content (LI-Tg, 6–12 I atoms/dimeric molecule) produced very few Abs to LI-Tg, T3, or T4. A modest response was, surprisingly, observed against HI-Tg, but in essence, these findings on Tg-specific B-cell reactivity paralleled those of Champion

et al. (1991). Subsequent studies confirmed the enhanced immunogenicity of HI-Tg in SJL mice (Dai *et al.*, 2002) at the B- and/or T-cell level. However, studies with the BB/W rat model of spontaneous thyroiditis yielded apparently conflicting results. Ebner *et al.*, 1992 showed that early challenge (before day 75 of age when spontaneous thyroiditis starts) of the highly susceptible NB rat subline with normal Tg induced thyroiditis in 31% of the animals, but similar challenge with LI-Tg did not cause disease. However, others (Allen and Thupari, 1995) demonstrated that splenic T-cells from 30- to 45-day-old unprimed BB/Wor rats responded equally well to HI-Tg, normal Tg and LI-Tg preparations, suggesting that spontaneously occurring Tg-reactive T-cells are not influenced by the iodine content of Tg.

The critical role of iodine in the enhancement of Tg immunogenicity can be best explained on a structural basis, i.e., by the formation of neoantigenic determinants containing either iodothyronines or iodotyrosyls. As described above, iodotyrosyl formation within a single T-cell epitope may have variable and unpredictable effects on Tg immunogenicity, as autoreactive clones are switched on or off by the neoantigenic determinant. A study from Burek's laboratory (Saboori *et al.*, 1998a, b), analyzing Tg immunogenicity, through the use of mAbs, has confirmed this concept at the B-cell epitope level. However, as the level of Tg iodination increases, the number of iodinated B- or T-cell epitopes may increase, tipping the balance toward a preponderance of T_{eff}-cell clones or B cells responding to the iodine-modified Tg. Enhancement of Tg immunogenicity by iodine can also be explained via an alternative, but not mutually exclusive, mechanism taking into account that the structure (Edelhoch *et al.*, 1969) and proteolytic degradation (Fouchier *et al.*, 1983; Lamas and Ingbar, 1978) of Tg have been shown to be affected by the iodine content. Iodination may alter the immunodominance hierarchy within Tg, rendering some cryptic peptides dominant. In support of this concept, it was shown that processing of HI-Tg in DC or macrophages from SJL mice selectively generates the *noniodinated* pathogenic Tg peptide p2495 (Dai *et al.*, 2002). If it applies to more epitopes, this process can be potentially very important in pathogenesis as it would facilitate the "spreading" of the immune response (Lehmann *et al.*, 1993) to a considerable number of noniodinated but pathogenic Tg determinants.

Regulatory T cells (Tregs) have been implicated in early studies of spontaneous development of thyroiditis in thymectomized rodents (Kojima *et al.*, 1976; Penhale *et al.*, 1975; Silverman and Rose, 1974). Their suppressive function was subsequently suggested by studies in which elevation of circulatory Tg levels, via injection of Tg (Kong *et al.*, 1982), or via TSH-mediated release of endogenous Tg (Lewis *et al.*, 1987), led to tolerance induction and prevention of Tg-induced EAT. It was then shown that Tg-induced tolerance was mediated by CD4⁺ T-cells (Kong

et al., 1989). Recent studies have further characterized Tregs in EAT as belonging to the CD4⁺CD25⁺ subset (Morris *et al.*, 2003; Morris and Kong, 2006; Yu *et al.*, 2006) and existing naturally in low numbers in both susceptible and resistant mouse strains (Morris *et al.*, 2005). Their induction has been proposed to occur either via injection of mTg-primed mice with GM-CSF (Gangi *et al.*, 2005), or challenge with Tg-pulsed, TNF- α -treated dendritic cells (Verginis *et al.*, 2005). The above studies (Gangi *et al.*, 2005; Kong *et al.*, 1982; Lewis *et al.*, 1987; Verginis *et al.*, 2005) support the view that *normally iodinated* Tg can be presented in a tolerogenic context, perhaps leading to the expansion of naturally occurring, specific Tregs. It has been suggested that Treg may recognize distinct tolerogenic fragments in Tg (Bagchi *et al.*, 1996) that may have a low iodine content (Gardine *et al.*, 2003), but the natural ligands for Tg-specific Treg have not been identified and it remains unknown whether they contain iodine.

Abs Recognizing Iodinated Determinants as Regulators of Tg-Specific T-Cell Reactivity

A study with Tg peptides as model antigens (Carayanniotis and Kong, 2000) has yielded valuable insights as to how Tg-reactive Abs can influence T-cell recognition of pathogenic determinants. At least two distinct mechanisms may be involved – first, Abs elicited against iodotyrosyl-containing peptides such as the I-p117 (Li and Carayanniotis, 2006), localized on the surface of the intact Tg molecule, may bind to normally iodinated Tg and form immune complexes. *In vitro* experiments have shown that processing of Tg bound to certain Tg-specific mAbs, such as 5D2 and 3C4, led to activation of T4(2553)-specific T-cells (Dai *et al.*, 1999). This effect was selective because it did not promote generation of other Tg peptides, nor was it observed with other Tg-specific mAbs. Enhancement of peptide presentation may result from a combined effect of increased Tg uptake by APC and altered Tg processing that converts cryptic pathogenic Tg peptides into dominant ones (Dai *et al.*, 1999). In principle, such a process may be facilitated by Abs directed to iodotyrosyl-containing determinants, and may further help in the "spreading" of the Tg-specific T-cell response during the later stages of the disease.

A second mechanism may operate via thyroid hormone-binding Abs, frequently found in patients with autoimmune thyroid disorders or animals developing EAT (Benvenega *et al.*, 1987; Sakata, 1994). The 55H8 mAb, representing this subset, has been shown to recognize the 5' iodine atom of the outer phenolic ring of T4 within the T4(2553) peptide, even after the peptide is bound in the MHC (A^k) groove (Dai *et al.*, 2005). 55H8 inhibits activation of T4(2553)-specific T-cells and significantly reduces

their capacity to transfer EAT to naïve mice. Protrusion of the thyroxyl side chain above the groove may cause a steric hindrance effect, explaining these observations. In this case, mAbs recognizing T4-containing ligands may cause suppression of autoreactivity. Interestingly, 55H8, simultaneously bound to Tg with 5D2 or 3C4, abrogated the enhancing effect of these mAbs on the formation of T4(2553), perhaps by preventing the loading of this peptide onto A^k molecules (Dai *et al.*, 1999).

Does Tg Iodination Play a Role in the Pathogenesis of Human Thyroiditis?

Ingestion of iodine has been clearly shown to increase the incidence and/or severity of thyroiditis in animals prone to develop spontaneous autoimmune thyroiditis, such as CS chickens (Bagchi *et al.*, 1985), BB/W and Buffalo rats (Allen *et al.*, 1986; Cohen and Weetman, 1988), and NOD.H2^{h4} mice (Braley-Mullen *et al.*, 1999; Rasooly *et al.*, 1996). Increased iodine intake has also been linked to the development of hypothyroidism in humans (Markou *et al.*, 2001). Since Tg is the only molecule that stores dietary iodine and HI-Tg demonstrates increased immunopathogenicity, it appears plausible that an iodine-induced dysregulation of homeostatic mechanisms may initiate via HI-Tg leaking from the thyroid. However, the formation of HI-Tg following exposure to an iodine-rich diet has so far been documented only in OS chickens (Sundick *et al.*, 1987), and remains to be confirmed in other animal models or in humans. Iodine-induced hypothyroidism may at times have no autoimmune basis, as has been recently shown in the studies of SJL mice (Li and Carayanniotis, 2007). Challenge of this strain with HI-Tg leads to severe EAT development, as well as strong B- and T-cell responses to HI-Tg (Dai *et al.*, 2002), yet their Tg obtained *ex vivo*, after placement on drinking water with 0.05% NaI for 10 weeks, is not enriched in iodine content (Li and Carayanniotis, 2007). This dietary regimen induces goitrous hypothyroidism associated with only focal mononuclear cell infiltrates and no autoreactive responses to Tg. Iodine may exert pleiotropic effects in various hosts, including direct thyroid cell injury (Mahmoud *et al.*, 1986), and it is not currently clear whether HI-Tg formation is commonly the precipitating factor in pathogenesis.

A study with “humanized” DR3-transgenic mice has provided valuable insights into the immunoregulation of thyroiditis. Treg control does not supersede MHC restriction in that, while depletion of Tregs exacerbates EAT severity, the MHC-mediated hierarchy in susceptibility remains (Morris and Kong, 2006). In DR3-transgenic, class II knockout mice on the NOD background, i.e., with non-MHC genes that influence EAT development after prolonged iodine intake (4–8 weeks) when a susceptible allele (A^{g7}) or H2^{h4} (A^k) is present (Braley-Mullen *et al.*, 1999; Hutchings

et al., 1999; Rasooly *et al.*, 1996), we likewise administered NaI orally for 8 weeks (Flynn *et al.*, 2007). In addition, we examined the influence of Tregs on this environmental factor by prior depletion of CD4⁺CD25⁺ T-cells. Figure 33.2 presents composite data from several experiments. They show that: (1) oral NaI intake over 8 weeks led to ~50% incidence of thyroiditis involving up to 30% of the thyroid; and (2) Treg depletion exacerbated NaI-induced thyroiditis with a higher incidence of 70%, and over half displayed thyroid destruction involving 40–80% of the thyroid. Treg depletion alone did not result in spontaneous thyroid infiltration over background. Our findings that Treg depletion exacerbates NaI-induced EAT are at variance with a recent report in NOD.H2^{h4} mice wherein Treg depletion prior to NaI treatment actually resulted in less thyroiditis development (Yu *et al.*, 2006). The class II allelic difference and other possible reasons for such discrepancies have been discussed (Flynn *et al.*, 2007).

Currently, it remains controversial whether human T-cells respond equally well to Tg preparations with varied iodine content (Rasooly *et al.*, 1998; Shimojo *et al.*, 1988). Furthermore, although HT is considered to be a T-cell-mediated disease, clinical studies examining the reactivity of intrathyroidal or peripheral T-cells to Tg peptides with established pathogenic potential in EAT are lagging. It is unlikely that thyroiditogenic processes revealed through the use of Tg peptides in animals will be devoid of clinical relevance, since some of them occur with hTg epitopes in HLA-transgenic mice (Flynn *et al.*, 2004b; Karras *et al.*, 2005). Genetic studies reporting association of Tg polymorphisms with susceptibility to human autoimmune thyroid disease (Ban *et al.*, 2003; Tomer *et al.*, 2002) have also helped to promote the view that Tg may be directly involved in HT pathogenesis and may prompt

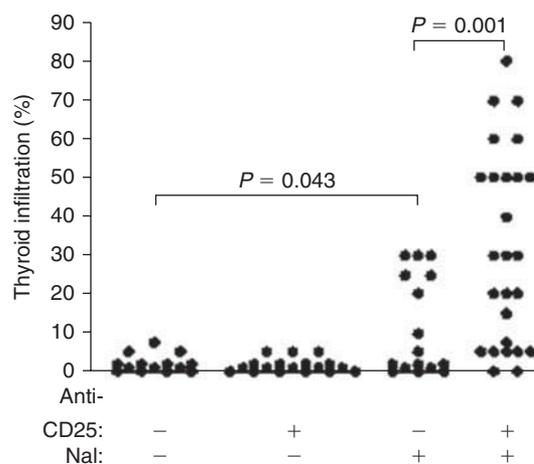


Figure 33.2 Mice were injected with 1 mg anti-CD25mAb (PC61), at weeks -2 and -1.5, before being given NaI (0.3% in drinking water) for 8 weeks. The extent of thyroid infiltration of individual mice is presented. Adapted from Flynn *et al.*, (2007), with permission from Blackwell Publishing Ltd.

investigations of events that initiate T-cell autoreactivity. More experimentation seems warranted with long-term exposure of susceptible animals to different ranges of iodine intake, and the growing map of pathogenic Tg peptides may aid investigations in this completely unexplored area of research.

Summary Points

- Tg, the storage protein of thyroid hormones, is an autoantigen associated with autoimmune thyroid disease. It induces EAT, a mouse model for Hashimoto's thyroiditis, which is the prevalent hypothyroid syndrome.
- Tg comprises a polypeptide chain of ~2750 amino acids and forms a 660 kDa homodimer; it has a unique role of facilitating enzyme-mediated organification of dietary iodine to form thyroid hormones at specific hormonogenic sites via intramolecular coupling of certain iodotyrosyl residues.
- Unraveling the role of iodine in Tg pathogenicity has been the focus of several studies, since an increase in dietary iodine intake leads to iodinated Tg with greater pathogenic potential in some genetically susceptible animals.
- Studies using synthetic peptides show that iodine atoms on the primary hormonogenic sites are secondary to the influence of amino acid composition in inducing EAT. In contrast, iodine added to tyrosyls on other Tg peptides may have enhancing, neutral, or suppressive effects on their EAT-inducing capacity.
- Several known pathogenic Tg epitopes do not contain tyrosyl residues and are unlikely to be iodinated or directly affected by the iodine content of Tg. For some of them, however, enhanced Tg iodination may exert indirect effects, promoting their formation by influencing the processing of Tg in antigen-presenting cells.
- It is currently unknown how iodotyrosyl formation is regulated in Tg, and what influences this process may have on the generation of effector T-cells mediating EAT, or Tregs suppressing the autoimmune response.

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Goiter in the Elderly: The Role of Iodine

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Abstract

The term goiter means thyroid enlargement, either diffuse or nodular. The incidence of goiter increases with age. The most common type of goiter in the elderly is multinodular goiter. Graves' disease is very rare in older adults. The most common cause of goiter worldwide is iodine deficiency. It is becoming less of a problem in developed countries, due to the addition of iodine to salt and bread. Goiter commonly presents as neck mass. Although most patients with goiter do not have any symptom other than neck swelling, occasionally patients complain of dysphagia, dysphonia, or pain. Many goiters do not cause any thyroidal hormonal abnormality (euthyroid status). Some can present with either excess thyroidal hormonal activity (hyperthyroidism) or deficiency of thyroid hormones (hypothyroidism). Proper work-up for elderly patients with goiter includes a thorough history, detailed clinical examination, thyroid hormone assays, e.g., TSH, T₄, T₃, and an ultrasound or CT scan. Most goiters are benign in nature and may not require tissue diagnosis. In some elderly patients, fine needle aspiration and biopsy may be considered to rule out malignancy. Most elderly patients with nodular goiter are treated best by surgical removal, while radioiodine therapy and/or antithyroid drugs are the most effective therapy for non-nodular goiter.

Abbreviations

CT	Computerized tomogram
FNA	Fine needle aspiration
MNG	Multinodular goiter
MRI	Magnetic resonance imaging
T ₃	Triiodothyronine
T ₄	Thyroxine
TSH	Thyroid-stimulating hormone

Introduction

Goiters are common in the elderly. Many elderly patients have asymptomatic thyroid enlargement, particularly the nodular variety, most frequently detected on autopsy or by an ultrasound performed for some other indication (Levey, 1991). The term goiter denotes physical enlargement of the thyroid gland, and does not incorporate the functional status of the thyroid gland. Patients with goiter may have a normal (euthyroid) or hypo- or hyperfunctioning of the thyroid gland (hypothyroidism or hyperthyroidism).

Elderly patients have a higher prevalence of goiter, ranging from 54% to 74% (Cooper *et al.*, 2006; Diez, 2005; Cavaliere *et al.*, 2002; Knudsen *et al.*, 2000), especially in low-iodine intake areas, where it is called *endemic goiter* (Laurberg *et al.*, 1998; Aghini-Lombardi *et al.*, 1999). Thyroid enlargement can be diffuse or nodular. Multinodular goiter (MNG) is the most common type of goiter in the elderly, and its incidence increases with age (Bagchi *et al.*, 1990). MNG affects 5% of the general population in nonendemic areas (Hegedüs, 2004) and 15% in endemic areas (Knudsen *et al.*, 2000). Thyroid carcinoma, though rare, can present as thyroid nodules (Correa and Chen, 1995), or may develop as a dominant nodule in a long-standing MNG.

Types and Causes of Goiters

The types and causes of goiters affecting the elderly are listed in Table 34.1. Table 34.2 lists the types of goiter in descending order of frequency. The most common type in elderly patients is MNG. Iodine plays a critical part in thyroid metabolism, and the most common cause of goiter worldwide is iodine deficiency (*endemic goiter*). Iodine deficiency commonly causes diffuse enlargement of the thyroid gland, and only occasionally causes a nodular enlargement. Introduction of iodized salt and bread

Table 34.1 Types of goiters

<i>Diffuse goiter</i>	<i>Nodular goiter</i>
Euthyroid	
1. Physiological (also called <i>simple goiter</i> to denote nontoxic or nonhyperfunctioning gland)	1. Benign cysts
2. <i>Endemic goiter</i> in areas of low iodine (usually euthyroid, could present with hypothyroid symptoms – see below)	2. Thyroid cancers <ol style="list-style-type: none"> Papillary carcinoma Follicular carcinoma Medullary carcinoma Anaplastic carcinoma Primary thyroid lymphoma Metastatic carcinoma (breast, renal cell, others)
Hyperthyroid	
1. Graves' disease	1. Solitary nodule or hyperfunctioning "hot" nodule
2. Autoimmune thyroiditis, e.g., Hashimoto's thyroiditis ^a	2. Multinodular goiter
3. Iodine-containing medications <ol style="list-style-type: none"> Lithium Iodides (Jod-basedow disease) 	3. Thyroid cancer (very rarely)
4. Goitrogens <ol style="list-style-type: none"> 1-5-vinyl-2-thio-oxazolidone (present in cabbage, turnips and soybeans) Cassava and millet contain goitrogenic substances 	
5. Herbs like kelp and sea weed (high iodine content)	
Hypothyroid	
Rarely, iodine deficiency and chronic autoimmune thyroiditis, e.g., Hashimoto's thyroiditis ^a can cause goiter	

Note: Main clinical types of goiters are summarized according to the hormonal status.

^aHashimoto's thyroiditis causes transient hyperthyroidism in the early stage of the disease. Patients will ultimately develop hypothyroidism due to thyroid gland destruction and atrophy.

Table 34.2 Frequency of goiter in the elderly^a

<i>Type</i>	<i>Percentage of goiters</i>
Nontoxic multinodular goiter	51.3
Toxic multinodular goiter	23.8
Solitary thyroid nodule	9.8
Toxic adenoma	5.0
Graves' disease	4.3
Chronic autoimmune thyroiditis (e.g., Hashimoto's thyroiditis)	3.9
Simple goiter	1.3

^aReproduced from [Rehman et al., \(2006\)](#), with permission of Future Medicine Ltd.

has reduced the incidence of endemic goiter worldwide, particularly in developed countries. The detail of iodine metabolism and its effect on thyroid has been described elsewhere in the book.

Nodular goiters are more common in the elderly and can be associated with euthyroid or hyperthyroid state,

Table 34.3 Mechanism of nodular goiter^a

1. Iodine deficiency
2. Chronic TSH stimulation
3. Genetic influences, e.g., mutation of TSH receptor gene
4. Growth-stimulating immunoglobulins
5. Goitrogens <ol style="list-style-type: none"> Food: Cassava and millet – contain goitrogenic substances Herbs, e.g., kelp Lithium Iodine and iodine-containing drugs like expectorants

Note: The table gives a list of clinical conditions and substances causing goiter formation.

^aModified from [Rehman et al., \(2006\)](#), with permission of Future Medicine Ltd.

but rarely with hypothyroid status. **Table 34.3** lists the mechanisms responsible for nodular goiter. Approximately 20–25% of patients with large cervical MNGs have hyperthyroidism ([Davis and Davis, 1984](#)). The most frequent cause of hyperthyroidism in the elderly is toxic MNG, especially in iodine-deficient areas ([Vitti et al., 2002](#)). Graves' disease, one of the causes of diffuse goiter in younger patients, is much less common in older patients. Autoimmune thyroiditis, such as Hashimoto's, causes hypothyroidism, and its prevalence increases with age but rarely presents with a goiter ([Cooper et al., 2006](#)). Thyroid cancers may present with a single nodule or a dominant nodule in a long-standing MNG. Only 5% of MNG have malignant nodules.

Clinical Evaluation

The algorithm for the evaluation of goiter is shown in **Figure 34.1**. Goiter most commonly presents with neck swelling in younger patients, since the thyroid gland usually grows outwards; elderly patients may not notice the enlargement for a while due to laxity and wrinkling of the skin as a result of the loss of subcutaneous fat. Other clinical manifestations of goiter vary with the size, location and thyroid hormonal status, i.e., hypo- or hyperthyroid state (**Table 34.1**). Symptoms of goiter are listed in **Table 34.4**. Substantial enlargement of the gland could cause displacement, or less often, compression of the trachea, esophagus, recurrent laryngeal nerve, or blood vessels. Elderly patients with MNG, the most common goiter, usually do not have obstructive symptoms as it usually grows very slowly over many decades ([Jauregui et al., 1977](#); [Jones, 2001](#)). Acute tracheal obstruction is uncommon, but can develop into a goiter in patients with acute hemorrhage ([Torres et al., 1983](#)). Chronic autoimmune thyroiditis may cause fibrosis, leading to concentric tracheal compression ([Katz and Vickery, 1974](#)). Painful goiters are caused by viral thyroiditis or hemorrhage and can develop into a thyroid nodule. Sometimes the thyroid gland grows through the inlet into

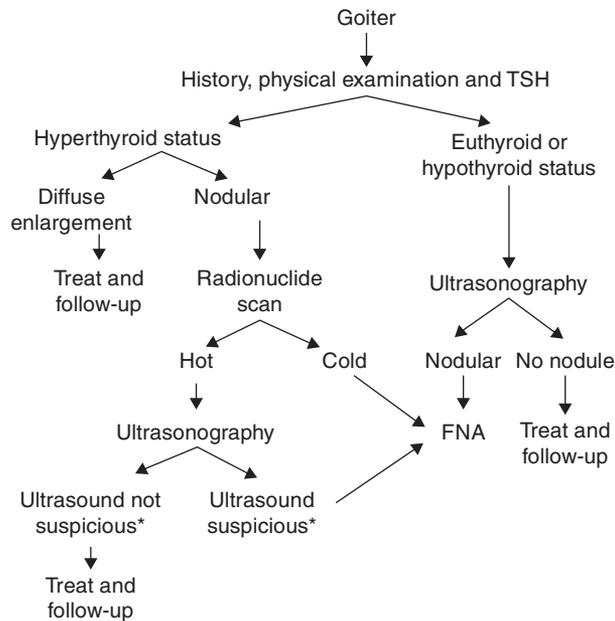


Figure 34.1 Diagnostic approach to patients with goiter. Evaluation of patients with goiters. TSH, thyroid-stimulating hormone; FNA, fine needle aspiration. *FNA should be performed on all hypoechoic nodules ≥ 10 mm with irregular margins, chaotic intranodular vascular spots, a more tall than wide shape, or microcalcifications. Ultrasongraphic findings suggestive of extracapsular growth or metastatic cervical lymph nodes warrant an immediate cytologic evaluation, irrespective of the size of the lesions. Reproduced from Rehman *et al.*, (2006), with permission of Future Medicine Ltd.

Table 34.4 Symptoms of goiter

- Painless and asymptomatic in nonobstructive goiter
 - Swelling in the neck
 - Incidentally found on routine examination
- Obstructive symptoms in obstructive cervical or retrosternal goiter
 - Common symptoms
 - Difficulty in breathing
 - Cough
 - Wheezing and/or stridor (when tracheal diameter is ≤ 5 mm)
 - Choking sensation in the throat
 - Uncommon symptoms
 - Pain
 - Dysphagia
 - Hoarseness
 - Horner's syndrome caused by the compression of the cervical sympathetic chain
 - Rare symptoms
 - Jugular vein compression or thrombosis
 - Cerebrovascular steal syndromes
 - Superior vena cava syndrome
 - Phrenic nerve paralysis causing diaphragmatic paralysis

Note: Goiters can present with any of the symptoms listed in the table.

the thoracic cavity, resulting in retrosternal goiter. Most retrosternal goiters grow in the anterolateral mediastinum, but about 10% are located primarily in the posterior mediastinum (Katlic *et al.*, 1985). A study by Diez (2005)

Table 34.5 Features associated with high likelihood of thyroid nodule being malignant^a

- History
 - Men over 70 years
 - History of neck irradiation
 - Rapid nodular growth
 - Family history of thyroid cancer
- Physical examination
 - Firm, nontender nodule
 - Local lymphadenopathy
 - Recurrent laryngeal nerve palsy

Note: The above features are associated with high likelihood of thyroid nodule being malignant.

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has shown that male patients and the elderly have a higher incidence of retrosternal goiter and tracheal deviation. Clinical examination of the thyroid gland is important, as most patients with retrosternal goiter also have visible goiters (Katlic *et al.*, 1985).

Most obstructive goiters, either cervical or retrosternal, are benign. However, the incidence of thyroid cancer in patients with retrosternal goiter has ranged from 10% to 16% (Torre *et al.*, 1995). Differentiated thyroid cancers usually grow slowly and rarely cause obstructive symptoms. Anaplastic carcinoma is the most common thyroid cancer affecting elderly individuals (Finucane and Anderson, 1995). Anaplastic thyroid carcinomas or primary lymphomas of the thyroid, though rare, can present with a rapidly enlarging neck mass, and may cause obstructive symptoms. Some patients with thyroid cancer may present with symptoms of metastases, such as bone pain. It is important to rule out a malignancy in patients with goiter. The features listed in Table 34.5 are associated with high likelihood of the thyroid nodule being malignant (Belfiore *et al.*, 1992; Hamming *et al.*, 1990). Thyroid malignancies are discussed elsewhere in this book.

Thyroid hormone assays should be performed in every patient who presents with goiter to determine the thyroid functional status, because elderly patients may not present with classical symptoms and signs of thyroid disorders. In younger adults the classical symptoms of thyroid dysfunction are obvious, e.g., symptoms of hypothyroidism such as cold intolerance, weight gain, dry skin, constipation and a drop in mental or physical activity. However, in the elderly the diagnosis is often overlooked or misdiagnosed, as the above-mentioned symptoms are often subtle, absent, mistaken for normal aging, attributed to commonly present coexisting illnesses, or can be blamed on the medications prescribed for those coexisting conditions (Rehman *et al.*, 2005). Another example is that of an elderly man receiving betablocker for atrial fibrillation; he may also have concomitant or underlying hyperthyroidism, and the signs of tachycardia and tremulousness

may be either blamed on to atrial fibrillation, or masked by the betablockers. In another example, an elderly patient receiving calcium channel blocker for hypertension develops hypothyroidism; his complaints of constipation, weight gain and fatigue, are erroneously attributed to the medication. A review of clinical evaluation of hormonal dysfunction of thyroid in the elderly is available elsewhere (Rehman *et al.*, 2005).

Physical examination of the thyroid gland in the elderly may not be helpful. The thyroid gland normally shrinks with age, despite the fact that the hormonal function of the gland is usually intact even in the ninth decade of life. Wrinkling and laxity of the skin resulting from the loss of subcutaneous fat may decrease the visibility of goiter. While examining the thyroid gland, the shape, size, mobility, consistency and the presence of nodules in the goiter should be recorded. Many elderly have degenerative joint disease, along with bone loss, causing kyphoscoliosis. The presence of kyphoscoliosis may push the gland down into the retrosternal area, making it less visible and difficult to palpate in the neck. Usually the thyroid examination is done while the patient is sitting on the examination couch or a chair. If the lower end of the thyroid gland could not be identified in this position, examination of the thyroid should be conducted while the patient is lying down with a pillow under the shoulders to hyperextend the neck to make the thyroid gland palpable. When it is difficult to identify the lower end of the thyroid gland, the possibility of substantial retrosternal extension should be considered. Elderly patients in these situations should be asked to lift their arms vertically above the head for 1 min; if they develop facial plethora, if their neck veins become distended or if they become dyspneic, they may have a retrosternal goiter (Pemberton's maneuver). Rarely, thyroid gland could become impacted in the thoracic inlet by performing this maneuver (thyroid cork) (Blum *et al.*, 1974). Acute neck flexion could also cause the "thyroid cork" phenomenon.

Graves' disease is suggested by the presence of bruit in a diffusely enlarged, firm goiter. Tracheal deviation is frequently visible or palpable if the goiter is asymmetric. Rarely, dilated neck veins can be seen when there is an obstruction on the neck veins by the enlarged thyroid gland.

Serum thyroid-stimulating hormone (TSH) and thyroid hormone levels should be measured in any patient with a goiter to determine the hormonal status. TSH concentrations increase with age, but the levels remain within the normal range in the healthy population throughout life up to 100 years of age (Canaris *et al.*, 2000; Mariotti *et al.*, 1995). See Chapter 106 by Diez and Iglesias on "Hypothyroidism in the Middle Aged and Elderly: Clinical Aspects" for details of thyroid hormone changes in the elderly. Serum calcitonin levels are not needed unless there is a family history of medullary thyroid cancer or multiple endocrine neoplasia (MEN) type 2.

Table 34.6 Indications for fine needle aspiration biopsy of a goiter^a

1. Prominent discrete nodules
2. If there is a history of rapid growth, pain, or tenderness
3. Hard or unusual firmness in one region of the goiter raise the suspicion of cancer, particularly anaplastic carcinoma or thyroid lymphoma

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A chest X-ray should be obtained if obstructive symptoms are present or if retrosternal goiter is suspected on clinical examination. Computed tomography (CT), magnetic resonance imaging (MRI), or ultrasonography will identify the exact size and extent of the goiter and its effect upon surrounding structures. CT scan of the thyroid should be done without iodinated radiocontrast agents, as the excess iodine may worsen the symptoms of hyperthyroidism (Mintzer and Cattani, 1992). Thyroid ultrasound is a sensitive method of determining the nodule size and whether the nodule is cystic or solid (McHenry *et al.*, 1999), but it is not satisfactory for imaging of posterior neck structures or the retrosternal region. Thyroid radionuclide imaging with radioiodine can define areas of autonomous function; however, some retrosternal goiters are not identified by this technique because they take up radioiodine poorly, and the radioactivity is attenuated by the sternum and clavicles (Park *et al.*, 1987).

Radionuclide thyroid scans can provide information about the function of the nodules, but are not a reliable indicator of malignancy. "Cold spots" are nonfunctioning areas of the thyroid gland and may suggest malignancy, whereas "hot spots" are mostly benign thyroid adenomas or MNGs. However, a small proportion of hot nodules could be malignant (Hoving *et al.*, 1981; Miller, 1980; Patel and Vanderpump, 2005), and may require a fine needle aspiration (FNA) biopsy. Indications for FNA are listed in Table 34.6 (Kojic *et al.*, 2004).

Treatment

Treatment of goiter is dependent on the type of goiter, i.e., diffuse vs. nodular goiter.

Management of diffuse goiters

Physiological goiters are rare in the elderly in the developed world. An intervention is not needed in most patients if they are euthyroid. These patients should be reassured and prescribed a high iodine diet. Some patients present with symptoms of hypothyroidism. Thyroxine replacement is indicated in these patients, and goiter diminishes in size in 4–6 months. The starting dose of thyroxine in the

elderly is lower than that in younger patients, as the standard replacement doses of thyroxine can cause or precipitate cardiac complications, such as angina, infarction and arrhythmias. Hypothyroidism in the elderly develops over a prolonged period; hence, the accomplishment of euthyroid status should be achieved slowly. The usual starting dose of thyroxine in the elderly is 25 µg; if there is coexistence or uncertainty regarding cardiac status, a starting daily dose should be 12.5 µg. The patient should be monitored for tachycardia and other signs of thyroxine overdose (Singer *et al.*, 1995; Ladenson, 1990). TSH should be monitored every 3–4 weeks to assess the response to therapy. Since TSH changes occur over 2–4 weeks, due to the delay in down-regulation of TSH secretion, the replacement therapy can be adjusted every 2–4 weeks. If early monitoring is needed, thyroid hormone levels may be obtained. A slight elevation of TSH in a euthyroid elderly patient early in the course of thyroid replacement may not indicate the need for further increase in thyroid hormone dose (Helfand and Crapo, 1990). TSH may ultimately fall to normal levels over a period of months without further increase in thyroxine. If the thyroxine dose needs to be adjusted, the increments must be in the range of 12.5–25 µg. The replacement therapy should be stopped for several days to weeks if thyroid replacement therapy causes cardiac instability, such as heart failure, angina, or arrhythmia. The half life of T₄ in elderly patients is sufficiently long that interruption to therapy for several days will not cause any clinical problem. Most elderly will require a physiologic dose of approximately 75 µg daily.

Graves' disease can be treated by one of the three treatment options: radioiodine, antithyroid therapy (carbimazole or propylthiouracil), or surgery. Radioactive sodium iodide (¹³¹I) is considered the best treatment for most elderly patients with Graves' disease, as it is the safest and most efficient therapy (Becker, 1984). It can be administered easily in the office setting and it avoids any perioperative complications of surgery. Antithyroid drugs (such as propylthiouracil or methimazole) are very effective in reducing the symptoms and suppressing the thyroid hormone surge rapidly. Additionally betablockers (or calcium channel blockers, e.g., diltiazem, if betablockers are contraindicated) are used to control tachycardia and tremors. Some patients with hyperthyroidism may require anticoagulation for atrial fibrillation. None of these agents would affect the natural history of the disease; hence, these agents are not considered a definitive therapy for Graves' disease. These medications can be used for a short period in the elderly; once the patient is euthyroid and stable, definitive treatment with radioiodine should be considered. Antithyroid agents are associated with a high relapse rate after the drug is stopped, and should not be used alone in the management of Graves' disease. Surgery is rarely needed for patients with Graves' disease.

Management of thyroid nodules and multinodular goiter

While radioiodine is the best treatment for Graves' disease, it may not be effective in the presence of multinodular toxic goiter or a large single hyperfunctioning nodule. Surgery is the treatment of choice for patients with MNG. Several studies demonstrated the safety and efficacy of surgery in patients over 75 (Bliss *et al.*, 1999). For patients at high risk for surgery, large and repeated doses of radioiodine can be considered (Hennemann *et al.*, 1986). Since multiple doses and longer duration is needed if radioiodine is employed as the definitive treatment, a patient remains hyperthyroid many months after the diagnosis and antithyroid drugs and/or betablockers should be given to control the symptoms. Antithyroid drugs do not lead to permanent remission in patients with uninodular or multinodular toxic goiter. Surgery may also be necessary in those patients whose goiter causes obstructive symptoms, dysphagia and tracheal compression. Surgery is also indicated for substernal goiters, whether or not obstructive symptoms are present (Allo and Thompson, 1983; Hedayati and McHenry, 2002; Mehta and Savino, 1995). Surgery is the treatment of choice for any thyroid nodule if malignant disease is suspected. Some patients may want to remove a goiter for cosmetic reasons. Surgery is usually performed for autonomous nodules (also called hot nodules, as they light up on the thyroid scan); radioiodine therapy can be used in these patients if surgery is contraindicated. These patients should receive antithyroid drug therapy before starting the definitive treatment to make them euthyroid, since some patients may develop severe hyperthyroidism (thyroid storm), due to the release of preformed hormones during definitive therapy. Most patients with nonautonomous (cold) microfollicular adenomas should have surgery. The thyroid gland should be surgically removed if there is a suspicion of malignancy in a goiter. Some centers are using ultrasonographically guided ethanol injection and laser photocoagulation to treat benign thyroid nodules (Lippi *et al.*, 1996; Bennedbæk *et al.*, 1997; Zingrillo *et al.*, 1998; Bennedbæk and Hegedüs, 1999; Døssing *et al.*, 2002), but these procedures are not recommended as major therapy, due to the lack of controlled trials and nonavailability in many centers.

Hashimoto's and other thyroiditis causing hypothyroidism rarely cause goiter, as they are usually associated with atrophy of the thyroid gland. Nonetheless, goiters in these situations should be treated with thyroxine-replacement therapy. The replacement dose of thyroxine is lower in the elderly than in younger patients, and titration should also be slow in the elderly to avoid cardiac toxicity. Patients also require thyroxine therapy following surgical thyroidectomy or radioiodine ablation, to suppress serum TSH to below normal or undetectable levels to reduce the

risk of recurrence. Periodic assessment of thyroid function every 3–5 years is suggested due to the long-term risk of thyrotoxicosis.

Summary Points

- Goiters are common in the elderly.
- Multinodular goiter is the most common type of goiter in the elderly.
- Work-up for goiter includes a thorough history and physical examination; thyroid hormone assays, e.g., TSH, T4, triiodothyronine (T3); and an ultrasound or CT scan.
- Thyroid ultrasound is very helpful in determining the nodule size, number, type, and whether the nodule is cystic or solid.
- Surgery is the treatment of choice for MNG in elderly patients. The effectiveness and safety of surgery in the elderly has been demonstrated by many studies.
- Graves' disease, uncommon in the elderly, usually presents with a diffusely enlarged goiter with a bruit. The common symptoms of heat intolerance, tremor, tachycardia, and so on, may be erroneously attributed to normal aging or commonly present coexisting diseases in the elderly.
- Graves' disease can be treated by antithyroid therapy (carbimazole or propylthiouracil), radioiodine, or surgery. Radioiodine is the safest and most effective treatment for Graves' disease.
- Physiological goiters do not need treatment.
- Goiters associated with hypothyroidism can be regressed with thyroxine replacement therapy. Low doses of thyroxine should be used first and titrated in the elderly to avoid cardiac toxicity.
- Most thyroid nodules are benign, but there is a 5% chance of goiter being malignant. Fine needle biopsy should be considered to rule out malignancy.
- Anaplastic types of thyroid cancer are common in the elderly and may present as a single nodule, as a dominant nodule in a long-standing MNG, or with symptoms of metastases such as bone pain.

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Section 2.3

Nutrition and Dietary Aspects

Iodine Intake and Food Choice

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Abstract

A significant amount of iodine is found in few food groups, mainly fish and seafood. In many countries, milk has a high iodine content due to iodine given to dairy cattle in mineral supplements. Milk and dairy products are ingested in relatively high amounts in some countries; therefore, this food group would be the most important source of iodine in these countries. Drinking water may contribute significantly to iodine intake in some countries. Populations having a high dietary consumption of fish or other seafood will have a high intake of iodine. Furthermore, the iodine intake depends on the market share of iodized products and their level of fortification. This makes it difficult to make any general conclusions about food choice and iodine intake.

Abbreviations

Cr Creatinine
h hour

Introduction

Because a significant amount of iodine is found in relatively few food groups the intake of these foods is important for iodine intake. Many countries iodize household salt and/or salt used by the food industry. The intake of fortified food influences iodine intake, depending on the iodization level and the market share of iodized salt. However, even in countries with iodine fortification, the intake of other iodine-rich foods may have a significant impact on total iodine intake.

Iodine Content in Food

Iodine is mainly found in marine material. Salt water fish, shellfish, and seaweed are food sources with the highest natural iodine content. Seaweed has a very high iodine content; one bowl of soup made by tangle, which is a common soup

in the Japanese diet, can contain 5 mg iodide (Nagataki, 1993). Iodine content in fish and shellfish varies from less than 5 to more than 200 µg/100 g (Julshamn *et al.*, 2001). Mean iodine content in milk in Northern Europe is at the level of 15–20 µg/100 g (Rasmussen *et al.*, 2000; Dahl *et al.*, 2003a; Bader *et al.*, 2005). The iodine content in cows' milk depends on the amount of iodine given in mineral mixtures, and partly on the use of teat dips and udder washes containing iodophors. Further, goitrogenic substances, such as glucosinolates, result in decreased uptake of iodine in the thyroid gland (Gaitan, 1990), and the same mechanism seems to work in the mammary gland (Hermansen *et al.*, 1995). Thus, the type of fodder influences the iodine content in milk.

The iodine content in soil is highly dependent on its geological origin. Therefore, the iodine content of soil and drinking water varies appreciably within different geological localities; in some localities water is a significant iodine source. Denmark covers a relatively small geographical area; however, the iodine content in drinking water varies from less than 2 µg/l to more than 30 µg/l, and even reaches more than 100 µg/l in one small town (Pedersen *et al.*, 1999; Rasmussen *et al.*, 2000).

Geographical differences in iodine content in drinking water also exist in other countries. For instance, in Finland the iodine content in tap water from 21 cities was found to vary from 0.3 to 9.1 µg/l (Häsänen, 1970). Variations from about 2 µg/l in the south to about 12 µg/l in the north (Felgentraeger, 1984) and from 0.7 to 5.5 µg/l (Dahl *et al.*, 2003a) have been found in Germany and Norway respectively. Iodine intake, expressed as iodine excretion, in different geographical areas with significant variations in iodine content in tap water can be seen in Table 35.1.

Main Dietary Sources of Iodine

The main source of dietary iodine may vary between countries, and even between different areas of the same country.

Table 35.1 Iodine intake, expressed as iodine excretion, in various geographical areas with different iodine content in tap water

	<i>Iodine excretion in areas with low iodine content in tap water</i>	<i>Iodine excretion in areas with medium iodine content in tap water</i>	<i>Iodine excretion in areas with higher iodine content in tap water</i>
Denmark ^a	98 (76–174) $\mu\text{g}/24\text{-h}$		155 (108–224) $\mu\text{g}/24\text{-h}$
Denmark ^b	166 (123–245) $\mu\text{g}/24\text{-h}$		204 (152–285) $\mu\text{g}/24\text{-h}$
Germany ^c	16–26 $\mu\text{g}/\text{g Cr}$	24–35 $\mu\text{g}/\text{g Cr}$	37–45 $\mu\text{g}/\text{g Cr}$

Note: The above table shows the influence of the iodine content in drinking water on iodine intake.

^aUnpublished results from the Danish Investigation of Iodine Intake and Thyroid Diseases before iodine fortification was introduced. Results are median values with 25th and 75th percentiles in parentheses.

^bUnpublished results from the Danish Investigation of Iodine Intake and Thyroid Diseases after iodine fortification was introduced. Results are median values with 25th and 75th percentiles in parentheses.

^cBauch *et al.*, 1993, results from before iodine prophylaxis was introduced. h, hour.

Table 35.2 Percentage contribution of iodine from various food sources from different countries

	<i>Milk/dairy products</i>	<i>Fish</i>	<i>Egg</i>	<i>Bread</i>	<i>Beverages</i>	<i>Other</i>
Norway ^a men/women	57/56	23/24	5/5		2/2	19/18
Denmark ^b	44	15			24	14
England ^c	35	8	5		16	26
Switzerland ^d	21	5	7	41		26
Finland ^e men/women	22/23	9/8		16/14	2/2	51/47

Note: The above table shows the data for adults.

^aData from the National Dietary Survey based on a food frequency questionnaire (FFQ). Dahl *et al.*, (2003a).

^bData from the Danish Investigation of Iodine Intake and Thyroid Diseases based on a FFQ before iodine fortification was introduced in Denmark. Food with a low iodine content was not included, iodine intake from other sources is a little underestimated and iodine from milk, fish and eggs consequently a little overestimated. Rasmussen *et al.*, (2002).

^cData from the Dietary and Nutritional Survey of British Adults. Lee *et al.*, (1994).

^dBased on per capita exposures from food using statistics on food consumption. Haldimann *et al.*, (2004).

^eMännistö, S., Ovaskainen, M.-J., Valsta, L. The national findiet 2002 study. Publications of the National Public Health Institute, Helsinki 2003. Results based on 48-h recall.

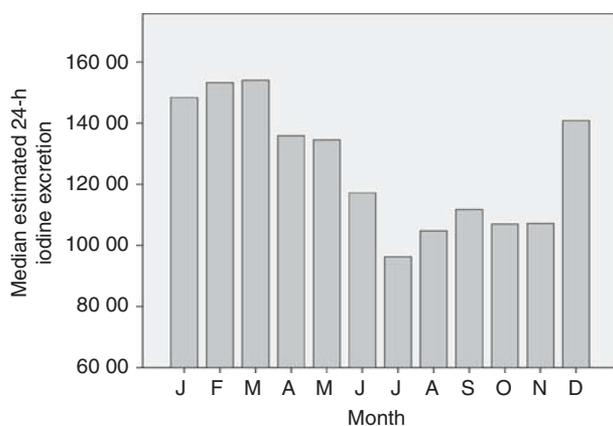


Figure 35.1 The figure shows the seasonal variations in iodine excretion in Danes with a milk intake of one or more glasses per day, $n = 993$ (unpublished results from the Danish Investigation of Iodine Intake and Thyroid Diseases).

The main contributors to iodine intake in various countries are shown in Table 35.2.

Contributions from dietary supplements are not included in the table. The food source that contributed

most to the iodine intake is generally milk and other dairy products. Despite the high iodine content, fish is not the most important source in any of the countries included in the table, because of its relatively low intake.

The iodine content in cows' milk constitutes a considerable part of the iodine intake in humans. It is important to consult nutritionists and others working with human health if a change in the iodine supplement to cows is being considered.

The iodine content in milk shows seasonal variations, with the highest content during winter and the lowest content during summer (Hetherington & Smyth, 1993; Rasmussen *et al.*, 2002; Dahl *et al.*, 2003c). This is reflected in a seasonal variation in iodine excretion, emphasizing the importance of milk as a source of iodine in some countries (Figure 35.1).

Intake in Different Countries with Different Dietary Characteristics

In some countries, e.g. Iceland and Japan, intake of fish and/or seaweed is quite high, which is reflected in the iodine excretion (Sigurdsson and Franzson, 1988; Nagataki, 1993.

Table 35.3 Iodine intake in some countries and the dietary characteristics of these countries

Country/area	Dietary characteristic	Iodine intake
Iceland ^a men/ women	High fish intake compared with Denmark	395/270 μg/day
Denmark ^b women	Low fish intake but otherwise comparable with Iceland	118 (81–197) μg/day
Japan ^c	Intake of seaweed common	660 ± 550– 1960 ± 2370 μg/g Cr

Note: The above table shows the iodine intake in some countries and the dietary characteristics of these countries.

^aSigurdsson and Franzson (1988), based on iodine in 24-h urine samples, mean values.

^bRasmussen *et al.*, (2002), based on iodine in 24-h urine samples, median values (25–75 percentiles).

^c(Negataki, 1993), results from different studies, based on casual urine samples, mean values ± SD.

As can be seen in Table 35.3, the iodine intake in some parts of Japan is very high. This high intake is caused by a regular intake of seaweed.

Arctic Food Choice and Iodine Intake

The traditional Inuit diet mainly consists of seal, whale, wild fowl, fish, reindeer, musk ox, hare and some walrus and polar bear. The iodine content of traditional food items ranged from 0.4 to 19.5 μg/100g (Andersen *et al.*, 2002). The average iodine content of blubber was 13 μg/100g, whereas it was around 7 μg/100g for viscera and 2.1 μg/100g for meat from both seal and whale. For other marine animals it varied between 0.9 and 19.5 μg/100g, with an iodine content of 138 μg/100g in one sample of cod flesh. Terrestrial animals had the lowest iodine content, with 1 μg/100g or less. Thus, marine mammals and fish had a higher iodine content compared to terrestrial and imported food items, but it was not excessive (Andersen *et al.*, 2002).

Greenland was kept secluded until around 1960. The subsequent transition of Greenlandic societies to a modern way of living has occurred at different rates in different parts of Greenland. In parallel, traditional Inuit food items have decreased in importance, with an increase in the intake of imported food items (Andersen *et al.*, 2005). The change in dietary habits is illustrated in Figure 35.2.

One study demonstrated the iodine intake in four different population groups in Greenland (Andersen *et al.*, 2005). The four groups were: rural Inuit in remote settlements in East Greenland living on hunting; intermediate Inuit in the main town on the east coast of Greenland, with access to some imported foods; urban Inuit in the capital Nuuk, with access to a wide variety of imported food items and restaurants serving fast food and Asian foods, and non-Inuit in

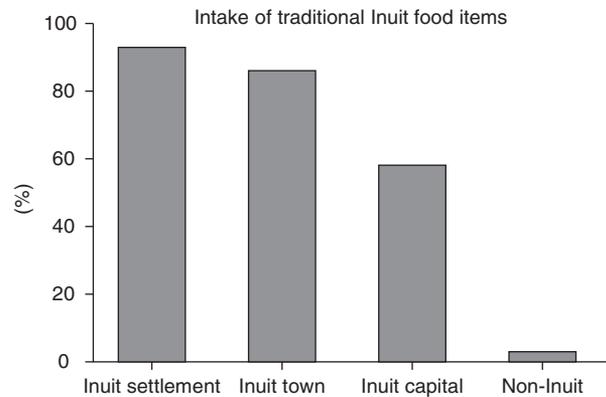


Figure 35.2 The figure shows individuals as four population groups in Greenland on four different levels of transition from hunter-gatherer to a modern way of living: rural Inuit in remote settlements in east Greenland, intermediate Inuit in the main town on the east coast of Greenland, urban Inuit in the capital Nuuk, and non-Inuit with the most westernized way of living. Adapted from Andersen *et al.*, (2005).



Figure 35.3 The figure shows iodine excretion in groups with different use of traditional Greenlandic food items, including sea mammals, fish and wild fowl. The differences in food choice influence urinary iodine excretion, with a gradual decrease towards the group that lives mainly on imported foods. Adapted from Andersen *et al.*, (2005).

Nuuk with the most westernized way of living. The change in way of living is associated with decreasing iodine intake, in parallel with the change in dietary habits (Figure 35.3).

Thus, a diet based on traditional Greenlandic food items contributed to an iodine intake of the order of 200 μg/day. The continuing changes in food choice in the circumpolar populations influence iodine intake, and may impact the occurrence of thyroid disorders.

Iodine Intake in Subjects with Different Dietary Patterns within a Country

As mentioned above, milk is an important source of iodine in many countries. Fish is the food group with the highest

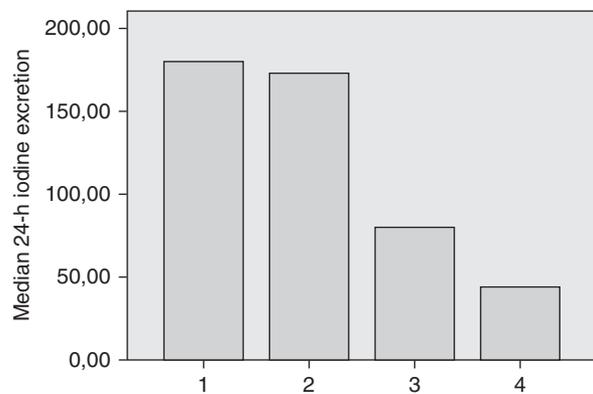


Figure 35.4 The figure shows iodine excretion with various intakes of milk and fish. Iodine excretion in a group of Norwegians in relation to their intake of fish and milk; 1, >150g milk and >60g of fish per day; 2, >150g milk and <60g of fish per day; 3, <150g milk and >60g of fish per day; 4, <150g milk and <60g of fish per day. Based on Dahl *et al.*, (2003b).

iodine content, but the intake of fish is generally lower than the intake of milk. However, food choice, of course, also affects iodine intake within a country. In [Figure 35.4](#), iodine excretion with various intakes of fish and milk in a small Norwegian sample can be seen. Iodine excretion is highest when the intake of milk and fish is the highest. Likewise, an increased iodine excretion with increased intake of milk and fish has been seen in a Danish study ([Rasmussen *et al.*, 2002](#)).

Iodine Intake in Subjects with Special Diets

Vegan diets do not contain meat, fish, milk, or other dairy products. Therefore, a vegan diet is, in general, low in iodine, except if seaweed or dietary supplements with iodine are included ([Lightowler and Davies, 1998](#); [Waldmann *et al.*, 2003](#)). In some countries, e.g. England ([Appleby *et al.*, 1999](#)) and Germany ([Remer *et al.*, 1999](#)), a lower intake of iodine in vegetarians (with inclusion of milk in their diet) than in nonvegetarians has been found, whereas in Denmark, there is no difference between the iodine intake in vegetarians (93 (58–149) µg/day, $n = 77$) and nonvegetarians (90 (59–157) µg/day, $n = 4492$) ([Rasmussen *et al.*, 2002](#)).

Conclusions

Iodine intake depends on food choice. In many developed countries, a high intake of milk is the main determinant of iodine intake, whereas fish intake is less important. However, in countries or populations with a tradition of a high fish intake, or especially of a high intake of seaweed, the iodine intake is high. Furthermore, iodine intake

depends on the market share of iodized products and their level of fortification. Hence, it is difficult to make any general, conclusions about food choice and iodine intake.

Summary Points

- In many European countries, milk is the most important iodine source.
- In some countries or populations, high intake of fish or seaweed results in a high iodine intake.
- In some countries iodine intake depends partly on the iodine content in drinking water.
- Various iodine fortification levels and the market share of iodized salt and other iodized products influence iodine intake.
- The continuing changes in food choice in Arctic populations influences iodine intake.

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Iodine Mineral Water and its Therapeutic Use in Health Resorts: Iodine Consumption from Natural Mineral Waters and its Effect on the Body

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Abstract

Treatment with native mineral waters is still offered therapeutically in many spas. Complex low-mineralized electrolyte solutions are preferentially used for this purpose. Occasionally higher mineralized spring waters, some of which have considerable iodine content, are used therapeutically. For daily use, low-mineralized mineral waters with low iodine content are utilized without requiring medical supervision. For the application of high iodine doses a justified clinical indication, exact dosage and medical controls are required. The present state of knowledge regarding the effects of different iodine doses on the thyroid will be discussed. Also, in consideration of the clear contraindications, the individual risk/benefit requires evaluation. Iodine is an essential trace element for the production of thyroid hormones. However, it is also in abundance in other organs, the importance of which fact is still unknown.

Abbreviations

GSH-PX	Glutathione peroxidase
NIS	Sodium iodine symporter
TAS	Total antioxidant status
TPO	Thyroid peroxidase

Introduction

Since the Middle Ages, if not before, iodine-containing mineral waters have been used for the treatment of goiters. It was the characteristic smell, probably, which stimulated this application, because other substances with a similar smell were used long before the discovery of iodine to reduce goiters, such as the sea grass (*Sargasso*) and *Laminaria japonica tresch* in Chinese medicine.

A long time ago spa doctors observed that large goiters rarely occurred in the environment of an iodine spring. This has been confirmed by Kopf (1952) using systematic analysis.

Iodine-containing mineral waters are found all over the world. From the geological point-of-view they are of marine origin; therefore, these waters contain many other electrolytes, above all sodium chloride or hydrogen carbonate. The concentrations of iodine and the other electrolytes vary strongly, ranging from less than 100 µg/l to up to 100 mg/l, but are usually about 30 mg/l (Table 36.1). Highly-mineralized mineral waters are used for treatment in medical health resorts for specific applications, while weaker-mineralized waters are sold in the market for daily use.

Iodine uptake by drinking mineralized iodine waters and complex treatment applications

Stronger mineralized iodine waters are used in spas for drinking treatments, almost always with other applications such as baths, inhalations and packs. The duration of such treatments varies from 2 to 3 weeks. The medically prescribed solution is drunk once or twice per day.

The quantities drunk always have been limited by their sodium chloride content; however, this formulation has been diminishing over the last few years. More diluted solutions, with the addition of carbonic acid, are being used increasingly nowadays, and the daily iodine supply varies from 13 mg in nondiluted waters down to 800 µg in reduced iodine waters (Figure 36.1).

The intake of iodine during complex treatment in health resorts was determined by measuring iodide excretion in urine over 24 h (Figure 36.2). A stay in an iodine spa without special iodine therapy causes only slightly increased iodine excretion. But if applications such as iodine baths,

Table 36.1 Chemical analyses of high- and low-mineralized iodine-containing mineral waters

Date of analysis	Tassilo mineral spring (1998)	Radenska mineral spring (1998)	Sichelsdorfer mineral spring (1998)	Eniva® Iodine Mineral Water (2007)
Content (mg/l)				
Potassium	26.95	64.0	93.0	
Sodium	5661.12	390.0	1210.0	
Calcium	163.70	230.0	171.0	
Magnesium	100.26	87.0	75.0	
Chloride	9210.75	44.0		
Bromide	96.87			
Iodide	33.12	0.120	0.800	0.150
Hydrogen carbonate	271.72	2370.0	3440.0	
Sulfate	<0.2	76.0		
Silicid acid	11.15			
Carbonic acid	48	3500	2380	

Note: Analyses of four typical iodine-containing mineral waters, shortened: high-mineralized iodine spring (Tassilo spring Bad Hall), low-mineralized iodine spring with a nonperceptible taste of iodine (Radenska classic, SL), low-mineralized mineral spring with perceptible taste of iodine (Sichelsdorfer Josefsquelle, A), purified water with iodine (Eniva® Iodine Mineral Water, USA). Abbreviations: A, Austria; SL, Slovenia; USA, United States of America.

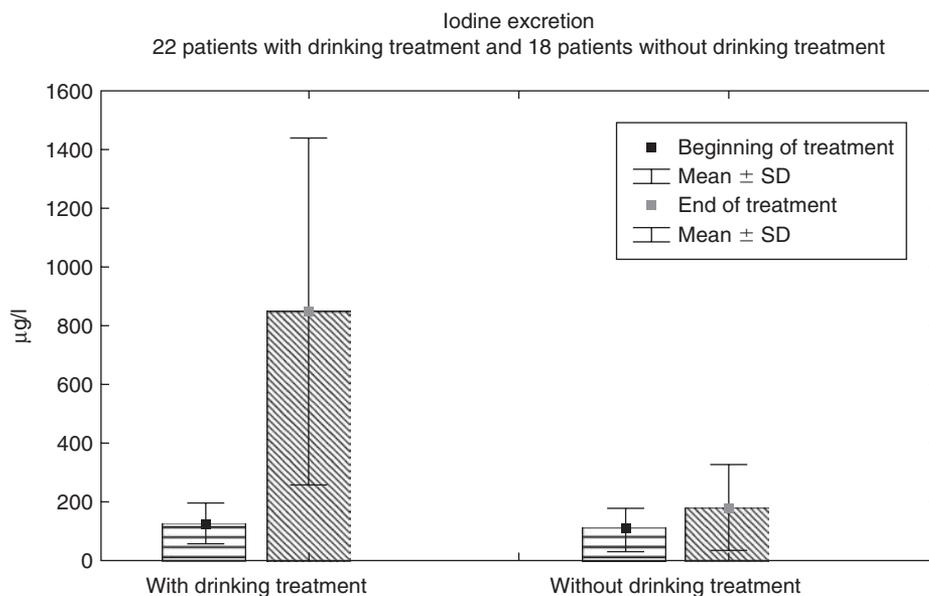


Figure 36.1 Iodine supply by treatment with higher-mineralized iodine-containing waters. Iodine excretion at the beginning and at the end of a 21 days' health resort treatment with administration of diluted Tassilo mineral spring water "Tassilo light" (left pair of columns) and a control group without prescription of iodine (right pair of columns). Iodine concentration in the morning urine was determined with the Sandell-Kolthoff method, modified by Wawschinek *et al.*, (1985) in the hospital of Barmherzigen Brüder. Permission of G. Thieme Verlag to use the illustration from previous source. Klieber and Winkler, (2006).

iodine inhalations, iodine iontophoreses, or iodine packs are used, iodine excretion increases noticeably after a 3-week treatment. Iodine excretion following iodine baths, inhalations or packs varies from 91 to 168 µg/g creatinine. More iodine is resorbed from the mucous membrane of the eye. The excretion then increases from 89.7 µg/g creatinine, at the beginning, to 361 µg/g creatinine at the end of the treatment.

After drinking treatment with medicinal iodinated waters, much higher iodine amounts are resorbed. Following treatment with highly-mineralized undiluted brines, which are not in current practice, 2450 µg/g creatinine, on average, could be found in urine. In comparison, the excretion values after using diluted brines for drinking therapy are expected to be significantly lower (Figures 36.1 and 36.2).

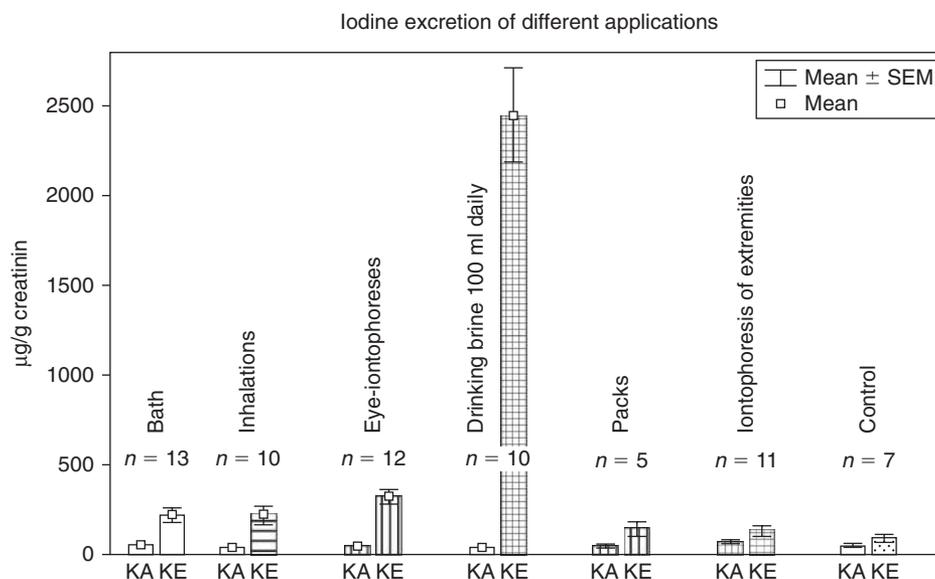


Figure 36.2 Iodine supply by single daily applications in the course of complex treatment programs. The iodine supply was given only in the course of the specified application. Iodine concentrations in the morning urine are in relation to creatinine at the beginning (KA) and at the end (KE) of the health resort treatment. Iodine determined with the Sandell–Kolthoff method in the Intern- and Chemical Section of the Paracelsus Department, Bad Hall by *Becherer et al.*, (1990). KA, beginning of treatment in health resort; KE, end of treatment in health resort. Permission of G. Thieme Verlag to use the illustration from previous source. *Klieber and Winkler*, (2006).

Prophylaxis and treatment of iodine deficiency goiter by drinking iodine-containing mineral waters

Currently many health resorts offer iodine-containing brine drinking therapies, using iodine waters, for the treatment and prevention of goiters. Here, sometimes relatively large total intake of iodine is still prescribed and followed. Indeed, a goiter caused by iodine deficiency in younger patients can be treated successfully by iodide; hence this would actually be a definitive therapy.

In addition to this high iodine dose therapy, low doses are given routinely however, there is a trend in iodine spas to strongly reduce these doses. In Bad Hall, we practice a method by diluting the local cure medium (Tassilo-brine diluted 1:10 with purified water, then called “Tassilo light”) to achieve iodine doses in a range that approaches the pharmacological therapeutic value in thyroid outpatient departments, where combinations of 100 µg iodide with 100 µg thyroxine are used for a long duration, with high success rates, as evidenced by *Pfannenstiel* (1988) in a multicenter study.

For goiter prophylaxis, the low-mineralized waters sold commercially can also be used for permanent intake. These mineral waters contain between 120 and 800 µg iodine/l, resulting in an iodine intake of 30–400 µg/day, if 250–500 ml of this mineral water is consumed. At concentrations of up to 200 µg/l, the iodine is not noticeable by taste; the iodine taste, which is unpalatable for many people, occurs

when the iodine concentration exceeds 500 µg iodine/l. If we compare the small amounts of iodine that is contained in our food, plus that supplied in daily drinking of iodine mineral waters, the consumption appears to be comparable to medically prescribed therapy (*Table 36.2*). On the other hand, the question arises whether changes in iodine consumption by drinking additional iodine-containing mineral waters may be a risk for thyroid disease.

The risk of causing thyroid disturbance by health resort treatments using higher-mineralized brines

A low or even a moderate single dose of iodide causes no or only a minor, reduction of hormone production in a normal thyroid. An excessive single dose of iodide leads, via inhibition of the thyroid peroxidase (TPO) mediated iodination (the Wolff–Chaikoff effect), to a transient decrease of intrathyroidal hormone concentration. This was first demonstrated by *Wolff and Chaikoff* (1949). When high plasma levels of iodide are sustained by repeated iodine administration, the inhibiting effect disappears and normal organic iodine levels in the thyroid are restored, with a decrease in thyroid sodium symporter, as demonstrated by *Eng et al.* (1999). This effect was demonstrated by *Haydl and Waldhäusl* (1975) in the course of health resort treatments administering iodine-containing mineral waters.

Table 36.2 Iodine supply by daily drinking of low- and high-mineralized iodine-containing mineral waters compared with the main iodine sources in our food

Common salt iodized (20 mg KI/kg salt)	4–5 g salt	60–80 µg iodine
Milk compounds	100–200 g milk products	50–100 µg iodine
Sea fish	150–200 g fish	200–300 µg iodine
Seaweed in Sushi (50–5000 iodine/kg algae)	5 g Nori	400 µg iodine
Low-mineralized iodine mineral water (containing 120 µg iodine/l)	250–500 ml daily	30–60 µg iodine
High-mineralized iodine mineral water (containing 33 mg iodine/l)	100–400 (usually applied 2–3 weeks)	3.3–13.2 mg iodine

Note: The daily uptake of iodine for the most important nutritive sources of iodine, compared with iodine uptake for drinking iodine-containing mineral waters.
Abbreviation: KI, potassium iodide.

Higher iodine doses, supplied by bolus or continuous therapy, can lead under certain circumstances to hyper- as well as to hypofunction. Statistically, the average risk of acquiring hyperthyroidism by iodine supplementation is low (0.25–0.43%); however, even this risk has to be considered when prescribing iodine-containing waters.

The incidence of iodine-induced hyperthyroidism is dependent on the iodine dose and the prevalence of goiter and, in connection with this, the occurrence of autonomies.

The iodine concentrations used in drinking treatments with highly-mineralized, unreduced medicinal waters are such that pharmaco-dynamic effects can be expected on consumption, especially as the electrolytes are present at a low oxidation level, are unbound and therefore are easily resorbable and thyroid-active. If we compare the risk of developing physiological thyroid disturbances with those of other medical measures, for example for X-ray contrast media and Polyvidone-iodine, only a portion of the administered iodine is in the thyroid-active form, and therefore thyroid disturbances are rare. In the case of amiodarone, where the organic iodine dose is very high and treatments are of long duration, more disturbances of the thyroid are seen (Table 36.3).

To reduce the risk of overprescribing iodine supplementation to individuals attending health resorts, the prescriptions now usually contain only small doses of iodine for a temporary period (2–3 weeks). Physicians also prescribe higher concentrated iodine mineral waters because the portion of spa patients aged >50 years is rather high, and

Table 36.3 Release of thyroid-active iodine by diagnostic and therapeutic treatments in medicine

X-ray diagnosis with contrast media		
Mainly used contrast media:		
	lopamidol 300 mit 300 mg organic iodine/ml	
	lopromid 300 mit 300 mg organic iodine/ml	
	iodixanol 320 mit 320 mg organic iodine/ml	
Released thyroid-active iodine		
Coronarangiography (24–98 g combined iodine)		15–59 mg
Cranial CT (35 g combined iodine)		8 mg
ERCP (6–12 g combined iodine)		1.5–2.5 mg
IVP (18 g combined iodine)		4 mg
Phlebography (14 g combined iodine)		3 mg
Angiography of peripheral legs (61 g combined iodine)		14 mg
Therapeutic iodine application		
Amiodaron 200 mg tablets	Loading dose 1000 mg/day	370 mg in 10 days
75 mg combined iodine	Continuous therapy 100–200 mg/day	1.4–2.8 g in 1 year
Potassium iodide 100 µg tablets	9 months in pregnancy	30 mg in 9 months
Polyvidone-iodine lotion standardized	2 ml daily	98 mg in 2 weeks
Drinking treatment with high-mineralized iodine waters (Tassilo spring Bad Hall)		
Mineral spring water natural (33 mg iodine/l)	2 × 200 ml daily for 3 weeks	277 mg in 3 weeks
Mineral spring water prepared (mineral spring water 1:10 diluted with pure water and carbonic acid added)	2 × 1/8 l daily	17 mg in 3 weeks

Note: Uptake of free thyroid-active iodine from the thyroid gland at a mean dose and duration of diagnostic and therapeutic measures, compared with drinking treatment in our health resort. The list made up by evaluation of publications of Fassbender *et al.*, (2001a, b), Fritzsche *et al.*, (1993), Hintze *et al.*, (1999), Mann *et al.*, (1994), Mönig *et al.*, (1999), and Mutzel *et al.*, (1989).

Source: Permission of G. Thieme Verlag to use the table from previous source. Klieber and Winkler, (2006).

Abbreviations: CT, computer tomography; ERCP, endoscopic-retrograde-cholangiography; IVP, intravenous-pyelography.

therefore it has to be assumed that goiters will exist more frequently. In 20% of these patients nodular alterations of the thyroid, and with them autonomies, are also frequently found.

Beside iodine, bromides are also sometimes present at higher doses in iodine brines. Bromide reduces iodine uptake in the thyroid, which may be of clinical importance, especially in those with iodine deficiency, as demonstrated by Pavelka (2004).

Sensitivity to small doses of iodine develops in Hashimoto's thyroiditis, which is an autoimmune disease

of the thyroid. Several studies have suggested that small amounts of supplementary iodine (250 µg) cause changes in thyroid hormone function and perhaps antibody production in predisposed individuals. These changes are generally mild, leading to subclinical hypothyroidism in some individuals, caused by changing the natural course of autoimmune thyroiditis (Reinhardt *et al.* (1998)). Therefore, we recommend the stabilization of thyroid hormone function for a period of 6 weeks after the end of such a treatment, or after modified iodine supply through iodine-containing mineral waters.

Extrathyroidal Effect of Iodine Treatment

The extrathyroidal effects of iodine are of interest to physicians, due to its possible functions in many other organs. Iodine appears to be a vital trace element that is found in higher concentrations not only in the thyroid, but also in other tissues, such as salivary glands, lacrimal glands, stomach mucous membrane, plexus choroideus, lactating mammary gland, pancreas, Langerhans' islets and ciliar muscle of the eye. The distribution is not random, but it is related to the action of a specific active transport mechanism, the sodium iodide symporter (NIS).

A wide body of research has been performed in an attempt to elucidate the role of iodine in other organs within the body.

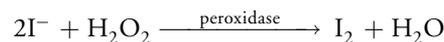
Venturi *et al.* (2000) assume that iodine compounds have an ancient role for life itself, as iodide uptake is present in algae, plants and nonvertebrate animals, but without showing any hormonal action. Three modes of iodine action have been postulated on the basis of phylogenesis and embryogenesis:

1. an ancient and direct action, where inorganic iodides probably act as antioxidants;
2. a recent and direct action of inorganic iodides on fetal pre-hormonal thyroid and on above-mentioned tissues; and
3. a recent and direct action of the iodinated hormones of the thyroid on all vertebrate cells.

Theoretical model to explain the antioxidative effect of iodine

The predominant environment of the human body is one in which there is a redox potential that enhances the reception of electrons, resulting in a prevailing presence of iodide (I⁻) as inorganic form of iodine. Thus, in many enzymatic reactions iodine can act as electron transmitter. Principally – according to the actual chemical status of iodine – enhancing, as well as inhibiting, influences are possible. The participation of I⁻ in peroxidase reactions is

well-known, which eventually promotes reactions as below (schematically):



Peroxidases are a group of nearly ubiquitous enzymes, which, on the one hand, catalyze the oxidation of numerous organic and inorganic substrates and, on the other hand, simultaneously reduce noxious hydroperoxides to harmless alcohols or water by electron transfer (Thomas and Aune (1978)).

Iodide can also inhibit the oxidation of sulfhydryl groups (e.g., in enzymes and other proteins) because of its antioxidative and reductant property, according to:



a reaction that is proposed by Gauri (1979) in the case of disulfide cross-linking of eye lens proteins. But, conversely, oxidative cross-linking can also be enhanced by I₂ or other oxidant forms of iodine, leading to the inactivation of enzymes and the destruction of proteins.

Another possibility, through which iodide can act as an electron donor, is proposed by the reaction of I⁻ with free radicals according to the scheme:



Here, R[•] usually represents the hydroxyl radical $\bullet\text{OH}$. This reaction means scavenging and transformation of the extremely reactive and tissue-damaging $\bullet\text{OH}$ into harmless hydroxyl ions (OH⁻).

The antioxidative reaction of I⁻ can also be directed against peroxides, especially lipid peroxides. Hence, the emerging iodine can be reduced to iodide by the relatively alkaline environment of the body or incorporated into organic compounds.

In an *in vitro* model to prove the antioxidant effect Winkler *et al.* (1989) showed that iodide can exert an anti-radical and preventive effect at concentrations of 3 µM.

In studies on spa patients with diabetes type II done by Moser *et al.* (1991), the effect of treatment with and without iodine brine, with special regard to antioxidant enzymes, was analyzed (Table 36.4). The activities of plasma catalase and glutathione peroxidase (GSH-PX) were increased significantly in the group with iodine drinking treatment. This result is an indication of an antioxidant effect of iodine consumption in balneotherapeutic doses. Similar results were found by a determination of the total antioxidant status (TAS) in the lacrimal fluid: in the case of dry eye syndrome, the antioxidant status of the lacrimal fluid is significantly increased in patients after iodide-iontophoresis, a treatment that is successfully utilized in Bad Hall. Thus, an improvement in the defense capacity of the tear film against oxidative stress seems to be possible, as interpreted by Rieger *et al.* (2000).

Table 36.4 Antioxidant effect of iodide

	<i>With iodine brine</i>		<i>With sodium chloride</i>	
	<i>Before</i>	<i>After</i>	<i>Before</i>	<i>After</i>
SOD ($\mu\text{g/g}$)	3.0 \pm 0.5	2.3 \pm 0.5*	3.6 \pm 0.8	2.7 \pm 0.7*
Catalase (Act./ml)	1.1 \pm 0.1	1.4 \pm 0.1**	1.3 \pm 0.1	1.2 \pm 0.3
Plasma GSH-PX (U/l)	201.6 \pm 8.5	213.3 \pm 11.5**	201.1 \pm 10.8	198.2 \pm 8.3
Erythrocytes GSH-PX (U/g Hb)	49.1 \pm 2.3	47.8 \pm 2.4	44.9 \pm 2.5	44.1 \pm 1.8
Malondialdehyde ($\mu\text{M/ml}$)	15.3 \pm 0.8	15.8 \pm 1.0	14.2 \pm 0.8	14.1 \pm 0.7

Note: The activity of antioxidative enzymes before and following drinking therapy with highly-mineralized iodine water or saline solution in diabetic patients. 43 type II diabetics received either iodine brine (9mg iodine per day) or pure saline solution with the same concentration of sodium chloride as iodine brine (0.9% sodium chloride). Changes in the activity of antioxidative enzymes, superoxide dismutase of erythrocytes (SOD), plasma catalase (Plasma cat.), glutathione peroxidase of plasma and erythrocytes (GSH-PX), and malondialdehyde could be interpreted as antioxidative effect.

Means \pm SEM, * $p < 0.05$, ** $p < 0.025$, each compared with initial value.

Source: Permission of Springer Verlag to use the table from Moser *et al.*, (1991).

Abbreviations: SOD, superoxid dismutase; GSH-PX, glutathione peroxidase; U/g Hb, units per gram hemoglobin; $\mu\text{M/ml}$, micromolar per milliliter; SEM, standard error of the mean.

Summary

- Mineral points
- Health resort
- Spa
- Balneotherapy
- Antioxidant effect

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The Iodine Content of Foods and Diets: Norwegian Perspectives

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Abstract

Dietary sources of iodine vary with country and population. Determination of the iodine content in Norwegian foods shows that fish and seafood, milk and dairy products, and eggs have the highest iodine concentration. Dietary studies in the Norwegian population show that the amount and frequency of intake of these iodine rich sources are of great importance to achieve sufficient dietary iodine intake. Several studies carried out by sampling urine or calculating dietary iodine intake conclude that the majority of Norwegians have an iodine intake within the recommended ranges. However, subgroups of the population are in danger of having an insufficient dietary intake, i.e., pregnant women, adolescents, or subjects with irregular consumption of fish and dairy products. The contribution from iodized salt is considered to be insignificant, because only some brands of table salt are iodized and industrial salt is not. Increase in the iodine content of milk and dairy products through fodder supplement, and also the more widespread use of fish and seafoods, explains the eradication of iodine deficiency in Norway in the 1950s. To secure sufficient iodine intake in the population of Norway today, a continued monitoring of iodine concentration in foods, together with surveys of iodine nutrition and status in the population is necessary.

Abbreviations

MoBa study	Norwegian Mother and Child Cohort Study
TSH	Thyroid-stimulating hormone
TPOAb	Thyroid peroxidase antibodies

Introduction

Monitoring of iodine status is important to public health, as both low and high iodine intakes may cause adverse

health effects. In Norway, the public health implications of iodine intake have moved from the pre-World War II challenge of endemic goiter in some parts of the country to present-day concerns about excessive iodine intake (Frey *et al.*, 1993). Although iodization of household or industrial salt never has been mandatory in Norway, the population has been considered iodine replete for decades, i.e., having a daily iodine intake above the recommendation of 150 µg. This is explained by iodine supplementation of cow fodder since 1950 to protect animal health. Through fodder, milk and dairy products have become a substantial source of iodine in the Norwegian diet.

Dietary Sources of Iodine

In the Norwegian food composition table, iodine concentration in some food groups are reported in the appendix in Rimestad *et al.* (2001). The most recent data on iodine concentrations in Norwegian foodstuffs and drinking water are compiled in a study by Dahl *et al.* (2004) and include analyses of iodine in 1010 samples representing 102 different Norwegian foods. In Table 37.1, iodine concentrations in major food groups and drinking water are listed.

The highest iodine content was found in foods of marine origin, and the ranges of reported values in fish filets varied widely, both within a species and between species. In general, the mean iodine concentration in lean fish species like cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and saithe (*Pollachius virens*) (86 µg/100 g) was more than twice as high as in fatty fish species, such as farmed salmon (*Salmo salar*), herring (*Clupea harengus*) and mackerel (*Scomber scomber*) (40 µg/100 g) (Julshamn *et al.*, 2001; Dahl *et al.*, 2004).

Norwegian milk and dairy products also contain relatively high amounts of iodine. Analysis of the iodine concentration in milk (i.e., low-fat milk, 1.5% fat) showed seasonal variation. The average iodine content of low-fat

Table 37.1 Iodine concentration ($\mu\text{g}/100\text{g}$) of Norwegian foods, mean, minimum and maximum

Food group	n	Iodine ($\mu\text{g}/100\text{g}$)		
		Mean	Min.	Max.
Lean fish	393	86	3	1270
Fatty fish	313	40	5	161
Fish products	44	59	8	176
Fish as sandwich spread ^a	21	33	7	82
Other fish products ^b	17	12	3	36
Milk, all types ^c	70	15	2	37
Yoghurt	9	18	12	24
Cream and cream products	10	11	6	17
Cheese, white	20	38	13	75
Cheese, whey	11	129	10	200
Eggs	4 ^d	45	39	52
Meat and meat products	12	2	<1	7
Bread and cereals	32	3	<1	9
Potatoes	4 ^e	2	<1	4
Vegetables	24 ^f	2	<1	5
Fruits and berries	8 ^g	2	<1	4
Fats and oils	3	2	<1	6
Water	15	0.2	0.05	0.55

Note: n = number of samples analyzed.

Source: From Dahl *et al.*, (2004), with permission.

^aMackerel in tomato sauce, sprat in tomato and in oil, pickled herring, caviar, smoked trout and mackerel, crab patè and cod liver patè.

^bShrimp, fresh water trout and tuna canned in oil.

^cFull-fat milk, low-fat milk, extra low-fat milk, skimmed milk and curdled milk, from summer and winter season.

^dEach sample consisted of 90 eggs.

^eThree samples consisted of 4 subsamples and one sample consisted of 25 subsamples.

^fEach sample consisted of 2–9 subsamples.

^gEach sample consisted of 2–17 subsamples.

milk in the winter season was $231 \pm 34\mu\text{g}/\text{l}$ and $93 \pm 17\mu\text{g}/\text{l}$ in milk produced in the summer (Dahl *et al.*, 2003b). The significantly higher iodine content of milk sampled in the winter season compared to milk sampled during the summer season is consistent with a previous report about Norwegian milk sampled in 1972 (Rena and Staveland, 1974). The same seasonal variation was found in organic low-fat milk, although the iodine concentrations from both summer and winter seasons in general were lower compared to non-organic milk.

No differences were found between the average iodine content within the same season for different types of milk, e.g., skimmed milk versus full-fat milk (Dahl *et al.*, 2003b).

The higher iodine concentration of winter milk is explained by the use of cow fodder fortified with iodine (Dahl *et al.*, 2003b), and these findings are in accordance with a number of studies from other countries (Varo *et al.*, 1982; Pennington, 1990; Lee *et al.*, 1994; Phillips, 1997; Ministry of Agriculture, Fisheries and Food, 1997, 1999, 2000; Larsen *et al.*, 1999; Rasmussen *et al.*, 2000). The iodine content of animal feed is controlled by legislation in Norway. The fortification of cow fodder with $2\text{mg}\cdot\text{l}/\text{kg}$

as calcium iodate $\text{Ca}(\text{IO}_3)_2$, to protect animal health (Ministry of Agriculture, 2002), has also provided a source of iodine in human diets (Frey, 1986). In 1996, a new regulation was passed ensuring that cattle should spend at least 8 weeks each summer outdoors, grazing naturally (Ministry of Agriculture, 1996). The regulation not only contributes to better animal welfare, but also makes the animals' feed more vulnerable to local soil-mineral conditions.

Eggs are another source of iodine in the diet, as its content was found to be $45\mu\text{g}/100\text{g}$ (Dahl *et al.*, 2004). The fodder for hens is controlled by legislation (Ministry of Agriculture, 2002) and iodine is normally added at $0.5\text{--}0.6\text{mg}\cdot\text{l}/\text{kg}$. The iodine content in hen fodder is shown to influence the content of iodine in eggs (Garber *et al.*, 1993).

The mean iodine concentration in other foodstuffs, e.g., meat and meat products, bread and cereals, vegetables, potatoes, fruits and berries, and fats and oils, is $2\text{--}3\mu\text{g}/100\text{g}$ and their contribution of iodine to total iodine intake are assumed to be limited (Dahl *et al.*, 2004). As a consequence of the high iodine concentration in eggs, eggs are of equal importance to total iodine intake as the sum of bread, cereals, vegetables and fruits.

The iodine content in drinking water in Norway is in the range of 0.5–5.5 µg/l. The iodine concentrations of drinking water sampled along the coast were higher (2.0 µg/l) compared to drinking water sampled from inland areas (0.8 µg/l). The highest iodine concentration in drinking water (5.5 µg/l) was found on the west coast of Norway (Stavanger). The lowest iodine concentration in drinking water (0.5 µg/l) was also found at the coast, however, in the northern part of Norway (Tromsø). With an average iodine concentration of 2 µg/l, the contribution of iodine from drinking water is negligible in Norway (Dahl *et al.*, 2004).

Iodized salt is the most important source of iodine worldwide, and is also the agreed strategy for achieving iodine sufficiency (WHO/UNICEF/ICCIDD, 2001). Although Norway has never had mandatory iodization of salt, some brands of table salt are fortified with iodine, and regulations permit the addition of 5 µg of iodine per gram of NaCl (Ministry of Health, 2002). Industrial salt used in food production is not supplemented with iodine (Frey, 1986).

Iodine Recommendations

The recommended daily intake of iodine is 150 µg for adults, whereas the lowest and maximum intakes per day are 70 µg and 1 mg, respectively, in the Nordic countries. The recommended daily intake of iodine is 90 µg for children 2–5 years, 120 µg for children 6–9 years, and 150 µg for children 10 years and older and adults in Norway (NNR, 2004). Pregnant women are recommended a daily intake of 175 µg, and lactating women 200 µg of iodine. These recommendations are in line with the World Health Organization (WHO, 2001).

Studies Assessing Iodine Nutrition

Iodine intakes assessed in studies in Norway have mainly used urinary iodine excretion to evaluate iodine status. An overview of the studies is given in Table 37.2. Areas with endemic goiter existed in Norway before World War II, and the well-known goiter district Modum was extensively examined in 1934–1935 (Devold *et al.*, 1937). The prevalence of goiter was very high, especially among school children in the community (80%). Higher prevalence of goiter was found among subjects not consuming fish, compared to subjects that consumed fish (Devold *et al.*, 1937). The conclusion from the reinvestigation of school children in Modum in 1977 was that the goiter rate in children was 1.5%, and it is not a problem in Norwegian children (Frey *et al.*, 1981). Iodine intake was considered not only to be sufficient due to the high iodine content of milk, but also due to the more widespread use of saltwater fish (Frey *et al.*, 1981). Studies carried out in men in 10 locations sampled in 1971–1972 (Halvorsen and Muri, 1974; Frey *et al.*,

1974) and in 1985 showed satisfactory urinary iodine excretion and the dietary intake of iodine of Norwegians was suggested to be 150–250 µg iodine per day (Frey *et al.*, 1993). In a study from a coastal town of northern Norway, including 63 men and women in 1999, the median daily iodine intake was calculated to be 162 µg and the median urinary iodine concentration was 117 µg/l. The estimated median iodine intake of men (187 µg) was above the recommended daily intake of iodine, whereas the iodine intake of women (114 µg) was below the recommendations (Dahl *et al.*, 2003a). In a selected population sample of 44 adults (80% women) carried out in 2001, both iodine intake and iodine excretion were in the range of mild iodine deficiency, with median daily iodine intake of 89 µg and median urinary iodine excretion per 24-h of 96 µg (Dahl *et al.*, 2003a). The daily iodine intake varied from 30 to 427 µg and the urinary iodine excretion per 24-h varied from 16 to 316 µg. However, the participants in this study were selected on the basis of either low or high intake of fish and dairy products, so a wide range of intakes and excretion was expected. The only study carried out in representative samples of Norwegians calculated iodine intake on the basis of food intake by a food-frequency questionnaire (Dahl *et al.*, 2004), and Tables 37.3 and 37.4 show the results. The mean dietary intake of iodine was within the recommendation for men (176 µg/day), while among women the mean dietary intake was slightly below the recommendation (136 µg/day). Only a minor percentage (7%) of the adult population had a daily intake of iodine below the lowest recommended intake of 70 µg/day. The food intake of subjects with iodine intake less than 70 µg/day was probably underestimated, as the daily energy intake was about 3 MJ below the mean energy intake of all subjects in the study. Furthermore, the study showed that none of the adults had an intake of iodine above 1 mg/day. This indicates that the dietary intake of iodine in adults are unlikely to exceed the tolerable upper intake level set by the Nordic countries (NNR, 2004). It should be commented that the contribution from vitamin and/or mineral supplements is not included in the above mentioned study.

The largest study of iodine intake in Norway until now has been conducted on 40108 women participating in the Norwegian Mother and Child Cohort Study (MoBa Study) (Magnus *et al.*, 2006). The study group is not entirely representative of the whole pregnant population of Norway, being somewhat better educated and with a lower percentage of smokers than the overall population of pregnant women. Thus, it is all the more remarkable that 5% of the study group has an intake level of less than 50 µg/day from the diet alone, estimated on the basis of a food-frequency questionnaire (Meltzer *et al.*, 2008). However, 82% of the women use some sort of dietary supplement, so one has to await the results from estimation of the whole diet, including iodine containing supplements, before a final evaluation can be done.

Table 37.2 Summary of iodine intake studies in Norway

Location, year of study	Method	Number of subjects and gender	Age (years)	Results	References
Telemark, 1918	Palpation of thyroidea	594 schoolchildren	7–14	58% of girls and 56% of boys diagnosed with goiter	Kjølstad, 1921
Modum, 1934–1935	Palpation of thyroidea	2074 men	0–50+	57% goiter, 78% in boys 7–14 years ^a	Devold <i>et al.</i> , 1937
		2234 women	0–50+	73% goiter, 80% in girls 7–14 years ^b	
Oslo, 1934–1935	24-h urine	29 men with goiter	19–71	23 (11–41) µg l/24h ^c	
		20 men without goiter	37–64	27 (15–37) µg l/24h ^c	
Oslo, 1934–1935	24-h urine	9 men (control)	17–31	35 (29–45) µg l/24h ^c	
Valle, Sætedal 1936–1938	Palpation of thyroidea	340 men	6–50+	4% goiter, highest in boys 7–14 years (13%)	Høye, 1941
		393 women	6–50+	35% goiter, 48% when 15–19 years, 53% when 30–39 years	
Vågå, Florø, Karasjok, and Vadsø, 1971–1972 ^d	24-h urine	116 men	19–57	199 µg l/24h ^f	Halvorsen and Muri, 1974
		Casual urine ^e	82 men	19–57	
Oslo, 1971–1972	Casual urine	213 men	20–70	248 µg l/24h ^g	Frey <i>et al.</i> , 1974
		76 women	20–70	173 µg l/24h ^g	
Modum, Gjøvik, Forsand, Valldal, and Herøy, 1972	Casual urine	171 men	20–70	260 µg l/24h ^f	
Modum, 1977	Palpation of thyroidea, serum TSH, T ₄ and T ₃ , Casual urine ^f	1418 boys and girls ^h	7–16	6 boys and 16 girls had possible palpation (1.5% goiter), TSH 0.4 ± 0.09 µg/l, T ₄ 107 ± 12.3 nmol/l, T ₃ 2.3 ± 0.2 nmol/l and 133 ± 113 µg l/g creatinine	Frey <i>et al.</i> , 1981
		Casual urine ^f	243 boys and girls (control)	7–16	
Oslo, Modum, Gjøvik, Forsand, and Valldal, 1985	Casual urine ^f	252 men		207 (53–925) µg l/24-h ^c	Frey, 1986 Frey <i>et al.</i> , 1993
Tromsø, 1999	Casual urine	32 men and 28 women	23–64	132 (38–572) ^j µg l/l in men, 112(57–314) ^j µg l/l in women	Dahl <i>et al.</i> , 2003b
				TSH and free T ₄	
Bergen, 2001	24-h urine	9 men and 35 women	21–49	140 (33–235) ^j µg l/24 h in men, 79(16–316) ^j µg l/24 h in women	
				TSH and free T ₄	
Norway, 2004	Dietary intake	1298 men 1374 women	18–79	176 µg l/day in men ^g 136 µg l/day in women ^g	Dahl <i>et al.</i> , 2004
Oslo, 2004	24-h urine	119 pregnant women	23–44	110 µg/24-h ⁱ in nonsupplement users, 190 µg/24-h ⁱ in supplement users	Brantsæter <i>et al.</i> , 2007
				Dietary intake	
Norway 2002–2005	Dietary intake	40 108 pregnant women	14–47	121 µg l/day (50, 247) ^k from diet alone	Meltzer <i>et al.</i> , 2008

^a842 boys.^b785 girls.^cFigure is mean with range in parenthesis.^d24-h urine was sampled during winter 1971–1972 and casual urine was sampled during summer 1972.^eIodine concentration in the casual urine sample was converted to iodine excretion per 24 h.^fFigure is mean from all locations.^gFigure is mean.^hResults are given for 22 participants.ⁱFigure is median with range in parenthesis.^jFigure is median.^kFigure is median with 5th and 95th percentile in parenthesis.

Table 37.3 Daily intake of food and iodine from different food groups by Norwegian adults

Food	Mean food intake ^a (g/day)		Iodine intake ^b (µg/day)		Proportion of intake (%)	
	Men	Women	Men	Women	Men	Women
Fish, total	72	58	40	33	23	24
Fish, lean	26	21	22	18		
Fish, fat	11	8	4	3		
Fish products	14	12	8	7		
Fish, sandwich spread	12	8	4	3		
Fish other	10	9	1	1		
Dairy products	577	416	101	76	57	56
Milk	518	363	78	54		
Cheese, white	23	22	9	8		
Cheese, whey	9	8	12	11		
Cream and cream products	27	23	3	2		
Eggs	19	15	9	7	5	5
Meat and meat products	125	87	3	2	2	1
Bread and cereals	317	227	9	6	5	4
Potatoes	147	100	3	2	2	1
Vegetables	123	146	3	3	2	2
Fruits and juice	218	225	4	5	2	4
Fats and oils	42	28	1	1	1	1
Beverages	1512	1370	3	3	2	2
Total intake			176	136		

Source: From Dahl *et al.*, (2004), with permission from Elsevier.

^aFood intake data from Johansson and Solvoll (1999).

^bMean iodine concentrations are found in Table 37.1.

Table 37.4 Intake of iodine by Norwegian adults^a

Percentile	Iodine (µg/day)		
	Men (n = 1298)	Women (n = 1374)	All (n = 2672)
5	72	58	63
10	89	71	78
25	121	96	105
50	166	129	146
75	219	168	191
90	274	206	246
95	315	236	283

Source: From Dahl *et al.*, (2004), with permission from Elsevier.

^aFood intake data from Johansson and Solvoll (1999).

In a substudy of MoBa, including pregnant women grouped as users or nonusers of vitamin and mineral supplements, median urinary iodine excretion per 24h was 190 µg and 110 µg, respectively (Brantsæter *et al.*, 2007). In the same study, dietary iodine intake was calculated by a food-frequency questionnaire and a food diary. The dietary intake of iodine among nonsupplement users was below the recommendations, whereas the dietary iodine intake of supplement users was above the recommendations. This demonstrates that supplements may contribute considerably to the total dietary iodine intake.

Dietary Iodine Intake in Children

Several nationwide surveys among children at different ages have been conducted to provide detailed information on their current dietary habits (Pollestad *et al.*, 2002; Øverby and Andersen, 2002). The food intake of children was reported for 4 consecutive days in a pre-coded diary with lists of the most common foodstuffs and beverages in Norway. The dietary intake of iodine for boys and girls aged 4, 9, and 13 years is given in Table 37.5. The mean daily iodine intake among the 4-year-old children was approximately 100 µg and was above the recommended daily intake of iodine. For children aged 9 years, mean dietary intake of iodine of the boys was in accordance with the recommendation, but in girls the mean iodine intake was below the recommendation of 120 µg/day. The iodine intakes among the adolescents (13 years) were found to be below the recommendation, especially among the girls.

Thyroid Disorders

As iodine intake is an important up- or downregulator of the activity of the normal thyroid gland, the iodine intake of a population should be brought to the level at which iodine deficiency disorders are avoided (Laurberg *et al.*, 2000). Thyroid dysfunction is known to be more common in women than in men. The frequency of thyroid abnormalities has been reported in several Norwegian studies

Table 37.5 Intake of iodine from different food groups among Norwegian children^a

Age	Iodine intake ($\mu\text{g}/\text{day}$)					
	4 years		9 years		13 years	
Food	Girls (n = 185)	Boys (n = 206)	Girls (n = 411)	Boys (n = 404)	Girls (n = 517)	Boys (n = 492)
Fish and fish products	12	14	13	14	11	14
Milk and dairy products	70	70	67	85	59	70
Eggs	4	5	4	5	4	5
Meat and meat products	1	1	2	2	2	3
Bread and cereals	5	5	7	8	7	9
Vegetables and fruits	5	5	6	6	6	6
Fats and oils	0	0	0	0	0	0
Beverages	1	1	1	1	1	2
Iodine intake ($\mu\text{g}/\text{day}$)	98	101	100	121	90	109

Source: From Dahl *et al.*, (2004), with permission from Elsevier.

^aBased on mean food intake from Pollestad *et al.*, (2002) and Øverby and Andersen (2002).

(Normann, 1955; Brochmann *et al.*, 1989; Bjøro *et al.*, 2000), however, the relationship to iodine intake has not been investigated. Nevertheless, the prevalence of diagnosed hypothyroidism, hyperthyroidism and goiter was 4.8, 2.5 and 2.9% in women, whereas the prevalence in men was 0.9, 0.6 and 0.4%, respectively, in a large health study performed in Nord-Trøndelag County in Norway (Bjøro *et al.*, 2000). The study also demonstrated that values of thyroid-stimulating hormone (TSH) were significantly higher in participants with positive thyroid peroxidase antibodies (TPOAb) than in those with negative antibodies. TPOAb is used as a marker of an autoimmune process in the thyroid. Furthermore, the study indicated that autoimmunity is an important factor in both hyper- and hypothyroidism in Norway, since the majority of females and males with hypothyroidism or hyperthyroidism had positive TPOAb.

Iodine Nutrition in Norway

Since the fortification of cattle fodder started in 1950, iodine deficiency has been assumed to be eradicated in Norway (Frey, 1986). Although there never has been systematic monitoring of iodine nutrition, several studies in the last decade have shown that the iodine intake in the majority of the population is in the range considered to be sufficient. The majority of studies conducted in Norway have focused on urinary iodine concentrations in selected groups of the population. Calculation of iodine intake based on a food-frequency questionnaire covering the habitual diet in a representative sample of adult Norwegians confirmed that milk and dairy products are a very important iodine source in the diet. The study showed

that milk and dairy products, together with fish and fish products, were main sources of iodine in the diet, contributing about 80% of the total iodine intake (Dahl *et al.*, 2004). Nevertheless, studies among children and adolescents show that the iodine intake can be lower than recommended levels in subgroups. Iodine intake among children aged 4 and 9 years and among men was at recommended levels. The MoBa Study points to the possibility that subgroups of pregnant women may also be in danger of a too low iodine intake.

The importance of milk and fish for total iodine intake in Norway may put certain groups of the population at risk of low intake of iodine, e.g., subjects with allergy to milk or fish, vegetarians who do not consume fish, milk and dairy products (vegans), and others with a low consumption of milk and fish. It should be emphasized, however, that even though other sources of iodine than fish and milk do not contribute substantial levels of iodine, other foods do provide some iodine, and for some subjects they are the only sources of iodine. Furthermore, the contribution of iodine from supplements should be taken into consideration. Data from a representative dietary survey showed that more than half of the female population and nearly 40% of the men used vitamin and/or mineral supplements (Johansson and Solvoll, 1999). In the MoBa, 82% of the women reported taking some sort of dietary supplement (Meltzer *et al.*, 2008). Data on children showed that less than 10% used supplements (Pollestad *et al.*, 2002; Øverby and Andersen, 2002). There is no available documentation as to the extent of, and in what amounts, the vitamin and mineral supplements contain iodine in Norway. However, it is reasonable to assume that about 25% of the most commonly used vitamin and mineral

Table 37.6 Iodine intake from milk ($\mu\text{g}/\text{day}$) during summer and winter^a

	Men (n = 1298)	Women (n = 1374)
Milk, summer ^b	48	34
Milk, winter ^c	120	84

^aBased on mean intake of milk (Johansson and Solvoll, 1999).

^bMean value of $93\mu\text{g}/\text{l}$. Dahl *et al.*, (2003a).

^cMean value $231\mu\text{g}/\text{l}$. Dahl *et al.*, (2003a).

supplements contain 70–150 μg iodine per tablet. Therefore, supplements containing iodine may contribute significantly to the total intake of iodine in the diet.

Knowledge about the variation in iodine concentration of milk and dairy products is important because regular, daily consumption of milk and dairy products is very common in Norway. It should be commented that the seasonal variation of iodine concentration in milk was not taken into consideration when calculations of iodine intake in representative samples of Norwegians were done (Table 37.3) (Dahl *et al.*, 2004). The seasonal variation of iodine content of Norwegian milk will give approximately 20% lower total intake of iodine during the summer compared to the winter season, assuming that there is small variation in the intake of milk during the year (Tables 37.3 and 37.6).

Iodine nutrition is not regarded as a public health issue, and is therefore not a concern of the Norwegian health authorities. Studies performed in the last decade more or less confirm that assumption; however, it is important to be aware of some subgroups in the population, i.e., pregnant women and adolescents, as studies in these groups have showed an average iodine intake below the recommendations. In future studies more information about the contribution of iodine from supplements should be obtained. More knowledge is also required about the impact of seasonal variation of iodine concentration in milk, and the wide range of iodine concentration in fish in relation to iodine status and incidence of thyroid disorders in the Norwegian population.

Summary Points

- Fish and seafood, milk and dairy products, and eggs have the highest iodine concentration in Norwegian foods.
- Dietary iodine intake of adults is in the range of 50–250 $\mu\text{g}/\text{day}$.
- Dietary iodine intake is in the range of the recommended level among children, and below the recommendation among adolescents and subgroups of pregnant women.
- Regular intake of fish and seafood and/or milk and dairy products is of great importance to achieve a sufficient iodine intake in the Norwegian diet.

- Urinary iodine concentration in small groups of adults indicates sufficient iodine intake.
- Mandatory iodization of cow fodder has been more important for iodine intake than iodized table salt in Norway.

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Correlation between Soy Phytoestrogens and Thyroid Laboratory Parameters: Implications for Iodine Nutrition

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Abstract

Iodine is the main component for thyroid hormone biosynthesis, hence, lack of its supplementation in diet may have severe consequences. Soy isoflavones are one of the nutritional factors that may affect thyroid hormone biosynthesis, transport, and actions in target tissues. Both isoflavones and isomeric flavones are widespread phytochemicals in the plant kingdom, whose individual varieties number in the thousands. The most important isoflavonoids, daidzein and genistein, are abundant in legumes, especially in soybeans and soy-based food and formulas. Soy foods carry a lot of significance in South- and East-Asian countries: a lower incidence of coronary heart diseases, certain forms of cancer, and osteoporosis in these countries has been ascribed to their purported beneficial effects on human health. Daidzein and genistein are weak estrogens due to their ability to interact with estrogen receptors (ER- β type), and have been used for estrogen replacement therapy. In addition, their estrogenic properties may also interfere with thyroid hormone actions. They are potent inhibitors of thyroid peroxidase, the principal enzyme of thyroid hormone biosynthesis. They also influence thyroid hormone transport and the metabolism of iodothyronines by deiodinases. The most recent knowledge concerning the isoflavonoid action is reviewed, including clinical trials on the effects of soy food and formulas on thyroid function. Two recent studies addressing this issue are also included. In the first of these recent studies carried out on a large group of school children ($n = 268$) in the Czech Republic, where soy food is not found in a common diet, we investigated whether free daidzein and genistein

levels correlate with thyroid laboratory parameters. In the second study, healthy young volunteers were followed to determine whether high soy consumption over the course of 1 week would: (1) influence thyroid hormone parameters, and (2) show correlation between isoflavone and thyroid hormone levels. In accordance with most other clinical trials, only modest and transitory effects of soy isoflavones on the thyroid were found, which may, however, be important in case of insufficient iodine supply.

Abbreviations

AbTg	Autoantibodies to thyroglobulin
AbTPO	Autoantibodies to thyroid peroxidase
ANOVA	Analysis of variance
ECLIA	Electrochemiluminescence immunoassay
ELISA	Enzyme-linked immunosorbent assay
ER	Estrogen receptor
fT ₃	Free triiodothyronine
fT ₄	Free tetraiodothyronine (thyroxine)
GC-MS	Gas chromatography–mass spectrometry
PTK	Protein tyrosine kinase
SHBG	Sex hormone-binding globulin
T ₃	Triiodothyronine
T ₄	Tetraiodothyronine (thyroxine)
TBG	Thyroxine-binding globulin
Tg	Thyroglobulin
TPO	Thyroid peroxidase
TSH	Thyroid-stimulating hormone, thyrotropin

Introduction

A diet lacking in iodine can have adverse effects on health, since iodine is essential for thyroid hormone biosynthesis. Many external and internal factors are known to influence the transport of iodine to thyroid follicular cells and thyroid hormone biosynthesis, metabolism, and actions in target cells. Soy isoflavones are among the nutritional factors that may exert an effect on these processes.

Soy Isoflavones

Soy isoflavones in nature

Soy isoflavones, daidzein and genistein, are low-molecular-weight compounds derived from isoflavone, an isomer of flavone (Figure 38.1). Both isoflavones and flavones are widespread phytochemicals in plant kingdom, which include thousands of chemical individuals. Daidzein and genistein are in abundance in legumes, especially in soybeans and soy-based food and formulas, but they are present in many other vegetables, fruits, nuts, peas, lentils, and seeds. A recent database is available providing the data on daidzein and genistein contents not only in plant raw materials, but also in various cereals, bakery products, milk, meat, and other food products. Most of the values reported are based on gas chromatography–mass spectrometry (GC-MS) measurements (Ritchie *et al.*, 2006).

In most available soy products, isoflavones are present predominantly as glucosides. There is evidence that they are not absorbed in this form by the gastrointestinal tract, and that their bioavailability requires initial hydrolysis of the sugar moiety by intestinal glucosidases. After absorption, isoflavones are reconstituted mainly to glucuronic acid and, to a lesser degree, to sulfuric acid. Free, unconjugated

aglycone has only been detected in small amounts in the blood (Rowland *et al.*, 2003). In addition, isoflavones are further extensively metabolized by the intestinal microflora. Considerable evidence is available that points to extensive interindividual variation in isoflavone metabolism (Rowland *et al.*, 2000). These facts are important from the point of view of their effect on thyroid hormone biosynthesis, since only free isoflavones exert biological activity.

Soy isoflavones and human health

Soy foods play an important role in the South- and East-Asian countries. A lower incidence of the most frequent diseases, such as coronary heart disease (Adlercreutz *et al.*, 2004; Weggemans and Trautwein, 2003; Nestel, 2003), certain forms of cancer, especially breast (Adlercreutz, 2002; Mishra *et al.*, 2003) and prostate cancer (Mishra *et al.*, 2003; Messina, 2003), and osteoporosis (Setchell and Lydeking-Olsen, 2003) in these countries has been ascribed to the purported beneficial effects of soy isoflavones on human health. Large-scale population screening and controlled dietary intervention studies have been undertaken over the past 15 years to address this issue; many excellent reviews have been reported weighing not only benefits, but also potential risks of high soy isoflavone intake (Rice and Whitehead, 2006; Messina *et al.*, 2006; Sirtori *et al.*, 2005; Cassidy, 2003). In addition, isoflavones, as their isomeric flavones, belong to a wide group of dietary antioxidants (Seifried *et al.*, 2007).

Soy isoflavones and hormone replacement therapy

Due to their mild estrogenic properties, soy isoflavones belong to dietary phytoestrogens and, as such, have been used as an alternative to classical estrogens in estrogen replacement therapy (Wuttke *et al.*, 2002). Their estrogenic potency is due to interaction with the ER β class (but not with the ER α class) of estrogen receptors (ERs) (Kurzer, 2003). Though their affinity to ER is by an order of magnitude less than classical estrogens, soy food consumers may reach levels which, from the point of view of their estrogenic potency, are comparable with endogenous estrogens (Whitten and Patisaul, 2001).

Effect of soy isoflavones on sex hormone regulation

The estrogenic potential of soy isoflavones raised the question of the impact of their intake on overall hormonal balance, particularly on female sex steroid actions. As early as in 1946, Australian veterinarians observed that phytoestrogens adversely affected reproduction in several animal species. The reproductive dysfunction in sheep known as

Formulas of flavone, isoflavone and major isoflavones

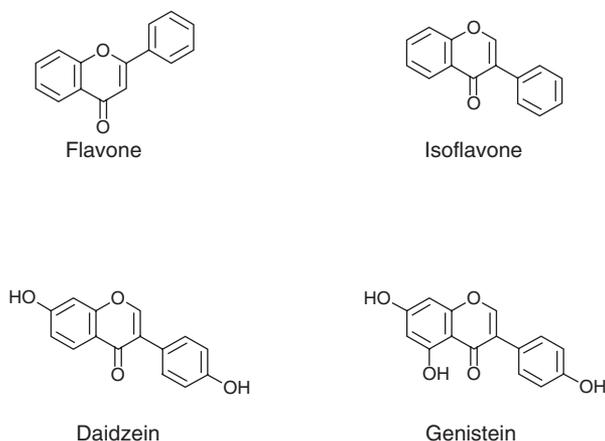


Figure 38.1 Formulas of flavone, isoflavone, and major soy isoflavones daidzein and genistein.

clover disease was attributed to isoflavones (Setchell *et al.*, 1997). These authors also used GC-MS for the determination of the levels of daidzein, genistein, and their metabolites, to show that plasma levels of isoflavones in children fed with soy-based infant formulas reached values that were tens of thousands times higher than plasma estradiol concentrations in early life. It should be emphasized, however, that only a small portion of isoflavones in circulation are in a free form. Therefore, it is not surprising that human intervention studies dealing with the effect of various soy-based diets or tablets containing isoflavones on pre- (Duncan *et al.*, 1999b; Watanabe *et al.*, 2000) and postmenopausal women (Duncan *et al.*, 1999a; Persky *et al.*, 2002) revealed only modest and transitory effects on estrogen status.

As for as androgenic status and isoflavone are concerned, most of the reports dealt with isoflavone's potential to reduce the risk of prostate cancer. Isoflavones, and especially genistein, along with other endocrine-disrupting chemicals, have been reported to inhibit several steroidogenic and steroid-metabolizing enzymes (e.g., 17 β -hydroxysteroid dehydrogenase, steroid glucuronyltransferase), and thus modulate steroid hormone availability (Deluca *et al.*, 2005; Whitehead and Rice, 2006). Generally, targets for phytoestrogens are very broad and may comprise steroid receptors (especially ERs), steroid metabolizing enzymes, various elements of signal transduction cascades including apoptosis, and even DNA-processing machinery (topoisomerases) (McCabe and Orrenius, 1993). In this connection, a well-known effect of isoflavones on sex hormone-binding globulin (SHBG)

should be mentioned, since the latter considerably influences the bioavailability of sex hormones (Pino *et al.*, 2000), and a link exists between this hepatic protein and thyroid hormones (Pugeat *et al.*, 1996).

Effect of soy isoflavonoids on thyroid hormone function

In regard to iodine utilization, the most significant effects of isoflavones are on thyroid function, including their antithyroid or even goitrogenous potential, due to their effects on iodine incorporation in thyroid hormone biosynthesis. Consumption of soy isoflavonoids may lead not only to benefits, but also to some risks (Chen and Rogan, 2004). As early as in the late 1950s and 1960s, goitrogenic effect in children fed with soy formula was reported, which could be reversed by iodine supplementation; for a review of the literature, see Cassidy (2003). This finding evoked a number of investigators to search for the cause of this effect. In their review, based on a series of animal as well as human experiments, the Arkansas authors (Doerge and Sheehan, 2002) have shown that the goitrogenic effect of soy isoflavones was predominantly caused by the inhibition of thyroid peroxidase (TPO) in thyroid hormone biosynthesis. Isoflavones may also influence thyroid hormone metabolism, including deiodinases, thyroid hormone transport, and various thyroid hormone-mediated signaling pathways. The possible sites of isoflavone effect on iodine utilization and metabolism in thyroid are shown in Figure 38.2.

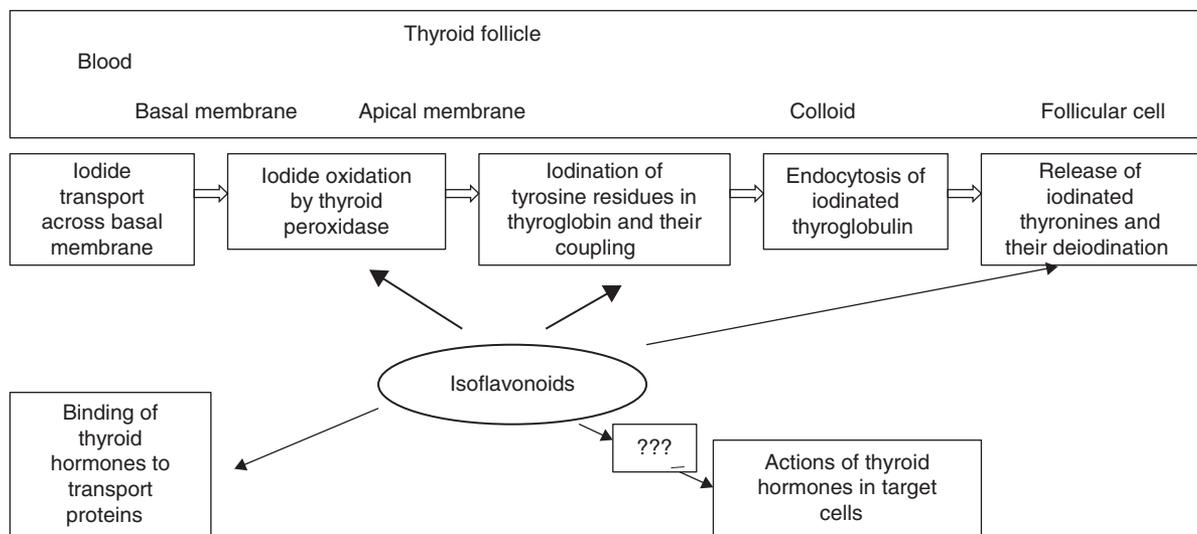


Figure 38.2 Possible sites of inhibitory actions of soy isoflavonoids on iodine utilization and thyroid hormone biosynthesis and actions. Soy isoflavonoids, genistein and daidzein, inhibit oxidation of iodide by thyroid peroxidase at the apical membrane of thyroid follicular cells, followed by iodination of tyrosine residues in thyroglobulin and their coupling in colloid. In addition, they may affect deiodination of iodothyronines and interfere with thyroid hormone binding to transthyretin. Full arrows indicate the sites of inhibition. So far, only few reports concern the effect of thyroid hormone actions in target cells.

Isoflavonoids as Inhibitors of Thyroid Peroxidase

Since the 1980s, it has been known that many flavonoids of plant origin act as potent inhibitors of various enzymes, including TPO. The literature on this issue was reviewed in 1996 (Divi and Doerge, 1996). These authors tested various flavonoids as inhibitors of purified porcine TPO *in vitro*. Among the 13 compounds tested one was an isoflavone, biochanin A, which differs from genistein only by a methoxygroup on carbon 4'. The inhibition constants including that for biochanin A were mostly in the micromolar range. The results led the authors to focus on soybean isoflavonoids, because of their widespread use in food products. Using the same *in vitro* assay system as in the case of flavonoids, they found that both genistein and daidzein in the presence of iodide ion blocked TPO-catalyzed thyroxine formation by acting as alternate substrates, and proposed the mechanisms for genistein inhibition of TPO-catalyzed reactions. Genistein also inhibited thyroxine synthesis when using human thyroglobulin (Tg) as a substrate for conjugation (Divi *et al.*, 1997). In their next study, they addressed the question to what extent may soy food influence thyroid hormone biosynthesis *in vivo*, starting from *in utero* life (Chang and Doerge, 2000). The main goal was to evaluate the possible antithyroid effect of soy-based formulas given to children, newborns, and their mothers. In their experiments they used rats exposed to soy-free or soy-fortified food in three different doses, given to animals during weaning through the 140th postnatal day. After this period, the total body genistein and daidzein serum levels were measured, along with intrathyroidal isoflavone concentrations and the activity of microsomal TPO. Though TPO was reduced significantly after isoflavone exposure, the remaining enzymatic activity was sufficient to maintain thyroid homeostasis, provided that iodine supply was not reduced. Decreased TPO activity in soy-fed animals did not lead to a hypothyroid state, as characterized by concomitant decreased triiodothyronine (T₃)/thyroxine (T₄) ratio and increased thyroid stimulating hormone (TSH) levels.

All these experiments were reviewed 2 years later (Doerge and Chang, 2002), with the following major conclusions: (1) isoflavone deactivation of TPO is a general phenomenon across mammalian species; (2) in the absence of iodide, genistein and daidzein are suicide substrates for TPO and also other peroxidases that share structural characteristic with TPO and functional properties, e.g., lactoperoxidase; (3) in the presence of iodide, the above isoflavones act as alternative substrates that block tyrosine iodination through preferential formation of iodinated isoflavone derivatives; and (4) though soy isoflavone intake caused a significant decrease in TPO activity, at least in animals, it does not necessarily lead to hypothyroidism, probably due to a large reserve of TPO in the apical membrane of thyroid follicular cells.

Soy Isoflavonoids and Deiodinases

The bioavailability of active thyroid hormones depends on the activity of deiodinases in thyroid follicular cells and in target tissues. *In vitro* experiments with purified enzyme revealed that some dietary flavonoids in micromolar concentrations inhibited thyroid type 1 iodothyronine deiodinase activity (Ferreira *et al.*, 2002). Among the compounds tested was an isoflavone metabolite, biochanin A. So far, a few reports using various cell cultures (Mori *et al.*, 1996) or animals (cats) (White *et al.*, 2004) have confirmed that genistein at least is capable of inhibiting 5'-deiodinases.

Effect of Soy Isoflavonoids on Thyroid Hormone Transport

In addition to affecting thyroid hormone biosynthesis and metabolism and thyroid hormone signaling (see below), soy isoflavones were also shown to affect their transport by interaction with the second thyroid hormone-binding protein, transthyretin. Both daidzein and genistein displaced labeled thyroxine from transthyretin in a competitive way in concentrations that occur in sera from soy-diet consumers. This binding was specific, because they bound to neither thyroxine-binding globulin (TBG) nor albumin (Radovic *et al.*, 2006). Interaction of genistein with transthyretin may have another physiological consequence: through its binding, genistein inhibits transthyretin tetramer dissociation and amyloidogenesis, which is known to be associated with severe neurodegenerative diseases (Green *et al.*, 2005).

Soy Isoflavones and Hormone Signaling

Genistein has been used for years as a model inhibitor of protein tyrosine kinases (PTKs). This ubiquitous enzyme, which is also present in thyroid follicular cells, is involved in phosphorylation of tyrosyl residues of membrane-bound receptors leading to signal transduction. One of the signal transduction cascades leads to topoisomerase II, which participates in DNA replication. Blocking PTK activity is one of the mechanisms believed to be responsible for the anticancer potential of genistein. For reviews addressing this issue, which are beyond the scope of this chapter, see Peterson (1995), and Ravindranath *et al.* (2004).

Soy consumption and thyroid hormone levels in humans

Clinical Trials Potential benefits and risks of high soy food consumption, the use of soy isoflavonoids in food supplements, as well as previous observations of the influence of soy-based formulas on hormonal balance and

overall health status, tempted several authors to undertake clinical trials, retrospective studies, and case reports addressing this issue.

One of the first retrospective epidemiologic studies of teenage children demonstrated an association of soy consumption in early infancy and a higher prevalence of autoimmune thyroid diseases (Fort *et al.*, 1990). This work was criticized, in part because children were often fed with soy formulas in order to prevent allergic disorders, known to be associated with autoimmune diseases (Chandra, 1997).

So far, 15 clinical trials have been reported on the influence of soy food or isoflavone-rich diet on thyroid hormone levels. Thirteen trials undertaken up until 2005 were reviewed recently (Messina and Redmond, 2006) with a general conclusion that with two exceptions, both from Japanese authors (see below), only modest, if any, hormonal effects occurred following the consumption of a soy-rich diet. Using roasted soybeans given to subjects of both sexes, the Japanese authors reported increased TSH (but still remaining in the normal range), while thyroid hormones were unaffected (Ishizuki *et al.*, 1991). The effects were determined by comparing final to baseline levels, as there was no control group. Another group of Japanese authors (Watanabe *et al.*, 2000), using tablets containing both major isoflavones as main components, found a decreased T_3/T_4 ratio in soy-supplemented women.

In most instances, the above-mentioned trials used isolated soy protein or soy milk with known content of isoflavones. The modest impact of soy consumption on thyroid laboratory parameters was also confirmed by the most recent crossover study (Dillingham *et al.*, 2007) on healthy young men, which also correlated the data of thyroid hormones with the urinary excretion of both major isoflavones. It is to be noted that, with only the latter exception, the effect on thyroid parameters was not the primary health outcome of these investigations.

Case Reports A few case reports showed only a transitory increase of TSH during soy-based food consumption. Jabbar *et al.* (1997), Conrad *et al.* (2004), and Bell and Ovalle (2001) report on higher thyroxine substitution required by hypothyroid children consuming soy-based formulas. The latter authors concluded that concurrent administration of thyroid hormone with a soy dietary supplement leads to decreased absorption of levothyroxine and the need for higher oral doses to attain therapeutic thyroid hormone levels.

How Thyroid Hormone Levels Correlate with Actual Soy Isoflavone Concentration in Children

So far, reported trials concerned the effect of soy food or soy isoflavone supplements on thyroid hormone levels.

In most instances, the data that we compared with control groups of age- and sex-matched subjects, or crossover design, was applied.

In our recent nonintervention study, we have addressed the question as to whether an association exists between thyroid hormone parameters and actual free isoflavone levels in normal middle-European populations for which soy food is not part of a regular diet (Milerova *et al.*, 2006). School children were used as a model because they are unaffected by thyroid and other endocrine disorders typical of old age. The Czech Republic belongs to geographic areas with a relatively low iodine intake, but where mean iodine intake has increased over the past years (Vanderpump *et al.*, 1995). Serum samples from screening of iodine deficiency in one region of the Czech Republic were used for the determination of thyroid laboratory parameters and actual levels of isoflavones, taking advantage of sensitive radioimmunoassays for daidzein and genistein, enabling us to measure subnanomolar concentrations of free (unconjugated) isoflavones in human sera.

The group consisted of 268 school children (139 girls and 129 boys) aged 8–15 years, without apparent medical disorders, including allergic symptoms, within the last 3 months. Among the girls, 33 were postmenarchial in the age groups 12 years and older. Written informed consent for blood withdrawal and participation at the screening was obtained from the parents. All children or their parents were also asked to complete a questionnaire concerning their dietary habits. One question dealt with soy-food intake during the last 24 h: “Did you eat yesterday some soy-containing food as soy beans or soy milk or other soy products (and if so, what)?” The screening was performed in agreement with the local ethical committee of the Institute of Endocrinology.

The screening of the thyroid function included assessment of thyroid volume by ultrasonography, a battery of basic serum laboratory tests of thyroid function, i.e., TSH, free thyroxine (fT_4), free triiodothyronine (fT_3), Tg, autoantibodies to thyroid peroxidase (AbTPO) and thyroglobulin (AbTg), and determination of iodine concentration in urine. Blood was collected from the cubital vein between 8 and 9 a.m. Serum was separated and stored frozen at -20°C until analyzed. TSH (normal range 0.27–4.20 mIU/l), fT_4 (12.0–22.0 pmol/l), fT_3 (2.80–7.10 pmol/l), and Tg (normal levels below 85 $\mu\text{g/l}$) were determined by electrochemiluminescence immunoassays (ECLIA) (Roche Diagnostics GmbH, Mannheim, Germany), using a commercial Elecsys System 2010, AbTg and AbTPO (physiological levels below 250 and 25 U/l, respectively) were assessed by enzyme-linked immunosorbent assay (ELISA) (Milenia Biotec, Bad Nauheim, FRG). Urine iodine was measured by alkaline ashing with subsequent Sandell–Kolthoff’s reaction, i.e., 2Ce^{4+} (yellow colour) + $\text{As}^{3+} = 2\text{Ce}^{3+}$ (colourless) + As^{5+} . The reaction was terminated by the addition of brucine, which

Table 38.1 Basic statistical data on soy isoflavone levels and major laboratory parameters of thyroid function in children

Parameter	Unit	Mean \pm S.E.M	Median	Minimum	Maximum
Daidzen	nmol/l	0.76 \pm 1.40	0.43	0.02	10.7
Genistein	nmol/l	0.82 \pm 0.92	0.38	0.03	5.24
TSH	mU/l	3.10 \pm 1.50	3.10	0.31	11.59
fT ₄	pmol/l	15.58 \pm 1.98	15.77	12.06	23.6
fT ₃	pmol/l	7.05 \pm 1.17	7.07	4.39	7.04
Tg	μ g/l	14.14 \pm 12.00	11.34	0.10	106
Anti-TPO	U/l	10.7 \pm 3.75	0.015	0	694
Anti-Tg	U/l	43.8 \pm 3.21	39.2	0	486
Ioduria	μ g/l	207 \pm 73.4	153	53.3	339
Thyroid volume	ml	4.68 \pm 0.12	4.31	1.77	13.87

Note: 268 school children aged 8–15 years were screened for iodine deficiency in one region of the Czech Republic. Actual serum levels of free daidzein, genistein, thyrotropin (TSH), free thyroid hormones, autoantibodies to thyroid peroxidase (AbTPO) and thyroglobulin (AbTg), and ioduria, along with thyroid volume, were measured. S.E.M. shows standard error of the mean.

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reduced the remaining Ce⁴⁺ ions, producing a reddish oxidized chinoid form suitable for spectrophotometric detection. Serum daidzein and genistein were measured by original radioimmunoassays developed in the authors' laboratory (Lapcik *et al.*, 1997, 1998). The reported physiological levels for omnivores were 0–2.1 and 0–1.7 nmol/l for daidzein and genistein, respectively.

One-way analysis of variance (ANOVA) was used for assessment of the differences among groups and subgroups of subjects. Spearman's rank order correlations were used for analyses of the relationships between isoflavones and thyroid hormone parameters.

Table 38.1 shows the basic statistical data of the measured parameters. Since sex hormones may influence thyroid parameters, the data from the whole female group were compared with those of 33 postmenarchial girls, and with the subgroups aged 12–14 years and above 14 years, without finding any significant differences in either thyroid parameters or phytoestrogen levels.

All the parameters were then mutually correlated. Since the distribution of the data was not Gaussian, Spearman's correlations were used. The extract from the correlation matrix showing only significant correlations between phytoestrogen levels on one side and thyroid parameters on the other is given in Table 38.2. The expected correlations reflecting mutual relations between individual pairs of thyroid parameters, as well as for both phytoestrogens, are not shown. The multiple regression analysis using a multiple linear regression model to describe the relationships between each phytoestrogen and the other seven variables measured (see Table 38.1) revealed that, at least in the case of genistein, there was a statistically significant relationship between the variables at 99% confidence level; for details see Milerova *et al.* (2006), and Vanderpump *et al.* (1995). The principal component explaining genistein variability was fT₃, followed by TSH and fT₄.

Table 38.2 Significant Spearman's correlations between phytoestrogen levels and thyroid parameters in children

Correlated pairs of parameters	<i>n</i>	<i>r</i>	<i>p</i>
Genistein/anti-Tg	257	0.2636	0.0000
Daidzein + genistein/anti-Tg	257	0.2226	0.0004
Daidzein/TSH	257	0.1873	0.0027
Genistein/thyroid volume	257	−0.1695	0.0067
Genistein + daidzein/thyroid volume	257	−0.1389	0.0263

Note: The data from Table 38.1 were mutually correlated. The table shows only significant correlations on at least 95% level. *n* is the number of correlated pairs, *r* the coefficient of correlation, and *p* the probability.

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Finally, the group was divided into subgroups of children who did or did not eat food containing soy within the last 24 h. The results are shown in Table 38.3. No significant differences were found between the groups, with the exception of fT₄ and genistein, which were significantly higher in soy-food eaters (*p* = 0.0032 and 0.0062, respectively).

The finding of a positive significant correlation of daidzein but not genistein with TSH is rather surprising, because daidzein is a weaker inhibitor of TPO-catalyzed iodination and coupling. On the other hand, a multiple regression analysis considering both phytoestrogens as explained variables revealed a weak but significant relationship only between genistein and thyroid parameters. In the recent soy-eaters, we observed higher fT₄ accompanied with only insignificantly higher levels of both TSH and fT₃ (Table 38.3). This may suggest that their actual serum levels reflected long-term dietary habits rather than the last food consumption.

In conclusion, only modest association was found between actual phytoestrogen levels and parameters of thyroid function in a sample of children in a population from a region where soy consumption does not belong in a typical

Table 38.3 Differences of phytoestrogen levels and selected thyroid parameters in the subgroups of children who did or did not eat soy food within the last 24 h

Parameter	Soy food eaters in the last 24 h			Non-soy food eaters in the last 24 h			Significance of differences between groups (P)
	n	Mean	S.E.M	n	Mean	S.E.M	
Daidzein (nmol/l)	36	1.352	0.362	229	0.664	0.081	0.0062
Genistein (nmol/l)	36	1.068	0.188	229	0.773	0.059	0.0775
Daidzein + genistein (nmol/l)	36	2.421	0.418	229	1.437	0.110	0.0025
fT ₃ (pmol/l)	36	7.186	0.175	229	7.031	0.078	0.4690
fT ₄ (pmol/l)	36	16.48	0.285	229	15.42	0.133	0.0032
TSH (mU/l)	36	3.593	0.326	229	3.188	0.162	0.3584
Ioduria (μg/l)	36	198.6	16.8	229	209.4	7.98	0.6194
Anti-Tg (U/l)	36	58.30	12.64	230	41.19	3.21	0.0738
Anti-TPO (U/l)	36	21.12	13.48	230	7.82	3.59	0.2074

Note: The children screened for iodine deficiency (see Table 38.1) were divided into subgroups who did or did not eat soy food within the last 24 h. The symbols are the same as in Tables 38.1 and 38.2. Significant differences between the subgroups are in bold characters.

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diet. On the other hand, we have demonstrated that even small differences in soy phytoestrogen intake may influence thyroid function, and this could become important when iodine intake is insufficient.

The Effect of Short-Term Soy Consumption on Thyroid Hormone Levels

In our last experiment (Hampl *et al.*, submitted for publication), we tested the short-term effect of controlled soy consumption on major laboratory thyroid parameters. In contrast to the intervention studies, reviewed above, lasting from 4 weeks to 2 years, the subjects ate soy-rich food only for 1 week. As in the previous study, one of the major tasks was to investigate whether thyroid parameters correlate with actual free isoflavone levels.

The studied group consisted of 86 university students, 54 females and 32 males aged 18–25 years (mean age 20.7 ± 0.9 years). All of them were without overt thyroid disease; their basal TSH levels were within the physiological range. None of the subjects reported having allergic disorders for at least 3 months before the study. There were nine females with basal positive titers of antithyroid autoantibodies (anti-TPO above 25 IU/l or anti-Tg above 125 IU/l). They were deliberately not excluded from the study in order that the group reflected the middle-European population as much as possible, where up to 10% of subjects display positive titers of antithyroid antibodies.

After basal investigation including anthropometrical evaluation, and determination of serum sex steroid hormone and lipid levels, the subjects were asked to eat 2 g of unprocessed boiled natural soybeans (Alfa Bio Slovakia) for every kilogram of body weight (approximately 140 g per day for

males and 120 g per day for females) for a period of 7 consecutive days. The unprocessed soybeans contained 1.2–4.2 mg of isoflavones per 1 g of dry weight. The amount of soybeans consumed was chosen to approximate or exceed the amount of isoflavones in the Asian diet (20–150 mg of isoflavones per day). All the subjects were omnivores, for whom soy food was not part of the daily diet. They signed a form in which they were instructed not to ingest any additional soy-containing or soy-derived products or food supplements and vitamins, to reduce physical and irregular sport activities, and to abstain from sex to reduce its influence on hormonal levels.

Morning peripheral blood was collected on the day of introductory investigation (= before), on the 8th day (the next morning after last soybean consumption = at the end), and 7 days after termination of soy food consumption (after). All the subjects were asked to keep a dietary record and note physical and sexual activities in case they were not avoided during the study. The study protocol was approved by the Ethical Board of the School of Medicine, Comenius University, Bratislava, Slovak Republic. All the subjects signed a written informed consent.

TSH, fT₄, fT₃, AbTPO, AbTg, and free unconjugated daidzein and genistein were measured by the same methods as in the previous study.

The changes of observed parameters were evaluated using repeated-measures ANOVA with Bonferroni's corrected *post hoc t*-test for multiple comparisons of dependent variables. Pearson's correlation coefficient and test were used for the evaluation of dependence of quantitative variables. *p*-values less than 0.05 were considered significant. All statistic analyses were performed using the software package GraphPad Prism 4.0.

Actual levels of thyroid hormones and major antithyroid autoantibodies, along with free isoflavones daidzein

and genistein, were measured before, at the end, and after soy consumption. Two statistical approaches were applied: (1) the changes from the basal values of the studied parameters were compared to evaluate the significance of the effect of soy consumption, and (2) the data in each group and stage of the experiment were mutually correlated, particularly with respect to the relationship between

isoflavone levels on one side and thyroid parameters on the other.

Figures 38.3 and 38.4 show actual levels of daidzein and genistein before, at the end, and 7 days after termination of soy consumption. Both phytoestrogens increased significantly ($p < 0.0001$) during soy diet consumption and, after 7 days, fell back almost to the initial values.

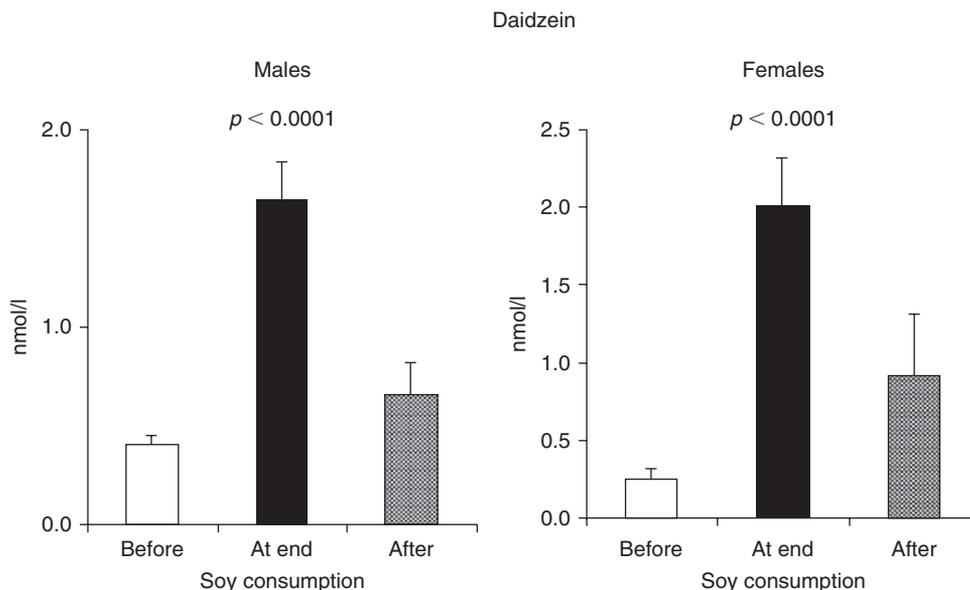


Figure 38.3 The effect of short-term soy food consumption on serum levels of daidzein in young men and women. The columns show serum daidzein levels before, at the end, and 7 days after termination of soy consumption in 86 healthy university students (54 females and 32 males). The vertical bars represent standard deviations while p value shows the significance of differences determined by analysis of variance (ANOVA).

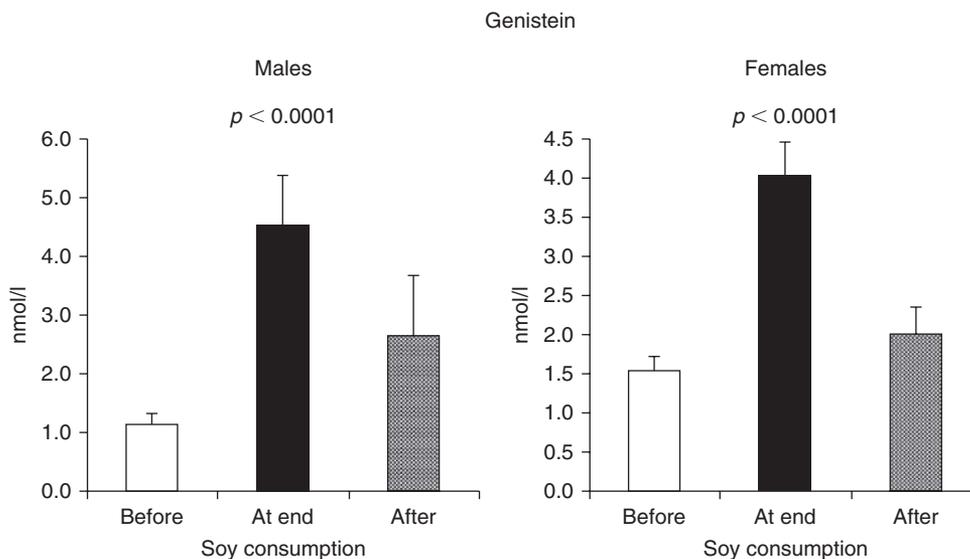


Figure 38.4 The effect of short-term soy food consumption on serum levels of genistein in young men and women. The columns show serum genistein levels before, at the end, and 7 days after termination of the soy consumption in 86 healthy university students (see Figure 38.3). Symbols as in Figure 38.3.

The corresponding levels of TSH are given in [Figure 38.5](#). As expected, the levels of both phytoestrogens increased significantly at the end of soy consumption, but a week after termination of soy consumption they returned to the basal levels. The consumption of a phytoestrogen-containing diet resulted in only a transitory increase of TSH in males at the end of soy consumption ($p < 0.0001$), while no significant changes were found in the female group. TSH increase in males was accompanied by an insignificant decrease of fT_3 and an increase of fT_4 .

No differences were found at any stage of the experiment between phytoestrogen levels in the subgroups of subjects with or without positive titers of antithyroid autoantibodies, which did not change significantly during the experiment.

[Table 38.4](#) provides the extract from Pearson's correlation matrices before, at the end, and 7 day after controlled soy food consumption in females and males. Only data from significant correlations ($p < 0.05$) in at least one subgroup of subjects and in at least one stage are shown.

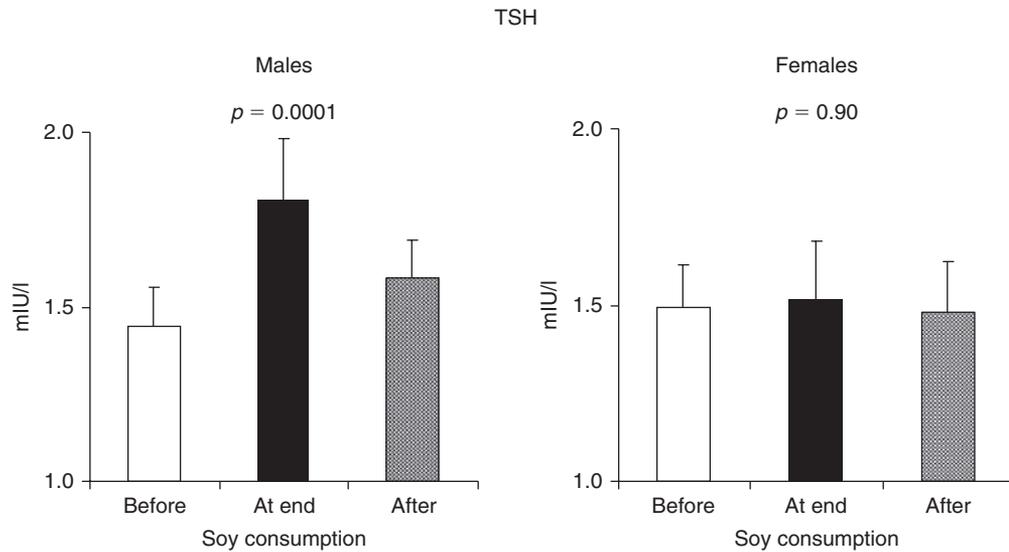


Figure 38.5 The effect of short-term soy food consumption on serum levels of thyrotropin in young men and women. The columns show serum TSH levels before, at the end, and 7 days after termination of the soy consumption in 86 healthy university students (see [Figures 38.3](#) and [38.4](#)). Symbols as in [Figure 38.3](#).

Table 38.4 Correlations between actual soy isoflavones and selected thyroid laboratory parameters in healthy young subjects consuming soy food

Correlated pair	Before		At the end of soy consumption				One week after termination					
	Females		Males		Females		Males		Females		Males	
	r	p	r	p	r	p	r	p	r	p	r	p
Daidzein/genistein	0.637	0.001	0.601	0.001	0.617	0.001	0.640	0.001	0.924	0.001	0.806	0.001
Daidzein/TSH	NS		0.599	0.001	NS		NS		NS		NS	
Daidzein/ fT_4	NS		NS		0.287	0.037	NS		NS		NS	
Daidzein/AbTg	NS		NS		NS		NS		NS		0.433	0.013
TSH/AbTPO	NS		NS		NS		NS		NS		0.362	0.049
TSH/AbTg	0.209	0.034	NS		0.313	0.023	NS		NS		NS	
fT_3/fT_4	0.449	0.001	NS		0.498	0.001	NS		0.588	0.001	NS	
AbTPO/AbTg	0.554	0.001	0.373	0.035	0.559	0.001	NS		0.727	0.001	NS	

Note: 86 university students (54 females and 32 males) ate 2g/body weight of unprocessed, boiled, natural soybeans for seven consecutive days. Actual serum levels of daidzein and genistein, TSH free thyroid hormones, and autoantibodies to thyroid peroxidase and thyroglobulin were measured before, at the end, and 7 days after controlled soy consumption. The table provides an extract from Pearson's correlation matrices. Data from significant correlations ($p < 0.05$) in at least one subgroup of subjects in at least one stage of the experiment. NS means not significant; other symbols are the same as in the previous tables. Source: Reproduced from [Hampfl et al. \(2007\)](#).

The correlation analysis confirmed the expected relations between both isoflavonoids, as well as among thyroid parameters. Of interest is the finding of a positive significant correlation of daidzein with TSH in the male group before soy food consumption. In our previous nonintervention study (see above) with school children on a typical diet, we also found a positive significant correlation of daidzein levels (but not genistein) with TSH. It may be speculated that actual TSH serum levels reflected long-term dietary habits rather than the last food consumption, as this may probably be the reason for correlation of free thyroxine with thyroid autoantibodies in the female group.

In conclusion, our data confirm that soy phytoestrogens possess only modest and transitory effect on thyroid hormone values, but, on the other hand, reveal that some thyroid hormone parameters do correlate with actual isoflavone levels.

General Conclusion

The available data from *in vivo*, as well as from *in vitro*, experiments clearly demonstrate that soy isoflavones act as inhibitors of TPO and thus may influence iodine incorporation in thyroid hormone biosynthesis. On the other hand, most of the clinical trials, including our own, have shown only modest and transitory effects of soy isoflavones on circulating thyroid hormone levels. However, they may be important in the case of insufficient iodine supply. Besides thyroid hormone biosynthesis, soy isoflavones may influence other events involved in thyroid function, such as iodine transport, thyroid hormone deiodination, and signaling pathways. So far, the data available on these topics is scarce and more experiments addressing these issues are needed.

Summary Points

- Soy phytoestrogens, especially isoflavone derivatives daidzein and genistein, are nutritional factors that, among many other effects, may affect iodine utilization.
- They form an important part of the South- and East-Asian diet.
- Based on large population studies, some beneficial effects, such as lower incidence of coronary heart diseases, some forms of cancer, and osteoporosis in these countries were ascribed to a diet rich in soy food.
- Because of their mild estrogenic properties, they have been used for estrogen replacement therapy.
- Studies revealed, however, that their abundant consumption may adversely affect thyroid function and may even be goitrogenous.
- Since the 1980s, many *in vitro* studies demonstrated that soy isoflavones are potent inhibitors of TPO.
- Clinical trials, including ours, have been undertaken to ascertain whether soy food consumption can influence thyroid function, especially actual thyroid hormone biosynthesis and actual thyroid hormone levels.
- In our first study on 268 children from a European region where soy food is not included in the daily diet, we correlated thyroid hormone levels with actual concentrations of both isoflavonoids. Though correlations were found, the differences between subgroups with low and high isoflavone levels were insignificant.
- In our second study on 86 healthy university students consuming soy-rich food for 1 week, we have found only transitory changes in TSH in males during soy consumption, and confirmed the correlations among the parameters studied.
- In conclusion, the clinical trials including ours have shown only modest and transitory effects of soy isoflavones on circulating thyroid hormone levels. However, they may be important in case of insufficient iodine supply.

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Knowledge of Iodine Nutrition

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Abstract

Knowledge of iodine nutrition among the various role players involved in a national salt iodization program could be considered as one of the important, but often neglected, components of such programs. Moreover, the scant information published on this topic shows variable levels of knowledge of iodine in different countries. Knowledge of iodine nutrition is considered one of the process factors operating either on its own, or via one of the other process indicators, which has an impact on the iodine status of people, and most likely plays a crucial role in the sustainability of salt iodization programs. Because of its importance, various groups or role players in any country, such as politicians and health ministerial staff, salt producers, wholesalers and retailers, consumers, producers of processed foods, and medical and health professionals, should be knowledgeable about iodine nutrition. The level of knowledge regarding iodine nutrition can be assessed in these groups using appropriately designed questionnaires.

Abbreviations

ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDD	Iodine deficiency disorders
IQ	Intelligence quotient
UNICEF	United Nations Children's Fund
WHO	World Health Organization

Introduction

"Knowledge is power"

Sir Francis Bacon, Religious Meditations, Of Heresies, 1597

Successful national salt iodization programs to eliminate iodine deficiency usually consist of a number of important components (WHO/UNICEF/ICCIDD, 2007). Depending on the country's situation, examples of these components may include: appropriate legislation; a functional national controlling body; regular monitoring of the iodine content of salt at production sites, at retail level, and in households; iodine laboratory facilities; a national database to track progress; and a program of public education and social mobilization. Invariably, the attention in national salt iodization programs is primarily focused on delivering adequately iodized salt to the consumer, and then on assessing the iodine status in vulnerable groups in the population, while comparatively little attention is directed toward some of the other components, such as improving the level of general knowledge regarding iodine nutrition in the population.

Only a small number of articles have been published in the scientific literature on issues related to the level of knowledge about iodine nutrition or iodine deficiency disorders (IDD) among the general population or in subgroups of the population. With few exceptions, these articles report a low level of knowledge regarding iodine nutrition or of IDD. For example, two studies in India showed very low levels of public awareness regarding the role of iodine in the prevention of IDD (Mohapatra *et al.*, 2001; Mallik *et al.*, 1998). There was a universal lack of knowledge about the cause of endemic goiter, which was believed to be one of the major factors for the unsuccessful results in the iodine control program in one of the areas (Mohapatra *et al.*, 2001). A low percentage of the people were aware of iodized salt, and an even lower percentage used it in the household.

Some variation in the knowledge pattern sometimes occurs in different areas. In the northeastern part of India, virtually all the families participating in a study were aware of goiter, but hardly any knew the cause of goiter

(Hazarika and Mahanta, 2004). A very worrying observation in this study was the fact that more than 90% of the heads of households neither considered goiter a health problem, nor had any knowledge of iodization of salt.

In a national study assessing the knowledge of iodine nutrition in 2164 adults in South Africa, representing the full range of socioeconomic strata, only 15% of respondents correctly identified iodized salt as the primary dietary source of iodine, 16% knew the thyroid gland needs iodine for its functioning, and a mere 4% considered brain damage as the most important health consequence of iodine deficiency (Jooste *et al.*, 2005). This situation was even worse in lower socioeconomic households, where respondents were considerably less informed about aspects of iodine nutrition compared to higher socioeconomic households.

In contrast to the low level of knowledge of iodine nutrition in some countries, people in other countries seem to be better informed. In the Philippines, awareness regarding the importance of iodized salt consumption was 81%, while consumption was much lower at 21% (Tuazon and Habito, 2007). Awareness rates were of similarly high magnitude in Iran, where it was also found that literate women were more likely to use iodized salt in the household than illiterate women (Sheikholeslam, 1996).

From these limited examples, it appears that the knowledge level of iodine nutrition varies virtually from one extreme to the other in different countries. These variable knowledge levels have the potential to impact not only on the iodine status of the population, but also on the sustainability of national salt iodization programs. Because knowledge of iodine nutrition is viewed as one of the important determinants in the success of salt iodization programs, we take an in-depth look at the fundamentals of iodine knowledge in this chapter.

Iodine Knowledge as a Process Indicator

Conceptually the whole field of iodine nutrition may be subdivided into two broad subdivisions, the process and the impact (or outcome) fields of iodine nutrition, each with its own assessment indicators. The impact side of the iodine nutrition field represents the response of the human body to the iodine delivered to and consumed by the consumer, and therefore follows the process phase sequentially. This response of the human body is usually assessed in terms of impact indicators, such as the median urinary iodine concentration, thyroid size, and blood constituents such as thyroid-stimulating hormone, thyroglobulin, or other thyroid hormones. The process side of iodine nutrition covers factors playing a role in the delivery of iodine to the consumer via iodized salt or via an alternative source, such as processed food, drinking (iodine-containing cleaning agents) used in the dairy industry water or iodophors.

In the case of iodized salt, the process phase starts at the point of salt iodization and comprises the whole chain of events, which includes storage, transport, and distribution at wholesale and retail levels, and extends to the point of consumption at the household level. Factors operating at any point in this chain of events and influencing or determining the amount of iodine eventually consumed at household level are considered process factors, and are assessed in terms of the process indicators. Of these indicators, the iodine content of iodized salt at the point of iodization, at the retail level and in the household represent the process indicators most commonly assessed in surveys.

This list of process indicators may be extended to include more indicators, such as the iodine content in processed food, the iodine concentration in drinking water, the contribution of using iodophors in the dairy industry and knowledge of iodine nutrition. Because knowledge of iodine nutrition may influence virtually all the process indicators, it carries a considerable potential of impacting on the outcome indicators as a process factor on its own, as well as operating via most of the other process factors. For example, a thorough knowledge of iodine nutrition may influence the decisions and behavior of several different groups of role players, such as health professionals, salt producers, retailers, and women providing and preparing food in the household. It may therefore be fruitful to take a closer look at how knowledge of iodine nutrition in these groups of role players may enhance and strengthen a national iodine program.

Iodine Knowledge of Role Players

Each of the several role players has a decisive role in the supply chain providing iodine to the consumer. Knowledge of iodine nutrition exerts its influence through these role players functioning at particular levels of the society. Their combined influence on the national iodine program may be responsible for a highly effective program or, in the case of poor iodine knowledge, for a weak or failing program. The role players are the following.

Politicians and ministerial health staff

In most countries, the point of departure of an iodine program is at the political and ministerial level. They have to be sufficiently knowledgeable not only about iodine nutrition, but also about the intricacies of designing and maintaining an effective IDD prevention and control program. All of this knowledge needs to be molded into appropriate iodine-related policy, legislation and monitoring mechanisms, creating a framework for other role players within which the national iodine program needs to be executed. Most of all, at the political and ministerial level, which includes program managers, a sound iodine knowledge-base is a prerequisite for formulating effective communication strategies

to convey the framework, goals, implementation, monitoring and evaluation of the entire program to all other role players.

Sufficient knowledge of iodine nutrition is the basis of decision-making at the political and ministerial level. Where iodine knowledge is lacking at this level, a vacuum exists at the top of the national iodine program and the program is at serious risk of failure. To meet the demands of a national iodine program, a knowledgeable person or group of people in the ministry of health should therefore direct the national iodine program.

Salt producers

Salt producers are obliged to meet the legal requirements related to iodized salt in any country, but their level of iodine knowledge is likely to have a crucial influence on their dedication and efficiency in meeting these requirements. They work and produce at the interface between a commercial enterprise and a health system, often without an understanding of the health issues involved in the iodization of salt. In the absence of knowledge of these iodine health issues, regulation and enforcement are frequently viewed as appropriate mechanisms to ensure proper iodization of salt, and as a way of circumventing weaknesses, such as a poor knowledge of iodine nutrition among salt producers. However, in practice, enforcement is seldom used as the only tool to achieve optimal iodization of salt, and it remains doubtful whether the approach of enforcement will have long-term favorable results of good compliance with legal requirements and accurate iodization.

Shortcomings in the level of knowledge of iodine nutrition were apparent in an assessment among salt producers in South Africa (Jooste, 2003). Instead of merely enforcing the legal specifications, the iodine knowledge level of these producers was improved through an educational campaign that consisted of intermittent mailing of IDD brochures, pamphlets and other material, workshops, personal visits and a PowerPoint presentation to senior staff at production plants. The benefit of this approach showed up in a reassessment of the iodine content of salt at the production sites, as well as in the iodine status of women and children in a subsequent national survey. For strengthening and successfully sustaining a national salt iodization program, it is believed that an educational campaign, enhancing the iodine knowledge of producers, is much more effective than a punitive approach, as was seen in the South African example.

Ideally, in view of the key position and role of salt producers in the iodized salt supply chain, they should form an integral part of the country network of role players sharing in the mutual flow of IDD and related information. Every opportunity should be utilized to improve and strengthen their iodine knowledge so that their commitment toward the production of accurately iodized salt is

based primarily on a combination of knowledge of iodine nutrition and social responsibility, and secondarily on meeting the legal requirement.

Small producers pose an even greater challenge to educate, because their subsistence operations with small turnovers and modest profits leave little room for additional expenses such as iodization. In addition, their salt is invariably of inferior quality, making iodization less effective. Despite these potential barriers, they should share in the iodine-related information flow and have exposure to educational activities that may improve their iodine knowledge and positively influence their attitude and perspective of iodine nutrition and iodization of salt.

Wholesalers and retailers

Hardly any information is available on the knowledge of iodine nutrition among wholesalers and retailers. As a group they are probably the least informed about iodine nutrition and the consequences of iodine deficiency or excess (Ling, 2004). Only in circumstances where repackaging occurs, and in wholesaler or large retailer companies with in-house quality control laboratories, may one find some awareness of iodine nutrition and an awareness of legal requirements and consumers' iodine health.

Theoretically, if knowledgeable about iodine nutrition, this group of role players may add value to the salt iodization program by verifying accurate iodization levels through laboratory titration tests. Without such knowledge, the wholesalers and retailers become merely a distribution depot for iodized salt, without playing a significant role or contributing toward the iodine health of the population or communities that they serve. In few countries, legislation exists specifying the required iodine concentration at this level. Under these circumstances the same considerations as for salt producers may also apply to wholesalers and retailers.

Consumers at household level

While the World Health Organization (WHO) reported a marked improvement in the iodine status of 54 countries over the past decade (Andersson *et al.*, 2005), there is little information in the literature to suggest that a concomitant improvement in knowledge of iodine nutrition at the household level occurred at the same time. The scant information available suggests, with few exceptions, a lack of iodine knowledge, and many reports call for educational campaigns and awareness creation as a means of boosting iodine programs to achieve the international goals.

For the past decade or more the emphasis in iodine messages at the international level has shifted from focusing on goiters as the most important abnormality resulting from iodine deficiency to brain damage in children (WHO/UNICEF/ICCIDD, 2007). However, the available

data suggests that this message has not been successfully conveyed to the household level, because only a small percentage of consumers in surveys were aware of some of the consequences of iodine deficiency (Mohapatra *et al.*, 2001; Mallik *et al.*, 1998), and an even smaller percentage knew that mental impairment might result from iodine deficiency (Jooste *et al.*, 2005).

An aggravating factor is the finding that knowledge of iodine nutrition is markedly lower in lower socioeconomic households than in higher socioeconomic households (Jooste, 2001). This lack of knowledge increases the vulnerability of lower socioeconomic sectors of the population to practices weakening salt iodization programs, such as the domestic use of noniodized agricultural salt, obtaining noniodized salt directly from producers, informal repackaging and marketing of noniodized salt, and others. The vulnerability of lower socioeconomic households showed up in the positive linear relationship between socioeconomic status and the iodine content of household salt (Jooste, 2001), where the iodine content of household salt was low in poor households and high in wealthy households.

Producers of processed foods

Iodine-containing processed foods, such as bread, fish sauce and other foods, play an increasingly significant role in dietary iodine intake in many populations. It has even happened that producers of processed foods use iodized salt unknowingly (Harris *et al.*, 2003). The practice of processed foods containing iodine has grown to the extent that, in some countries with voluntary salt iodization, iodine-containing processed foods may be the primary source of dietary iodine intake.

Irrespective of whether the use of iodized salt in the production of processed foods is compulsory or voluntary, the production of iodine-containing processed foods draws the producers of such foods into the iodine nutrition arena. It could therefore be expected that these producers are familiar with the basics of iodine nutrition, the benefits and risks involved, and how their product fits into the iodine nutrition program of the country. Particularly in countries where processed foods are the primary source, or are contributing a significant percentage of dietary iodine intake, these producers need to be educated and well-informed about iodine nutrition.

Medical and health professionals

During the training of medical doctors and health staff, the emphasis is usually on a clinical approach to diagnosis and treatment of thyroid abnormalities. Their training rarely covers the public health aspects of iodine deficiency and its disorders, such as brain damage and a loss of intelligence quotient (IQ) in children, neither does it cover the prevention and control of IDD programs on a population

basis. As a result, it is frequently found that medical and health professionals require additional training to gain a thorough understanding of iodine nutrition and its public health implications.

Assessment of Knowledge of Iodine Nutrition

Specifications for assessing the level of iodine knowledge do not exist, and surveys until now used locally-designed questionnaires to assess the level of knowledge in a descriptive way, to evaluate an intervention, or to find an answer for a specific need. In general, educational programs aiming to improve the iodine-related knowledge level of any specific target group should cover the basic fundamentals of iodine nutrition that may in turn feature in the assessment of the knowledge level. These fundamental issues may differ between groups, e.g., the issues that are important to salt producers may differ from those that are important to consumers.

Examples of fundamental issues that should be assessed in salt producers include:

- Motivation for the iodization of salt.
- Legal specifications for salt iodization.
- Understanding of the concept of “universal salt iodization.”
- Expected duration of salt iodization.
- Familiarity with the widely-used abbreviation “IDD.”
- Knowledge of the most important examples of IDD.
- Percentage of households in a country that should be using adequately iodized (>15 ppm) salt.
- Parameters to be measured in order to assess the impact of iodized salt in children.
- Potential health consequences of over-iodized salt.
- The influence of transportation between the producer and the consumer on the iodine concentration of iodized salt.

At the consumer level the expectation regarding iodine knowledge is different to that of the salt producer, and it is perhaps somewhat more basic. Examples of basic issues that should be assessed in surveys at the consumer or household level comprise:

- An awareness that salt is iodized or that iodine has been added to salt.
- An ability to identify whether iodine is a vitamin, mineral, micronutrient, something in the food that we eat, or something else.
- Knowledge of the main dietary sources of iodine.
- Knowledge of which part of the body requires iodine for its functioning.
- Knowledge of the most important harmful effects to health in the case of an insufficient iodine intake.

Additional questions may be phrased to assess the sources of iodine if certain sources provide only noniodized salt. The assessment could easily be extended to also assess the knowledge of issues other than that mentioned above, as well as aspects of attitude and behavior. Assessing the knowledge of other groups, such as politicians, ministerial staff, program managers, or wholesalers and retailers may utilize combinations of the above and may be extended depending on the local situation.

Discrepancy between Iodine Knowledge and Behavior

Improved knowledge about iodine nutrition and the benefit of iodized salt is expected to have a positive effect on behavior, except if inhibiting factors are preventing positive behavioral changes. A good example is the situation in the Philippines, where the awareness regarding the importance of iodized salt among consumers was 81%, while consumption of iodized salt was much lower at 21% (Tuazon and Habito, 2007). The reasons for the low consumption were due to an unavailability of iodized salt in some areas and a two- to threefold higher price of iodized salt compared to noniodized salt.

In addition, the supply of iodized salt in the Philippines was short because of a low production rate of iodized salt despite adequate capacity, which in turn was attributable to a low public demand for iodized salt, in a situation where abundant noniodized, cheaper salt was available. This example illustrates a number of factors that may play a role in the discrepancy sometimes seen between iodine knowledge level and behavior. More factors may be present in other countries, but the message for program managers is to be watchful for these factors that may weaken the iodine program, even when knowledge of iodine nutrition is satisfactory.

The Role of Iodine Knowledge in Mandatory and Voluntary Iodization Programs

In countries with voluntary or optional iodization of salt, housewives' choice of iodized over noniodized salt is usually based on their perception of the greater health benefit derived from using iodized salt, if there is no price differential. In these countries, varied educational strategies are required to ensure an adequate iodine knowledge level for individuals to choose correctly if given a choice.

However, in countries with mandatory iodization, only iodized salt is available on the shelf and the question may rightly be asked: "Is education, and therefore a certain level of iodine knowledge, really necessary in countries with mandatory iodization, since the iodine is in all the salt anyway?" The answer is unreservedly "yes" for a number of reasons.

Not only should people be informed about what fortificant is added to their salt and why it is added, but an understanding of the basics of iodine nutrition will strengthen the salt iodization program at all levels and contribute immensely to the sustainability of the program. Even in situations where the median urinary iodine concentration indicates a replete iodine status, it is known that median values at the national level may mask geographic pockets of persistent iodine deficiency or areas where the iodization program is not functioning optimally. These are typically the remote and lower socioeconomic sectors of a population that are difficult to reach with iodized salt. A basic knowledge of iodine nutrition among the general population in these circumstances may generate a demand for iodized salt, as has been seen in some African countries.

Moreover, a number of factors, such as the underiodization of salt by producers, the domestic use of noniodized agricultural salt, cross-border trade of noniodized salt, consumers obtaining noniodized cheap salt directly from producers, informal repackaging for household use of noniodized salt, and a variety of other malpractices, may weaken mandatory salt iodization programs in any country. A thorough knowledge of iodine nutrition and of IDD, from the program manager to the consumer, is likely to counteract these weakening factors to a large extent. These are also the factors responsible for not achieving the goal of 90% of households using adequately iodized salt. Improving the knowledge levels of all involved in the supply of iodized salt to the consumer may bring us closer to the goal and sustain a successful program.

Improving Knowledge of Iodine Nutrition

Experience in many countries has shown that legislation, even mandatory iodization of salt, does not automatically guarantee the successful elimination of iodine deficiency, unless it is accompanied by education and support to the salt producers. Legislation is a good start toward achieving this goal, but one of the biggest stumbling blocks in the implementation of a successful national salt iodization program is the inadequate education and lack of iodine knowledge of those involved. Dunn (1996) referred to inadequate education as one of the seven deadly sins in confronting endemic iodine deficiency. For an iodization program to be successful and sustainable, he emphasized that it is essential for all role players involved to have a thorough understanding of the importance of iodine deficiency, its consequences and the means for its correction.

While many reports call for iodine education and improvement of knowledge levels, very little is said about exactly how this should be achieved. The answer is not a

simple one, because of the variety of role players involved, which include the policy, legal, health providers, industrial, marketing, educational and even the consumer's interests. A multipronged approach aimed at informing and educating all parties at the different levels seems appropriate, but it needs to be tailored according to the requirements and priorities of any specific country or the local situation.

Mass media has been successfully used in Turkey to improve iodine knowledge and the percentage of households using iodized salt, particularly among women with higher levels of education (Çan *et al.*, 2001). Factors contributing to the success included the relative simplicity of the message and the fact that no major change in behavior was needed, merely substitution of salt by iodized salt.

In many countries the knowledge of iodine nutrition is available among program managers, scientists, academics and others, but the critical connection between knowledge, policy and practice is not made, or it was made in the past but not sustained (Haxton, 1996). Knowledge, therefore, cannot fulfill its role of stimulating action unless it is closely accompanied by an effective communication strategy. Communication channels must therefore be established and maintained to allow a mutual bidirectional exchange of knowledge and information among the relevant role players.

In summary, a sound knowledge of iodine nutrition and IDD is the key to realization that the intellectual, physical and economic health of a community, and indeed of a population, is closely linked to the effectiveness of the national iodine program and ultimately to the iodine status of its people. It is also one of the essential elements of all role players, from the political and ministerial, through the production, wholesale, and retail to the consumer level. Finally, iodine knowledge enhances the commitment and dedication toward not only achieving the goal of eliminating iodine deficiency, but also sustaining the elimination indefinitely.

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Iodine Metabolism and Parenteral Nutrition

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Abstract

The recommended intake of iodide during pediatric parenteral nutrition is 1 mcg/kg/day (Koletzko *et al.*, 2005). Parenteral nutrition solutions that are specific for infants and children reflect these recommendations, and if they are the sole source of iodide intake, this may be associated with deficiency. Iodine deficiency may compromise particularly extreme preterm infants as they are the group with the lowest endogenous iodide reserves; a scenario that is likely to contribute to the incidence of transient hypothyroxinemia. Paradoxically, the technologies associated with methods of delivery of parenteral nutrition may expose infants to uncontrolled iodide excesses, through the use of iodinated skin disinfectants and iodinated contrast media for the visualization of the position of central venous catheters, with the attendant risk of inducing transient hypothyroidism. Low thyroxine levels, whether secondary to transient hypothyroxinemia or transient hypothyroidism, risk compromising brain development and subsequent neurodevelopment. We recommend that exposure of infants to uncontrolled iodide excess is discontinued, noniodinated alternatives are available for skin disinfection, and alternative approaches exist for catheter visualization. In the ensuing absence of uncontrolled iodide exposures, we suggest that recommendations for the iodide content of parenteral nutrition for infants are urgently reviewed on the basis of best evidence available.

Abbreviations

FT4	Free thyroxine
rT3	Reverse triiodothyronine
TBG	Thyroid binding globulin
TSH	Thyroid-stimulating hormone
T3	Triiodothyronine

T4	Thyroxine
T4S	Thyroxine sulfate

Introduction

Iodide is essential for the synthesis of thyroid hormones, and thyroxine (T4) is necessary for normal brain development. The human fetus is critically dependent on a maternal supply of T4, as the fetal hypothalamic–pituitary–thyroid axis only begins to increase in function progressively after 20 weeks gestation (Fisher *et al.*, 1977). If maternal iodine deficiency in pregnancy is severe, irreversible fetal brain damage will occur and cretinism will result, with deafmutism, mental retardation and cerebral palsies (Delange, 2000). Mild and moderate deficiencies of maternal iodine intake are associated with neuropsychointellectual deficits in infants and children (see e.g., Tiwari *et al.*, 1996). Under optimal maternal conditions, the fetus obtains a sufficient iodine supply during gestation to support normal growth and development of the brain.

To sustain brain growth and development during the first two years of life, the infant and child requires a regular and adequate supply of iodide to support appropriate levels of T4. Preterm infants (<37 weeks completed gestation) are particularly vulnerable to iodide deficiency, as they have only a small reserve (of a few days) within the thyroid gland (van den Hove *et al.*, 1999), unlike adults (Delange, 2000) who have a substantial reserve (of several months). Although some iodide is available through the breakdown of thyroxine, most iodide is obtained through exogenous sources, such as enteral and parenteral nutrition; very limited or irregular amounts of iodide are present in some prescribed drugs.

Parenteral nutrition is the delivery of nutrients directly into the circulation, and aims to supply sufficient macronutrients (protein, carbohydrate, and fat) and micronutrients

(vitamins and minerals) with minimal adverse effects. In children, parenteral nutrition not only aims to supply the basic needs of fluid, energy and nutrients, but also must promote growth, especially brain growth, to allow an optimal neurodevelopmental outcome. Parenteral nutrition may provide all the nutritional requirements (total parenteral nutrition), or more usually, it is complemented by some enteral nutrition (partial parenteral nutrition). The most frequent recipients of parenteral nutrition support are infants, particularly extreme preterm infants (<30 weeks gestation) where enteral feeds cannot be established, or in the management of necrotizing enterocolitis, and in other surgical conditions, such as gastroschisis or following extensive bowel resection. The peripheral venous lines used to deliver parenteral nutrition have a limited life span, with consequent interruption of nutrient supply and the risk of extravasation of hypertonic parenteral solutions into superficial tissues, with the consequence of skin sloughing. Since the original description of use by Wilmore and Dudrick (1968), central venous lines have been used frequently to deliver parenteral nutrition. They require to be sited carefully in a large vein to avoid inadvertent malpositioning of the catheter, either in a small tissue vein or within a myocardial muscle bundle where there is the risk of extravasation, which, in the case of the heart, can result in a pericardial effusion and potentially fatal cardiac tamponade. Many of the commercially available central venous catheters are visible on plain X-ray films, but when the catheters have a particularly fine bore, visualization is augmented by the use of iodinated contrast media.

Iodine Metabolism and Requirements

The neonatal requirement for T₄, and hence iodide, is high (Delange *et al.*, 1984), as the calculated intrathyroidal pool of thyroid hormone is sufficient for only 1 day, or less (Vulsma, 1991). Renewal of the intrathyroidal pool of T₄ has therefore, to be very rapid and continuous to allow the preterm infant to maintain a postnatal serum thyroxine supply. This requires an appropriate supply of iodide, but the concentration of iodide and thyroglobulin in the thyroid gland of preterm infants is low (Ertling, 1977; Costa *et al.*, 1986; van den Hove *et al.*, 1991) and does not increase until 42 weeks postmenstrual age (van den Hove *et al.*, 1999). The iodide content of thyroglobulin in the infant thyroid gland appears to be related to maternal iodine status (van den Hove *et al.*, 1999), and thus potential reserves of iodide will be lower in infants of iodine-deficient mothers. Serum levels of T₄ are lower in preterm than in term infants, and this limits the potential supply of recyclable iodide for new hormone synthesis (Van den Hove *et al.*, 1999; Vulsma 1991). Increased T₄ content of the thyroglobulin in the thyroid glands of preterm infants who were prescribed thyroxine (van den Hove *et al.*, 1999),

suggests that iodide derived from exogenous thyroxine can be conserved and reused for iodothyronine synthesis.

Iodide is essential for the synthesis of T₄. Mild and moderate deficiencies of iodide are associated with neurodevelopmental deficits in infants and children (Lombardi *et al.*, 1995; Tiwari *et al.*, 1996). In 1987, the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) recommended an enteral iodide intake of 12 mcg/kg/day (Bremer *et al.*, 1987). However, subsequent iodide balance studies on enterally fed, healthy preterm infants up to 4 months of age concluded that an enteral intake was required of at least 30–40 mcg/kg/day (Ares *et al.*, 1999; Delange *et al.*, 1988). Iodine contents of a number of term and preterm formulae have since been increased (Ares *et al.*, 1999), but further increments in enteral intake to 40–50 mcg/kg/day have no additional beneficial effect on plasma thyroid hormone levels (Rogahn *et al.*, 2000).

Parenteral nutrition requirements of iodine for adults have not been defined (AMA, 1979; National Advisory Group on Standards and Practice Guidelines for Parenteral Nutrition, 1998) and, as a consequence, most of the total parenteral nutrition products for adults contain no iodine (Kelly, 2002).

Parenteral nutrition is routinely used immediately post-delivery in the UK in nearly all extreme preterm infants (<30 weeks completed gestation), and enteral feeds are gradually introduced with the speed dependent on the clinical condition of the infant. For example, parenteral nutrition provided 95% of the caloric intake of 23–27 weeks gestation infants who required intensive-care support on day 7 and 51% at day 28 (Williams *et al.*, 2005). In contrast to the recommended increments in iodine content of infant enteral feeds, the American Society for Clinical Nutrition in 1988 reduced the recommended iodine intake in parenteral nutrition regimens for infants and children to 1 mcg/kg/day (Greene *et al.*, 1988). Clinical exposure to excess iodine was common at that time, through the use of iodinated skin disinfectants (Moukarzel *et al.*, 1992) and exposure to iodinated contrast media for the visualization of central venous catheters. The most recent guidelines (2005) for pediatric parenteral nutrition by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), supported by the European Society of Paediatric Research (ESPR), have retained iodine intake for pediatric parenteral nutrition at 1 mcg/kg/day (Koletzko *et al.*, 2005). Because of the length of time since the origin of these guidelines in the 1980s, and consequent change in clinical practice and limited research evaluation, there needs to be a reassessment and a critical evaluation of parenteral iodine requirements for specific groups, such as extreme preterm infants (Ibrahim *et al.*, 2003), as well as a review of current exposures to iodide in contrast media and disinfectants in these groups of infants and children.

Iodide requirements in extreme preterm infants are particularly difficult to assess as they have very limited thyroidal iodine reserves (Etling, 1977; Costa *et al.*, 1986; van den Hove *et al.*, 1999), and they are also susceptible to iodine toxicity and hypothyroidism if too much iodide is given (L'Allemand *et al.*, 1987). There are no current published studies indicating the iodine requirements of extreme preterm infants, sick infants, or infants in the early neonatal period. Iodide requirements of these infants should therefore be assessed through carefully-controlled studies that avoid toxicity, but also test the efficacy of iodide supplementation in optimizing thyroid function.

In a previous study (Ares *et al.*, 1994), it was highlighted that the majority of a wide range of brand formulas, including those for preterm infants, contained less iodine (range 2–17 mcg/dl) than breast milk (10 mcg/dl), and in some there was a wide variability between manufactured batches of formulas. A more recent survey (Ares *et al.*, 1999) shows that the iodine content of a number of term and preterm formulae has since been increased. It has been argued that a further increase in iodine content of preterm formulae is not justified. For instance, this was the conclusion of a randomized controlled study of preterm infants, where one group received 12–16 mcg/kg/day and the other 40–50 mcg/kg/day, with no differences in subsequent T₄, free thyroxine (FT₄), triiodothyronine (T₃), or thyroid-stimulating hormone (TSH) sera values (Rogahn *et al.*, 2000). This study however, excluded sick, and parenterally fed infants, as the entry requirement at 2 weeks postnatal age was complete enteral feeding. This resulted in only more mature infants being included (mean gestation at birth 30 weeks), which are a group with lower risk for thyroid malfunction. Variances in iodine content of formula feeds, and the limited number of studies, emphasize the need for carefully conducted research to investigate the influences of gestation, postnatal age, intercurrent illnesses, and so on, on iodine homeostasis in preterm infants.

Iodine Balance and Parenterally Fed Preterm Infants

Parenteral fluids and nutritional support are an important part of the early management of most extreme preterm infants in the UK, and it is essential that iodine intake from these sources is sufficient to support brain growth and development.

Only one study (Ibrahim *et al.*, 2003) has reported iodine balances in a cohort of extreme preterm infants who were (initially) parenterally fed. Iodine intakes and urinary iodine outputs were determined for 13 infants over four separate 24h periods: at postnatal day 1, 6, 13 and 27. The types and volumes of all enteral and parenteral nutrition fluids used for each infant were accurately recorded.

All infants were under ≤ 30 weeks gestation (mean gestational age of 27 weeks) and recruited consecutively from the University Teaching Hospital in Dundee, Scotland.

All infants, on admission to the neonatal intensive care unit, were established on parenteral fluids within the first hour of day 1 at 80 ml/kg/day with a solution of electrolytes, dextrose 10%, amino acids (Vaminolact, Fresenius Kabi, Cheshire, UK) and a phosphate supplement (Addiphos, Fresenius Kabi, Cheshire, UK). Fluid intakes were thereafter managed on the basis of clinical requirements. On day 2 of life, and thereafter, the solution was further supplemented with water-soluble vitamins (Solvito N, Fresenius Kabi, Cheshire, UK) and trace elements (Peditrace, Fresenius Kabi, Cheshire, UK), to the levels recommended by the manufacturer. In tandem, a fat emulsion solution (Intralipid 20%, Fresenius Kabi, Cheshire, UK) with added fat-soluble vitamins (Vitlipid, Fresenius Kabi, Cheshire, UK) was infused, initially at 8 ml/kg/day, increasing maximally to 18 ml/kg/day by postnatal day 5. Enteral feeds were started, when the condition of the infant was stable, as hourly boluses of 0.5–1 ml/h. Thereafter enteral feed volumes were gradually increased as determined by the infants' clinical condition, with reciprocal reductions in the volume of parenteral nutrition infused. No infant progressed beyond hourly bolus feeds for the duration of the study.

The volumes and caloric intakes of parenteral and enteral feeding solutions were within the ranges expected for extreme preterm infants (Table 40.1). Parenteral nutrition solutions supplied 100% of the total caloric intake of the group at the day 1 balance period, and thereafter 70% (day 6), 58% (day 13) and 28% (day 27). The iodide content of the components of the parenteral nutrition solutions were estimated at each balance period. Although different batches of both dextrose–electrolyte–amino acid solution and fat emulsion solution were used throughout the study, most had iodide contents within a limited range of values (Table 40.2). In general, the mean iodide content per unit volume was higher in the fat emulsion solutions than in the dextrose–electrolyte–amino acid solutions (Table 40.2). The iodide content of breast milk, red blood cell concentrates and prescribed drugs varied, but were limited (Table 40.3).

All infants were in negative iodine balance on day 1; 12 remained in negative balance at day 6. By day 13, 6 infants were in negative balance, but by day 28 only 3 infants remained in negative balance (Figure 40.1). As a group, mean iodine balance became positive at day 13.

The mean iodine content of expressed breast milk produced by the mothers of the cohort was used to calculate individual iodine intakes for their infant. The mean value was 10.1 mcg/dl, with a wide range of individual iodine contents from 4 to 28 mcg/dl. It is possible that expressed breast milk with low iodide content came from mothers who themselves were iodine deficient. Pilot observations

Table 40.1 The types and volumes of enteral and parenteral nutrition fluids at balance days (Ibrahim *et al.*, 2003)

Postnatal age (days)	Parenteral nutrition		Enteral nutrition		Total nutrition	
	Mean [range] (ml/kg/day)	Mean [range] (kcal/kg/day)	Mean [range] (ml/kg/day)	Mean [range] (kcal/kg/day)	Mean [range] (ml/kg/day)	Mean [range] (kcal/kg/day)
Day 1	79 [56–96]	31 [24–38]	–	–	79 [56–96]	31 [24–38]
Day 6	110 [7–173]	63 [3–104]	42 [0–189]	27 [0–122]	152 [95–209]	91 [53–125]
Day 13	98 [0–152]	51 [0–93]	56 [0–204]	37 [0–133]	154 [101–204]	88 [56–133]
Day 27	65 [0–185]	28 [0–104]	101 [0–196]	65 [0–147]	166 [31–196]	93 [39–147]

Source: Permission from Ibrahim *et al.*, (2003).

Table 40.2 Iodine content of parenteral fluids on 4 days within the first month of life

	Number of infants	Mean (μg iodine/dl)	Range (μg iodine/dl)
Dextrose–electrolyte–amino acid solutions			
Day 1	16	1.2	0.4–3.6
Day 6	16	1.3	0.4–5.0
Day 13	14	2.1	0.4–11.2
Day 27	5	1.2	0.8–1.6
Fat emulsion solutions			
Day 6	15	4.4	1.0–12.0
Day 13	11	3.8	1.0–10.0
Day 27	3	4.0	2.0–8.0

Note: The dextrose–electrolyte–amino acid solution used Vaminolact (amino acids), Addiphos (phosphate), and from day 2, Solvito N (water-soluble vitamins), and Peditrace (trace elements). The fat emulsion solution used was Intralipid 20% with Vitlipid (fat-soluble vitamins). Fresenius Kabi, Cheshire, UK supplied all named products.

Source: Modified from Ibrahim *et al.*, (2003).

from pregnant women in this population (Tayside, Scotland) show that about 40% of pregnant women have a urinary iodine concentration that indicates a daily intake below 125 mcg per day (Barnett *et al.*, 2002), which is half the 250–300 mcg/day intake recommended in pregnancy and 225–350 mcg/day recommended during lactation (Ares *et al.*, 2005; Delange, 2004). The formula used in the study (Nutriprem I, Cow and Gate, UK) contained 21 mcg iodine/dl, which provides an iodine intake of around 30 mcg/kg/day based on an average intake of 150 ml/kg/day. Enteral iodine absorption from formula feeds appears to be at least 90%, based on stool iodine content and urinary iodine output (Ares *et al.*, 1997; Delange *et al.*, 1988). These levels for iodide absorption from formula feeds are high, even allowing for the general tendency of balance studies to overestimate (Heroux and Peter, 1975). We are not aware of similar studies using expressed breast milk, and the bioavailability of iodine in human milk may be different. Clearly, such studies are now required, especially if we are already feeding some of our preterm infants expressed breast milk with suboptimal iodine contents.

Table 40.3 Iodine content of expressed breast milk, red blood cell concentrates and selected drugs

Product	Mean iodine ($\mu\text{g}/\text{dl}$)	Range ($\mu\text{g}/\text{dl}$)	N
Cow & Gate, Nutriprem I Formula milk	20.2	15–24	5
Expressed breast milk	10.1	4–28	13
Red blood cell concentrates	7.1	4–10	10
Frozen plasma	0.7		1
Abidec (Warner Lambert)	8.0		1
Benzylpenicillin (Biochemie GmbH)	9.6		5
Caffeine (Tayside Pharmaceutical)	10.2		6
Ceftazidime (Glaxo Wellcome)	10.0		1
Dopamine (Abbot)	10.0		1
Furosemide (Martindale Pharmaceuticals)	7.5		2
Gentamicin (Hoechst Marion Rousssel)	11.3		4
Indomethacin (Merck Sharp & Dohme)	10.0		1
Insulin (Novo Nordisk)	9.0		1
Sytron (Link Pharmaceuticals)	1.0		1
Midazolam (Roche Products Ltd.)	13.0		1
Vancomycin (Faulding)	7.0		1
Hepsal (CP Pharmaceuticals)	3.5	0.8–10.4	8

Source: Modified from Ibrahim *et al.*, (2003).

Total parenteral nutrition at 150 ml/kg/day, with no enteral component, supplied the infants with a mean iodine intake of only 3 mcg/kg/day. The recommended enteral intake of iodide for preterm infants, based on balance studies, is 30 mcg/kg/day (Ares *et al.*, 1999; Delange *et al.*, 1988). Because (even in preterm infants) iodide absorption by the gastrointestinal tract is high, there should be a near equivalence of dosage by the enteral and parenteral routes to prevent iodine deficiency in infants totally parenterally fed by current standard regimens.

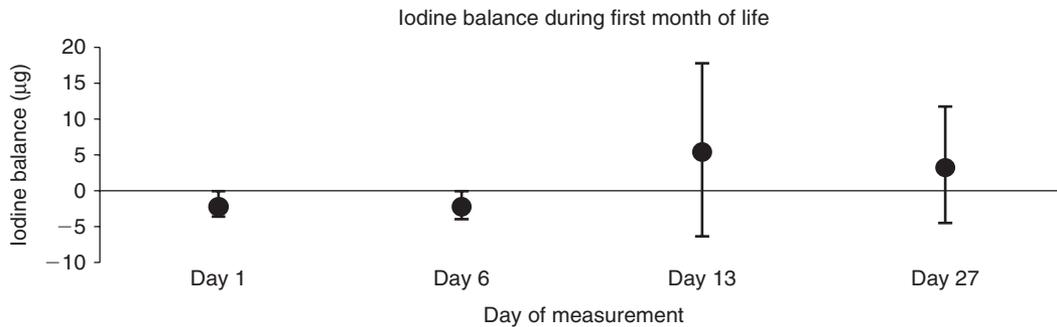


Figure 40.1 Iodine balances (mean and standard deviation) in preterm infants during the first 27 days of life. Modified from Ibrahim *et al.*, (2003).

Iodine Insufficiency

Is iodine insufficiency of current parenteral nutrition regimens potentially compromising the brain development of extreme and preterm infants when it is their only, or predominant, source of nutrition? The determination of the iodide requirements of extreme preterm infants, and the safe levels of iodide supplementation necessary to achieve optimal blood levels, requires carefully piloted and designed clinical trials so that iodine toxicity is avoided. We have started this program of work (Ibrahim *et al.*, 2003) and extended our knowledge through three further pilot studies in infants <30 weeks gestation.

Pilot studies

We piloted a randomized controlled trial of parenteral iodide supplementation on 17 infants, with iodide balance studies and iodothyronine measurements at postnatal days 1, 7, 14 and 28. Iodide intake was calculated daily to allow iodide supplementation to 30 mcg/kg/day. Under this regimen, supplemented infants were in positive iodide balance at all study times. It was very time-consuming for the nursing staff to calculate these levels on a daily basis for each infant. This step was necessary, however, to ensure that no infant was exposed to excess iodide and potential toxicity.

In the subsequent pilot study, we designed a simplified randomized controlled trial. Ten infants ≤ 30 weeks gestation were given a standard parenteral iodide supplementation of 30 mcg/kg/day, in addition to the iodide contained in their other routine enteral and parenteral solutions, including drugs. The maximum intake recorded by any infant in this study was 76 mcg/kg/day, which is well under the 100 mcg/kg/day recommended daily maximum allowance of iodide for infants (Delange *et al.*, 1988). We pooled data from these two pilots and from the work published earlier (Ibrahim *et al.*, 2003), and calculated mean urinary iodide excretions. Nonsupplemented infants excreted 64 mcg iodide/l compared to 269 mcg iodide/l in the iodide-supplemented group ($p < 0.006$).

In a final pilot study, we are streamlining the procedural elements of a randomized controlled trial that needs to be multicentered to achieve sufficient power to answer the effect of iodide supplementation on thyroid hormone levels and neurodevelopmental outcome.

Cochrane Reviews

There is no systematic review of iodide supplementation of parenteral or enteral nutrition for neonates. A recent Cochrane review of iodide supplementation for the prevention of mortality and adverse neurodevelopmental outcome in preterm infants found no eligible trials of iodide supplementation in parenterally fed infants (Ibrahim *et al.*, 2006). We found four papers that reported enteral iodide supplementation of neonates (Ares *et al.*, 1997, also Ares *et al.*, 1999; Ibrahim *et al.*, 2003; Delange *et al.*, 1988; Rogahn *et al.*, 2000), which have been incorporated into the preceding discussion.

Iodine Excess

The developing thyroid is inordinately sensitive to the inhibitory effect of excess iodide – the Wolff–Chaikoff effect (Wolff and Chaikoff, 1948) with the subsequent development of goiter and/or hypothyroidism (Roti and Vagenakis, 2000). Studies since the 1970s have highlighted that direct iodide overload of the newborn is caused by either skin disinfection agents or the use of iodinated contrast media for radiological examinations (e.g., Chabrolle and Rossier, 1978; Bühler *et al.*, 1973). Preterm infants are substantially more sensitive to the effects of iodine overload than term infants or adults (L'Allemand *et al.*, 1987; Delange *et al.*, 1984; Smerdely *et al.*, 1989). The fetus and newborn can also be exposed to high maternal iodine concentrations, either prenatally via transplacental transfer or postnatally through breast milk (Carswell *et al.*, 1970; Chanoine *et al.*, 1988). Iatrogenic iodine overload in fetal and neonatal life can result in transient hypothyroidism or hyperthyrotropinemia (Grüters *et al.*, 1983).

The daily enteral requirement of iodine for preterm infants is 30 mcg/kg/day (Ares *et al.*, 1997; Delange *et al.*, 1988), with no detrimental effects seen with intakes of 40 mcg/kg/day in preterm infants (Rogahn *et al.*, 2000). Infants may be exposed to very much higher and excess iodine intakes through the use of iodinated contrast media or iodinated skin disinfectants, through breast milk of mothers who were exposed to iodine during labor or cesarean section, or through vaginal disinfection (e.g., Weber *et al.*, 1998; Vorherr *et al.*, 1980; Novaes *et al.*, 1994).

Iodinated Contrast Media and Central Venous Catheters

Iodinated contrast media is the major source of excess iodide, as it is given in relatively high doses and may be repeated several times. Commercially available nonionic contrast media contain variable amounts of bound iodide (biologically inactive), but can liberate variable amounts of biologically active free iodide (Dembinski *et al.*, 2000). A single dose of contrast media, for example, Omnipaque, may expose an infant to up to 243 mcg/kg of free iodide in a single dose (L'Allemand *et al.*, 1987). The half life of iodide after a single dose of iodinated contrast media is 13.5 days in preterm infants (Ares *et al.*, 1995), and this explains why just one exposure (e.g., from contrast media or through breast milk in an exposed mother) can cause a prolonged effect on thyroid function.

Iodinated contrast media is used to visualize the placement of central venous catheters. Such catheters are widely used and essential for the delivery of parenteral nutrition, particularly to extreme preterm infants, i.e., the majority of infants <30 weeks gestation. Pericardial effusions are a serious complication arising from wrongly positioned catheters, and in 2001 the UK Department of Health Report, following a coroner's inquest into four deaths resulting from the cardiac tamponade caused by a misplaced catheter, recommended that the ideal position for the catheter tip "should be sited outwith the cardiac chamber" (DoH, 2001). Catheters are radiopaque, but accurate localization of the tip can be problematic on plain X-rays, especially those without a guide wire, or those of narrow gauge (e.g., Premicath 27G, Vygon, Cirencester, UK). Catheter tip positions can be more accurately localized on plain X-rays if they are injected with iodinated contrast media during film exposure; this method, although subject to interobserver variability, remains the "gold standard," as digital imaging remains equivocal, and color Doppler ultrasonography in neonatal units is a novelty. The implementation of X-ray image digitizing and manipulation may allow a more accurate placement of central venous catheters and requires wide application. The mechanical complication rate due to misplaced central venous catheters is reduced from 1.8 to 0.05% following visualization using iodinated contrast media (Pezzati *et al.*, 2004; Cartwright, 2004).

The list of recognized mechanical complications of wrongly positioned central venous catheters is largely based on individual case reports, with limited audits and no comparative studies. The most complete audit is a review of 2186 catheters in Brisbane and Women's Hospital from 1984 to 2002 (Cartwright, 2004). Iodinated contrast media was used to augment localization of the position of radiopaque and nonradiopaque catheters in plain X-rays with only one occurrence (0.05%) of a significant consequence of a wrongly positioned catheter. A smaller retrospective study of 258 central venous catheters with plain X-ray for localization, but without the use of iodinated contrast media, showed cardiac tamponade as the only complication in 1.8% of catheters (Pezzati *et al.*, 2004).

Iodinated Skin Disinfectants

Iodide-containing skin disinfectants also present a risk of iodide excess for infants. Maternal skin disinfection before cesarean section or vaginal disinfection prior to forceps delivery potentially exposes the infant to excess iodide, and because of the long half life, such exposure is exacerbated if the mother then breast-feeds her infant (McElduff *et al.*, 2005; Chan *et al.*, 2003; Vorherr *et al.*, 1980). If iodine-containing skin disinfectants are used with infants, especially preterm infants, there is again a risk of excess iodide exposure. There are alternatives, however, to the use of iodinated skin disinfectants, for example, chlorhexidine gluconate. In adults, a meta-analysis (Chaiyakunapruk *et al.*, 2002) reported an incidence of bloodstream infections with intravenous catheters of 1% following disinfection by chlorhexidine gluconate, compared to 2% with povidone-iodine (relative risk: 0.49, 95%CI: 0.28–0.88). Chlorhexidine gluconate was also more effective than povidone-iodine in reducing the number of positive blood cultures (38% vs. 79%) following elective surgery to the ankle or foot (Bibbo *et al.*, 2005), and in reducing blood culture contamination following phlebotomy (Mimoz *et al.*, 1999). In children, chlorhexidine gluconate is also effective in reducing bacterial colonization following skin disinfection prior to catheter placement: from 5.6 to 0.9 per 100 catheter days (Kinirons *et al.*, 2001) and from 9.3 to 4.7% (Garland *et al.*, 1995). Although it is an effective neonatal skin disinfectant, some neonatal units are reluctant to use chlorhexidine gluconate because the typical preparation is alcohol based and, if it is allowed to pool on the infants' skin, it may cause burns, a problem that is worsened by the extreme fragility of the skin of extreme preterm infants. Careful practice can minimize the risk of burns, or units could use aqueous chlorhexidine solutions with a preference for 0.5% over 0.05% (Lilley *et al.*, 2006). Chlorhexidine is a clear alternative to povidone-iodine. This position is supported by guideline recommendations of the British National Formulary for Children (British

National Formulary for Children, 2005). Povidone-iodine should be avoided in pregnancy for skin and vaginal disinfection, and also during breast-feeding. It is contraindicated for preterm infants under 32 weeks gestation; for infants whose body weight is under 1.5 kg; or for regular use in neonates (Weber *et al.*, 1998; Linder *et al.*, 1997; British National Formulary for Children, 2005).

Iodinated Contrast Media Exposure and Transient Hypothyroidism

In countries with established neonatal screening programs, all infants are screened for congenital hypothyroidism in the first week of life. In the UK, TSH is measured on the Guthrie card at postnatal days 5 to 6; if TSH levels are elevated, the infant is recalled immediately and plasma TSH and FT4 are measured. If TSH levels remain persistently high and FT4 levels low, the infant is diagnosed with permanent congenital hypothyroidism (incidence approximately 1:3500) with a need for lifelong thyroxine therapy. Transient hypothyroidism is defined by high TSH levels and low T4 levels after birth, and is treated with thyroxine therapy until resolution that may take up to 4 months (Köhler *et al.*, 1996; Larson *et al.*, 2003). Transient hyperthyrotropinemia is defined by elevated TSH levels, but normal T4 levels, and most resolve spontaneously without treatment (Köhler *et al.*, 1996).

The studies, to date, of iodide toxicity in preterm infants exposed to iodinated contrast media are limited in number, with small cohorts and restricted information on confounders; this is reflected in the wide range, 8–75%, of incidence of transient hypothyroidism. In the first study, a cohort of 13 preterm infants had features of transient hypothyroidism (Ares *et al.*, 1995). In the second study, a cohort of 12 infants had elevated TSH levels, but normal FT4 levels (Parravicini *et al.*, 1996); it is difficult to judge whether this was hyperthyrotropinemia alone or transient hypothyroidism, as T4 levels were not measured and FT4 levels can be exceptionally difficult to interpret in these infants (Williams *et al.*, 2004). In the third study six out of eight preterm infants had elevated TSH levels, with six suboptimal T4 levels (L'Allemand *et al.*, 1987), indicating the majority had transient hypothyroidism.

These studies suggest that transient hypothyroidism is common in preterm infants exposed to iodinated contrast media, and more frequent than complications arising from wrongly-positioned central venous catheters. We are unaware of any ongoing work in this area (e.g., there are none registered with National Research Register UK or the National Institute of Health USA). Because of the potential for permanent neurodevelopmental compromise in infants exposed to iodide toxicity, we believe that it is important to establish the true incidence of transient hypothyroidism associated with exposure to iodinated contrast media.

UK Use of Iodine in Obstetric and Neonatal Units

We surveyed 42 of the largest UK neonatal intensive care units in March 2006. The units were asked to describe the use of iodide in the obstetric and neonatal units. The majority (76%) used iodinated contrast media to visualize the placement of long lines, of which 36% used it on every occasion. Povidone-iodine was used for disinfection in 57% of the obstetric units for cesarean section and vaginal forceps delivery, and in 21% of the neonatal intensive care units. Infants are variably exposed to iodide during surgery or Gastrografin investigations, but these data are not routinely recorded by the units.

We asked the neonatal intensive care units whether they were prepared to change clinical practice based on current evidence, which is summarized on our web site (www.euthyroid.org). None of the units were prepared to change at that time, but all were anxious that we investigate the problem with a prospective study, and all agreed to support us by joining the project. The unwillingness to change was prompted by the lack of power in the previous studies, and the fear of causing a serious adverse event by misplacement of a long line. This attitude has been reinforced by the coroner's inquiry into four infant deaths secondary to cardiac tamponade (DoH, 2001). It is accepted by pediatricians that in term infants there is substantive evidence that congenital hypothyroidism is associated with a poor neurodevelopmental outcome if thyroxine substitution is not given soon after birth and in adequate amounts (e.g., Rovet, 2005; Bongers-Schokking *et al.*, 2000; Salerno *et al.*, 2002; Simoneau-Roy *et al.*, 2004). It is not known to what extent preterm infants may be affected by transient hypothyroidism, but it may last for several weeks (Köhler *et al.*, 1996; Larson *et al.*, 2003) during which time potential irreversible brain damage may occur. It is likely that the low serum levels of T4 as a result of iatrogenically induced transient hypothyroidism will have the same detrimental impact on neurodevelopment as the low levels of T4 resulting from transient hypothyroxinemia in preterm infants (Meijer *et al.*, 1992; den Ouden *et al.*, 1996; Lucas *et al.*, 1996).

For all intrapartum and postpartum uses of iodine, there are effective alternatives; and this is evidenced in Scotland's neonatal units, which have been largely free of excess iodine exposure from skin disinfectants and iodinated contrast media since the mid-1980s.

Brain Development and Thyroxine

Cerebral damage and neurodisability are common among extreme preterm infants. There are about 7000 such infants born per annum in the UK; 25% will have neurological abnormalities, 9% visual and 11% hearing impairments

(Bhutta *et al.*, 2002). The group as a whole has a reduction in IQ of 10 points (Bhutta *et al.*, 2002) and 50% will require specialist school support.

The etiology of brain damage and neurodisability in extreme preterm infants is multifactorial, and is associated with: intraventricular hemorrhage, periventricular leucomalacia, perinatal infection, hypoglycemia, hyperbilirubinemia, malnutrition, postnatal dexamethasone, and transient hypothyroxinemia, in addition to parental genetic endowment and lifestyle factors.

Thyroxine is essential for normal development of the human brain *in utero* and for the first 2 years after birth. Damage through deficiency of T₄ is irreversible, and at the extremes of deficiency, this results in cretinism. Transient hypothyroxinemia in preterm infants is common, evident in 41% of infants <28 weeks gestation and in 23% of infants 28–30 weeks gestation (Williams *et al.*, 2005), using one standard deviation below the mean as the criterion. Studies have linked low plasma T₄ in preterm infants with later deficits in motor and cognitive function (e.g., Reuss *et al.*, 1996; den Ouden *et al.*, 1996). The etiology of transient hypothyroxinemia is not clear and may have contributions from the withdrawal of maternal–placental thyroxine transfer (Vulsma *et al.*, 1989), hypothalamic–pituitary–thyroid immaturity (Murphy *et al.*, 2004), developmental constraints on the synthesis (Williams *et al.*, 2004; Thorpe-Beeston *et al.*, 1991; Hume *et al.*, 2004) and peripheral metabolism of iodothyronines (Pavelka *et al.*, 1997; Kester *et al.*, 2004; Richard *et al.*, 1998), iodine deficiency (Ares *et al.*, 1997; Ibrahim *et al.*, 2003), and nonthyroidal illness (e.g., Pavelka *et al.*, 1997; van Wassenaer *et al.*, 1997a; Simpson *et al.*, 2005).

Transient Hypothyroxinemia and Preventative Strategies

Universal thyroxine supplementation in extreme preterm infants does not improve neurodevelopmental outcome (van Wassenaer *et al.*, 1997a). However, subgroup analysis of the developmental scores of infants <27 weeks gestation in the thyroxine group, compared to placebo, were increased by 18 points, but decreased by 10 points in infants of 27–29 weeks. Thyroxine supplementation may be detrimental in some infants and there are no indications, apart from gestation, why this should be so. With this uncertainty, we believe that the correction of iodine deficiency is the first and safest approach to the correction of hypothyroxinemia.

Previous studies have used various combinations of T₄, T₃ and TSH levels at various postnatal days to define hypothyroxinemia. None of these definitions use serum levels adjusted for gestational age; this is crucial, as there are major developmental changes in cord and postnatal serum levels from 23 to 30 weeks gestation (Williams

et al., 2004; Hume *et al.*, 2004). To solve this problem, we describe postnatal T₄ values, equivalent for gestational age, based on cord levels that provide quantification of hypothyroxinemia for any postnatal value of T₄ up to the equivalent of term. An analysis of our data confirms that hypothyroxinemia is largely confined to infants ≤30 weeks gestation, which confirms other studies (Reuss *et al.*, 1996; van Wassenaer *et al.*, 1997b).

A wide range of serum thyroid hormones (T₄, FT₄, T₃, reverse triiodothyronine (rT₃), thyroxine sulfate (T₄S)), TSH and thyroid binding globulin (TBG) were analyzed in our studies; T₄, but not FT₄ levels were indicative of hypothyroxinemia (Williams *et al.*, 2004; Simpson *et al.*, 2005). In 23–27 week gestation infants, T₄ serum levels decrease to a nadir at 7 postnatal days and fail to increase in 28–30 week infants. This is in marked contrast to 31–34 and 37–42 week term infants, who show an increase in serum T₄ levels at day 7 (Williams *et al.*, 2004; Simpson *et al.*, 2005).

A major determinant of postnatal T₄ levels is nonthyroidal illness (Simpson *et al.*, 2005); 75% of infants <28 weeks gestation are characterized as severely ill, as defined by the British Association of Perinatal Medicine (BAPM), level 1 (Simpson *et al.*, 2005), whereas only 15% of infants 28–30 weeks gestation are so categorized.

Our research (e.g., Williams *et al.*, 2004, 2005; Murphy *et al.*, 2004; Hume *et al.*, 2004; Richard *et al.*, 1998; Ibrahim *et al.*, 2003; Simpson *et al.*, 2005) indicates that there are three potentially modifiable factors contributing to transient hypothyroxinemia: infection and other illnesses of prematurity, certain drugs and iodine insufficiency of parenteral nutrition. Of the three factors, primary correction of iodide deficiency is essential to allow physiologically appropriate responses before correction of other modifiable factors. As universal T₄ supplementation is detrimental in some groups (van Wassenaer *et al.*, 1997b), it is not until the modifiable factors have been corrected or minimized that a trial of T₄ supplementation is warranted in extreme preterms, and then only in those who are biochemically hypothyroxinemic. The necessary pilot work has been completed to allow a UK multicenter randomized controlled trial of iodide supplementation to take place safely.

Summary Points

- Thyroxine is critical for the developing human brain and it is likely to be compromised whether serum T₄ levels are reduced through transient hypothyroxinemia, with a contribution from iodine deficiency, or from transient hypothyroidism caused by iodine excess.
- In the only study that has investigated total parenteral nutrition in infants, intakes of 150 ml/kg/day, with no enteral component, supplied a mean iodide intake of

only 3 mcg/kg/day. The recommended enteral intake of iodide for preterm infants based on balance studies is 30 mcg/kg/day. Because (even in preterm infants) iodide absorption by the gastrointestinal tract is high, there should be a near equivalence of dosage by the enteral and parenteral routes to prevent iodine deficiency in infants totally parenterally fed by current standard regimens.

- Iodide requirements of sick and preterm infants should be assessed through carefully controlled studies that avoid iodide toxicity, but also test the efficacy of supplementation in optimizing thyroid function.
- Variances in iodine content of formula feeds, and the limited number of studies, emphasize the need for carefully-conducted research to investigate the influences of gestation, postnatal age, intercurrent illnesses, and so on, on iodine homeostasis in preterm infants.
- There is unexplained variation in levels of iodide in expressed breast milk, which may mean that some preterm infants have suboptimal iodine intake; and the bioavailability of iodide in human milk may be different from that of infant formula. Further studies are now required.
- Povidone-iodine should be avoided in pregnancy for skin and vaginal disinfection and also during breastfeeding. It is contraindicated for preterm infants under 32 weeks gestation; for infants whose body weight is under 1.5 kg; or for regular use in neonates.
- There are three potentially modifiable factors contributing to transient hypothyroxinemia: reduction of infection and other illnesses of prematurity, substitution of drugs that affect thyroid function, and iodine insufficiency of parenteral nutrition.
- If trials of iodide supplementation of parenteral nutrition show that supplementation improves neurodevelopmental outcome, there will be immediate implications for clinical management for neonates and children up to 15 kg (3–4 years of age).

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Iodine Content of Standard Infant Formula and Specialized Enteral Preparations

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Abstract

The aim of this chapter is to describe iodine intakes based on the calculated amounts for standard infant formulas available in the UK, and for all proprietary enteral preparations that can be used as standalone feeds for complete nutrition. There is appreciable variability in the iodine contents of both infant formula and specialized enteral preparations; with some providing well below and others providing well above the recommended daily allowances. No standard formula for term infants provides the recommended iodine intake of 90 µg/day at birth. Recommendations are met at 2–15 weeks, and thereafter are exceeded by 19–100%. Some formulas for preterm infants meet the criteria of an iodine intake of 30 µg/kg/day at birth and subsequently in the post-natal period. Pregnant and lactating women are vulnerable to iodine deficiency if specialized enteral preparations are their sole source of nutrition. Only 9 out of 44 specialized enteral preparations for adults provide the current requirement of 200 µg/kg day. If the WHO technical consultation presently under review is accepted, then only two preparations will meet the proposed allowance of 250 µg/day. This has particular consequences for the brain development of the fetus and infant if the mother is iodine-deficient during pregnancy and if she breast-feeds. Because of the variability of iodine content in specialized enteral preparations, the iodine status of individuals should be monitored with prolonged use of these products, with regular assessment of goiter, urinary iodine content and thyroid function tests. Such monitoring is also appropriate if specialized enteral preparations are only part of the total nutritional intake, particularly if protein- and iodine-rich foods are restricted.

Abbreviations

WHO	World Health Organisation
MRC	Medical Research Council
USA	United States of America

Introduction

The aim of this chapter is to describe iodine intakes based on the calculated amounts for standard infant formulas available in the UK, and for all proprietary enteral preparations that can be used as standalone feeds for complete nutrition. The proprietary preparations highlighted in this review are those that are listed in the British National Formulary, and the corresponding version for Children 2006 ([British National Formulary for Children, 2006](#)) mostly in the section Appendix A2 Borderline Substances; excluding those which we know have been discontinued (from 2007). Enteral supplements which are not the sole source of nutrition have not been included, as potential iodine intake will be variable and dependent on the proportion of enteral supplement consumed, as well as the amount and iodine content of nonsupplemented food.

Total protein and amino acid intakes were taken from current UK practice for the management of disorders of amino acid metabolism, as the majority of the specialized formulas are meant for these conditions ([Table 41.1](#)) ([Report of MRC Recommendations, 1993](#); [Dixon et al., 2001](#)). Recommendations of amino acid intake for teenagers are not available and, to maintain consistency, protein intakes are used throughout this chapter. Recommendations for daily iodine intake vary with age ([Table 41.2](#)) and are taken from the [WHO \(1996\)](#), while for preterm infants they are

Table 41.1 UK guidelines for amino-acid and protein intakes

Age (years)	Total protein (g/kg body weight/day)	Amino acids (g/kg body weight/day)
0–2	3.0	3.0
3–5	2.5	2.0
6–10	2.0	2.0
11–14	1.5	
>14	1.0	

Source: [Report of Medical Research Council on dietary management on phenylketonuria \(1993\)](#); [Dixon et al., \(2001\)](#).

Table 41.2 Current guidelines for daily iodine intake

Age (years)	Iodine ($\mu\text{g}/\text{day}$)
ICCIDD/WHO/UNICEF consensus ^a	
Birth–7 years	90
7–12 years	120
12+ years	150
Pregnant and lactating women	200
US National Academy of Sciences ^b	
0–6 months (AI)	110
7–12 months (AI)	130
1–8 years (RDA)	90
9–13 years (RDA)	120
14+ (RDA)	150
Pregnancy (RDA)	220
Lactation (RDA)	290

Note: AI, adequate intake; RDA, recommended daily allowance.

^a<http://www.iccidd.org/pages/iodine-deficiency.php>

^bhttp://books.nap.edu/openbook.php?record_id=10026&page=272.

from Delange *et al.* (1988) and Ares *et al.* (1997). The current recommendations for pregnant and lactating women are 200 $\mu\text{g}/\text{day}$ (WHO, 1996); it is likely that these will be increased to 250 $\mu\text{g}/\text{day}$ in the future, following a technical consultation by the WHO.

The daily protein intake per kg recommended for each age (Table 41.1) was used to calculate the total protein for a representative weight appropriate to the age (Child Growth Foundation, 1996); the derived amount of protein was then used to calculate the daily intake of iodine. The protein and iodine contents, and the specified recommended age range and indications for use of the enteral preparations, were taken from the manufacturer's literature. Readers should note that the temporal recommendations for daily iodine intake from birth through childhood, adolescence and into adulthood do not coincide chronologically with recommendations made for amino acid or protein intakes (Tables 41.1 and 41.2).

Weights of infants, children, adolescents and young adults up to 20 years were taken from growth charts (Child Growth Foundation, 1996); examples of weights were taken with age approximated to the 50th centile.

For the interpretation of the enteral iodine intakes given in Tables 41.3–41.12 we have used the most recent compilation of recommended intakes at the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) web site (<http://www.iccidd.org/pages/iodine-deficiency.php> 2007) which is a consensus of the opinions of the ICCIDD, the WHO and the United Nations Children's Fund (UNICEF; Table 41.2). The resultant intakes are: 150 μg for adolescents (12+ years) and adults, 200 $\mu\text{g}/\text{day}$ for pregnant and lactating women, 120 $\mu\text{g}/\text{day}$ for children aged 7–12 years and 90 $\mu\text{g}/\text{day}$ for birth to 7 years. Preterm infants are likely to be treated as a special group, and current evidence suggests that 30–40 $\mu\text{g}/\text{kg}/\text{day}$

is required by them to maintain a positive iodine balance (Ares *et al.*, 1997; Delange *et al.*, 1988).

The US National Academy of Sciences make different recommendations (http://books.nap.edu/openbook.php?record_id=10026&page=272). In the absence of evidence, the intakes that have been estimated and recommended from birth to 1 year are “adequate intakes.” The intakes recommended for children, adults, pregnant and lactating women vary slightly from those of the ICCIDD/WHO/UNICEF (Table 41.2).

Standard Formula for Term and Preterm Infants

No formula provides the recommended iodine intake of 90 $\mu\text{g}/\text{day}$ at birth for term infants (Table 41.3). Recommendations are met at 2–15 weeks, and thereafter are exceeded by 19–100%. Although manufacturers recommend that the formula may be taken until 1 year of age, we calculated intakes at an upper age of 6 months, as this is the age by which infants are recommended to be weaned.

There are two categories of formulas for preterm infants. The first category has formulas that are indicated for preterm infants from birth until they gain a weight of 2 kg, or are ready to be discharged home. Weights of 0.5 kg and 2 kg have been taken as exemplar weights, as these are the approximate extremes for these types of formulas. Two of the formulas (Nutriprem 1 Low Birthweight Formula and Milupa Pre-Aptamil) meet the criteria of an iodine intake of 30 $\mu\text{g}/\text{kg}/\text{day}$ (Ares *et al.*, 1997; Delange *et al.*, 1988) over the weight range (Table 41.3). SMA Gold Prem has approximately half the recommended amount.

The second category of formula is for preterm infants who have reached 2 kg in weight, but still require a specialized feed. Nutriprem 2 is the only formula in this category (Table 41.3). At a corrected age of 6 months, assuming a weight around the 50th percentile of 7.5 kg, the corresponding iodine intake is 225 $\mu\text{g}/\text{day}$ (the equivalent of 30 $\mu\text{g}/\text{kg}/\text{day}$). Had this infant been born at term, the iodine recommendation at 6 months of age would have been 90 $\mu\text{g}/\text{day}$, equivalent to 12 $\mu\text{g}/\text{kg}/\text{day}$. The issue of estimating requirements on a per kilo basis to a recommended daily intake as a preterm infant progresses towards term and beyond has not been resolved.

Specialized Feeds for Phenylketonuria

Phenylketonuria is an inherited disorder caused by the deficient function of the enzyme phenylalanine hydroxylase; this results in increased phenylalanine levels, which damage the developing brain and result in mental retardation. The aim of dietary management is to maintain a normal plasma phenylalanine level by restricting the routine

Table 41.3 Standard formula for term and preterm infants

<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (3.5 kg infant at birth) µg per day</i>	<i>Iodine intake (7.5 kg child at 6 months) µg per day</i>
TERM INFANTS					
Comfort first infant formula ^a	Birth–6 months	11.5	79	72	155
Organic first milk ^a	Birth–6 months	10.7	51	50	107
Cow & Gate Plus ^a	Birth–6 months	12.5	100	84	180
Cow & Gate Premium ^a	Birth–6 months	10.4	77	78	167
Milupa Aptamil First ^b	Birth–6 months	10.2	72	74	159
Milupa Aptamil Extra ^b	Birth–6 months	10.9	63	61	130
SMA Gold ^c	Birth–6 months	11.0	79	75	162
SMA White ^c	Birth–6 months	12.6	79	66	141
SMA Staydown ^c	Birth–6 months	12.4	78	66	142
<i>Product name</i>	<i>Age Indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (0.5 kg infant at birth) µg per day</i>	<i>Iodine intake (2.0 kg infant) per day</i>
PRETERM INFANTS					
Nutriprem 1 Low birthweight formula ^a	Preterm birth to 2 kg weight	(2.5)	(25)	15	60
Milupa Pre-Aptamil ^b	Preterm birth to 2 kg weight	(2.5)	(25)	15	60
SMA Gold Prem ^c	Preterm birth to 2 kg weight	(2.2)	(10)	7	27
<i>Product name</i>	<i>Age Indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (2.0 kg infant) µg per day</i>	<i>Iodine intake (7.5 kg infant) µg per day</i>
PRETERM INFANTS					
Nutriprem 2 ^a	2.0kg to 6 months corrected age	(2)	(20)	60	225

^aCow & Gate, Wiltshire, UK.^bMilupa, Trowbridge, UK.^cSMA Nutrition, Berkshire, UK.

dietary sources of phenylalanine, i.e. protein. Patients are given proprietary amino acid preparations, which are phenylalanine-free, and carefully controlled amounts of natural proteins to supply some necessary phenylalanine. The amounts of natural protein, and hence phenylalanine intake, that a patient can tolerate varies depending on the severity of their enzyme deficiency; in the UK, this is managed by means of a dietary exchange system. Occasional patients with the severest phenotype and lowest phenylalanine hydroxylase activity will be allowed no natural protein in their diet and will be reliant to a large extent on the phenylalanine-free amino acid preparation for adequate amounts of minerals and vitamins. The illustrative calculations of iodine intake in this chapter for phenylketonuric patients are based on this severe phenotype. Patients with less severe phenotypes can variably consume natural protein, although amounts are still severely limited. Foods with high or moderate iodine content such as shellfish,

sea-fish, milk, eggs and meat are also high in natural protein and phenylalanine content, severely limiting access to such sources in most phenylketonuric patients; this limited access renders the phenylketonuric susceptible to iodine deficiency. Foods with low protein content, such as fruits and vegetables, which in most instances are freely available to phenylketonuric patients, are also low in iodine content. Most of the salt used in the UK is noniodized. Given these constraints on the diet, it is possible that phenylketonuric patients who are more reliant on the proprietary phenylalanine amino acid preparations, which are supplemented with iodine, are also more likely to achieve recommended iodine intakes.

In all proprietary products, with the exception of the Phlexy-10 system, nonessential and essential amino acids are supplemented with minerals and vitamins directly by the manufacturer; iodine intake consequently varies according to amino acid or protein intake.

Table 41.4 Phenylalanine-free dietary preparations for the management of phenylketonuria

Product name	Age indications	Protein g/100g powder (or 100ml)	Iodine µg per 100g powder (or 100ml)	Iodine intake (3.5 kg infant at birth) µg per day	Iodine intake (10 kg infant at 1 year) µg per day
XP analog ^a	Birth–1 year	13	47	38	108
XP analog LCP ^a	Birth–1 year	13	47	38	108
PKU start ^b	Birth–1 year	(2.0)	(10)	53	150
Product name	Age indications	Protein g/100g powder (or 100ml)	Iodine µg per 100g powder (or 100ml)	Iodine intake (10 kg infant at 1 year) µg per day	Iodine intake (32 kg child at 10 years) µg per day
XP Maxamaid ^a	1–8 years	25	100	120	200 ^d
Minaphlex ^a	1–10 years	29	100	103	221
PKU gel ^b	1–10 years	42	120	86	183
Anamix ^a	1–10 years	29	100	103	221
Product name	Age indications	Protein g/100g powder (or 100ml)	Iodine µg per 100g powder (or 100ml)	Iodine intake (25 kg child at 8 years) µg per day	Iodine intake (60 kg nonpregnant, nonlactating woman at 20 years) µg per day
XP Maxamum ^a	Over 8 years ^c	39	107	137	165
Lophlex ^a	Over 8 years ^c	72	210	147	175
PKU express ^b	Over 8 years ^c	60	252	210	252
Lophlex LQ ^a	Over 8 years ^c	(16)	(46.7)	146	175
Easiphen ^a	Over 8 years ^c	(6.7)	(18.2)	136	163
PKU express cooler ^b	Over 8 years ^c	(11.5)	(48.5)	211	253
Product name	Age indications	Protein g/100g powder (or 100ml)	Iodine µg per 7g powder or five tablets	Iodine intake (35 kg child at 11 years) µg per day	Iodine intake (60 kg nonpregnant, nonlactating woman at 20 years) µg per day
Phlexy Vits powder ^{a,e}	Over 11 years ^c	0.3	150	150	150
Phlexy Vits tablets ^{a,e}	Over 11 years ^c	0	150	150	150

Note:^aSHS International Ltd. Liverpool, UK.^bVitafo International Ltd., Liverpool, UK.^cIncluding adolescents, adults and pregnant women.^dCalculated as for an 8-year-old weighing 25 kg.^ePhlexy Vits complement the Phlexy-10 exchange system, see text.

The phenylalanine-free infant feeds available for the first year of life, whether they are prepared from powder (e.g., XP Analog, SHS International Limited) or supplied by the manufacturer as a liquid (e.g., PKU Start, Vitafo), are similar in protein and amino acid content per 100 ml of feed. The suggested daily amino acid requirement recommended by the MRC (Table 41.1) for infants aged 0–2 years is at least 3 g amino acid per kg bodyweight. Therefore, the minimal daily amino acid requirement of a newborn term infant of average birth weight of 3.5 kg is approximately 10.5 g amino acids, which are contained in around 450 ml of the available phenylalanine-free feeds, such as XP Analog and PKU Start. On this regimen, the daily fluid intake would

be approximately 130 ml per kg, with a corresponding iodine intake of 38–53 µg/day. In addition, some natural protein must be given to supply phenylalanine in controlled amounts to allow normal growth, and this is provided initially by either breast milk or standard infant formula. A recent study reported that the median iodine content of breast milk in Boston, MA was 15.5 µg/dl (range 0.27–196.8 µg/dl) (Pearce *et al.*, 2007), whereas it is lower in Dundee, Scotland, 10 µg/dl (range 4–28 µg/dl) (Ibrahim *et al.*, 2003). This variability in the iodine content of breast milk may be related to differences in the iodine status of these populations. Standard infant formulas available in the USA have a wide range of iodine content (8.4–22.4 µg/dl)

Table 41.5 Phenylalanine and tyrosine free; and phenylalanine-, tyrosine-, and methionine-free dietary preparations for the management of tyrosinaemia

<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (3.5kg infant at birth) µg per day</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>
XPHEN TYR analog ^a	Birth–1 year	13	47	38	108
XPTM analog ^a	Birth–1 year	13	47	38	108
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>
XPHEN TYR Maxamaid ^a	1–8 years	25	100	120	200
XPTM Maxamaid ^a	1–8 years	25	100	120	200
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>	<i>Iodine intake (32kg child at 10 years) µg per day</i>
TYROFLEX ^a	1–10 years	29	100	103	221
TYR gel ^b	1–10 years	42	120	86	183
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
XPHEN TYR Maxamum ^a	Over 8 years ^c	39	107	137	165
TYR express ^b	Over 8 years ^c	60	166	138	166
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (7.5kg infant at 6 months) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
XPHEN TYR Tyrosidon ^a	Birth to adult ^d	77	0	0	0
XPTM Tyrosidon ^a	>6 months ^c	77	0	0	0

^aSHS International Ltd., Liverpool, UK.^bVitaflor International Ltd., Liverpool, UK.^cIncluding adolescents, adults, and pregnant women.^dFlavoring prohibited ≤6 months.

(Pearce *et al.*, 2007), which is similar to that in formulas in the UK (Table 41.3). No formula provides the recommended iodine intake of 90 µg/day at birth for term infants with phenylketonuria (Table 41.4). Recommendations are met at 13–31 weeks, and thereafter are exceeded by 20–67%. XP Analog and PKU Start are designed to supply the majority of nutritional requirements as the infant is weaned at around 4–6 months. Low-protein natural foods, such as pureed fruits and vegetables, are introduced using a protein exchange system. Proprietary low-protein foods, such as bread and milk substitutes, are available to add variety to an evolving mixed diet. Alternative phenylalanine-free amino acid supplements are available to meet the nutritional requirements after the first year of life.

XP Maxamaid, Minaphlex, PKU gel, and Anamix are proprietary phenylalanine-free powdered drinks or gel mixes suitable for children in the age range of 1–10 years, and contain essential and nonessential amino acids (as well as carbohydrate, vitamins and minerals) to meet the requirements of this age group (Table 41.4). Amino acid intake recommendations for children aged 1–2 years are 3 g amino acid per kg bodyweight, and thereafter 2 g amino acid per kg bodyweight up to 10 years of age (Table 41.1).

Currently, the recommended iodine intakes for children aged 1–10 years are 90–120 µg/day. Children aged around 1 year, with an assumed weight of 10 kg, and with no other sources of iodine other than from phenylalanine-free drinks or gels, have intakes of around 86–120 µg/day

Table 41.6 Leucine-, isoleucine- and valine-free dietary preparations for the management of Maple syrup urine disease

Product name	Age indications	Protein g/100g powder (or 100ml)	Iodine μg per 100g powder (or 100ml)	Iodine intake (3.5kg infant at birth) μg per day	Iodine intake (10kg infant at 1 year) μg per day
MSUD analog ^a	Birth–1 year	13	47	38	108
Product name	Age indications	Protein g/100g powder (or 100ml)	Iodine μg per 100g powder (or 100ml)	Iodine intake (10kg infant at 1 year) μg per day	Iodine intake (25kg child at 8 years) μg per day
MSUD Maxamaid ^a	1–8 years	25	100	120	200
Product name	Age indications	Protein g/100g powder (or 100ml)	Iodine μg per 100g powder (or 100ml)	Iodine intake (10kg infant at 1 year) μg per day	Iodine intake (32kg child at 10 years) μg per day
Mapleflex ^a	1–10 years	29	100	103	221
MSUD gel ^b	1–10 years	42	120	86	183
Product name	Age indications	Protein g/100g powder (or 100ml)	Iodine μg per 100g powder (or 100ml)	Iodine intake (25kg child at 8 years) μg per day	Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) μg per day
MSUD Maxamum ^a	Over 8 years ^c	39	107	137	165
MSUD express ^b	Over 8 years ^c	60	166	138	166
MSUD cooler ^b	Over 8 years ^c	(11.5)	(31.9)	139	166
Product name	Age indications	Protein g/100g powder (or 100ml)	Iodine μg per 100g powder (or 100ml)	Iodine intake (7.5kg infant at 6 months) μg per day	Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) μg per day
MSUD aid III ^a	Birth to adult ^d	77	0	0	0

^aSHS International Ltd., Liverpool, UK.

^bVitafo International Ltd., Liverpool, UK.

^cIncluding adolescents, adults, and pregnant women.

^dFlavoring prohibited ≤ 6 months.

of iodine. The recommendations for iodine intake may be exceeded if all daily amino acids are sourced only from proprietary phenylalanine-free drinks or gels; for example, a 10-year-old child weighing 30 kg could have iodine intakes of 183–221 $\mu\text{g}/\text{day}$.

Lophlex LQ, Easiphen and PKU express cooler are supplied as “ready to drink” phenylalanine-free liquids containing a mixture of essential and nonessential amino acids, carbohydrate, fat, minerals and trace elements for children over 8 years, adolescents and adults including pregnant women. The diet must be supplemented with some natural protein to meet phenylalanine requirements. The recommended total protein intake for an 8-year-old is 2 g/kg bodyweight/day, decreasing to 1 g/kg bodyweight/day for patients over 14 years of age (Table 41.1). Protein intake for an average 8-year-old child in the UK weighing 25 kg is 50 g/day, and is equivalent to Easiphen 750 ml/day or Lophlex LQ 300 ml/day, both supplying approximately 140 $\mu\text{g}/\text{day}$ of iodine if these drinks are the only source of

protein. It is unlikely that all dietary protein is supplied by Lophlex LQ and Easiphen, and with other natural protein sources it is possible that the recommended intake range of 90–120 $\mu\text{g}/\text{day}$ of iodine for children will be achieved. For adolescents and adults a protein intake of 1 g/kg bodyweight/day from Lophlex LQ or Easiphen would supply around 170 $\mu\text{g}/\text{day}$ of iodine (based on a weight of 60 kg and a protein intake of 1 g/kg/day) and, in the absence of other dietary iodine sources, approximates to the recommended intake of 150 $\mu\text{g}/\text{day}$ of iodine for an adolescent or adult, but not the 200 $\mu\text{g}/\text{day}$ of iodine required for pregnant or lactating women. Iodine intake using PKU express cooler is higher, with a corresponding intake of 211 $\mu\text{g}/\text{day}$ for the illustrative 8-year-old child and 253 $\mu\text{g}/\text{day}$ for the adult, with caveats similar to those for other products.

XP Maxamum, Lophlex and PKU express are proprietary phenylalanine-free powdered drink mixes suitable for children over 8 years of age, teenagers and adults including

Table 41.7 Methionine-, threonine-, valine- and isoleucine-free dietary preparations for the management of methylmalonic acidaemia and propionic acidaemia

<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (3.5kg infant at birth) µg per day</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>
XMTVI analog ^a	Birth–1 year	13	47	38	108
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>
XMTVI Maxamaid ^a	1–8 years	25	100	120	200
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>	<i>Iodine intake (32kg child at 10 years) µg per day</i>
MMA/PA gel ^b	1–10 years	42	120	86	183
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
XMTVI Maxamum ^a	Over 8 years ^c	39	107	137	165
MMA/PA express ^b	Over 8 years ^c	60	166	138	166
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (7.5kg infant at 6 months) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
XMTVI Asadon ^a	Birth to adult ^d	77	0	0	0

^aSHS International Ltd., Liverpool, UK.^bVitaflor International Ltd., Liverpool, UK.^cIncluding adolescents, adults, and pregnant women.^dFlavoring prohibited ≤6 months.

pregnant women. For a 25 kg child in the 8–10-year age range, requiring 2 g protein/kg/day and with no other source of protein or iodine other than phenylalanine-free drinks, the daily iodine intakes are in the range of 137–210 µg/day, which are higher than the current recommendations for children (90–120 µg/day of iodine). Similarly, for teenagers and adults, iodine intake is higher than the recommended amount (150 µg/day), for example, a 16-year-old weighing 60 kg requiring 1 g protein/kg/day has an iodine intake in the range 165–252 µg/day with no other source of protein or iodine other than from phenylalanine-free drinks. In contrast, the recommended iodine intake for the pregnant or lactating mother (200 µg iodine/day) would be met by just one of the proprietary products with higher iodine content, assuming a nonpregnant weight of 60 kg and an early pregnancy intake of 1 g protein/kg/day.

The Phlexy-10 System (SHS International Ltd., Liverpool, UK) is an interchangeable range of products for phenylketonuria consisting of a bar, drink mix, 10 tablets and 20 capsules, which provide 10 g of a mixture of essential

and nonessential amino acids, but are free from phenylalanine. The system allows flexibility, as patients can vary the type of protein substitute taken on a daily basis. Vitamins, minerals and trace elements are supplied separate from the protein substitute and in two forms. The first, Phlexy-Vits, is in the form of a powder, which is mixed with Phlexy-10 Drink Mix. The recommended amount for children over the age of 11 years' and adults is 7 g/day, which supplies 150 µg/day of iodine. The alternative is Phlexy-Vits tablets; five tablets per day are recommended for children over the age of 11 years and adults. This form also supplies 150 µg/day of iodine. The daily amount of iodine supplied using the Phlexy-10 System is recommended for an adolescent or adult, but is inadequate for pregnant or lactating women (200 µg/day) if other sources of iodine are limited (Tables 41.2 and 41.4). PK-Aid-4 (SHS International Ltd, Liverpool, UK) is similar to Phlexy-10, in that it is a phenylalanine-free powder drink mix with essential and nonessential amino acids, but without minerals and vitamins, which need to be

Table 41.8 Glutaric Aciduria Type I and dietary preparations used in treatment

<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (3.5kg infant at birth) µg per day</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>
XLYS, LOW TRY analog ^a	Birth–1 year	13	47	38	108
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>
XLYS, LOW TRY Maxamaid ^a	1–8 years	25	100	120	200
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
XLYS, LOW TRY Maxamum ^a	Over 8 years ^b	39	107	137	165
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (7.5kg infant at 6 months) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
XYLS, TRY Glutaridon ^a	Birth to adult ^c	79	0	0	0

^aSHS International Ltd., Liverpool, UK.

^bIncluding adolescents, adults, and pregnant women.

^cFlavoring prohibited ≤6 months.

Table 41.9 Isovaleric acidaemia and disorders of leucine metabolism, dietary preparations used in treatment

<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (3.5kg infant at birth) µg per day</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>
XLEU analog ^a	Birth–1 year	13	47	38	108
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>
XLEU Maxamaid ^a	1–8 years	25	100	120	200
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
XLEU Maxamum ^a	Over 8 years ^b	39	107	137	165
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (7.5kg infant at 6 months) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
XLEU Faladon ^a	Birth to adult ^c	77	0	0	0

^aSHS International Ltd., Liverpool, UK.

^bIncluding adolescents, adults, and pregnant women.

^cFlavoring prohibited ≤6 months.

Table 41.10 Homocystinuria and dietary preparations used in treatment

<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (3.5kg infant at birth) µg per day</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>
XMET Analog ^a	Birth-1 year	13	47	38	108
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>
XMET Maxamaid ^a	1–8 years	25	100	120	200
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>	<i>Iodine intake (32kg child at 10 years) µg per day</i>
HCU gel ^b	1–10 years	42	120	86	183
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
XMET Maxamum ^a	Over 8 years ^c	39	107	137	165
HCU LV ^a	Over 8 years ^c	72	168	117	140
HCU express ^b	Over 8 years ^c	60	166	138	166
<i>Product name</i>	<i>Age indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (7.5kg infant at 6 months) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
XMET Homidon ^a	Birth to adult ^d	77	0	0	0

^aSHS International Ltd., Liverpool, UK.^bVitafo International Ltd., Liverpool, UK.^cIncluding adolescents, adults, and pregnant women.^dFlavoring prohibited ≤6 months.**Table 41.11** Miscellaneous disorders of amino-acid metabolism and dietary preparations used in their treatment

<i>Product name</i>	<i>Indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (3.5kg infant at birth) µg per day</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>
XMET XCYS Analog ^a	Sulfite oxidase deficiency Birth–1 year	13	47	38	108
XLYS Analog ^a	Hyperlysinaemia Birth–1 year	13	47	38	108
XGLY Analog ^a	Nonketotic hyperglycinaemia Birth–1 year	13	47	38	108
<i>Product name</i>	<i>Indications</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>	<i>Iodine intake (25kg child at 8 years) µg per day</i>
XMET XCYS Maxamaid ^a	Sulfite oxidase deficiency 1–8 years	25	100	120	200
XLYS Maxamaid ^a	Hyperlysinaemia 1–8 years	25	100	120	200

^aSHS International Ltd., Liverpool, UK.

Table 41.12 Special foods for conditions of intolerance

<i>Product name</i>	<i>Age indications (conditions)</i>	<i>Protein g/100g powder (or 100 ml)</i>	<i>Iodine µg per 100g powder (or 100 ml)</i>	<i>Iodine intake (3.5 kg infant at birth) µg per day</i>	<i>Iodine intake (10 kg infant at 1 year) µg per day</i>
Infant formula					
Galactomin 17 ^a	Birth–1 year (1,2,3)	12.3	52	44	127
Galactomin 19 ^a	Birth–1 year (7)	14.6	55	40	113
Neocate ^a	Birth–1 year (4,5,6)	13	47	38	108
Infatrini ^c	Birth–1 year (11,12)	(2.6)	(15)	61	173
SMA High Energy ^c	Birth–1 year (12)	(2)	(14)	74	210
Pregestimil ^d	Birth–1 year (1,5,6,10)	(1.9)	(10)	55	158
Nutramigen ^d	Birth–1 year (1,13,14)	(1.9)	(10)	55	158
Enfamil Lactofree ^d	Birth–1 year (3)	(1.4)	(10)	75	214
Enfamil Prosobee ^d	Birth–1 year (1,3,13)	(1.7)	(10)	62	176
Locasol ^a	Birth–1 year (15)	14.6	78	56	160
Pepdite ^a	Birth–1 year (5,6,9)	13.8	47	36	102
Isomil ^e	Birth–1 year (1,2,3,4,13)	13.7	76	58	166
Caprilon ^a	Birth–1 year (17)	11.8	78	69	198
Monogen ^a	Birth–1 year (19)	11.4	40.6	37	107
MCT Pepdite ^a	Birth–1 year (5,6,17)	13.8	47	36	102
SMA Wysoy ^h	Birth–1 year (3,13)	(14)	(91)	68	195
SMA LF ^h	Birth–1 year (3)	(12)	(77)	67	193
Infasoy ^c	Birth–1 year (1,2,3,13)	14.2	102	75	215
Pepti-Junior ^c	Birth–1 year (4,5,6,8,10)	13.9	78	59	168
Pepti ^c	Birth–1 year (13)	12.4	79	67	191
Farley's soya formula ⁱ	Birth–1 year (1,2,3,4)	14.3	61	45	128
<i>Product name</i>	<i>Age indications (conditions)</i>	<i>Protein g/100g powder (or 100 ml)</i>	<i>Iodine µg per 100g powder (or 100 ml)</i>	<i>Iodine intake (10 kg infant at 1 year) µg per day</i>	<i>Iodine intake (20 kg child at 6 years) µg per day</i>
Fortini & multi fibre ^c	Children 1–6 years (10,12)	(3.4)	(15)	132	176
Nutrini & multi fibre ^c	Children 1–6 years (5,6,8,9,10)	(2.75)	(10)	107	145
Nutrini energy & multi fibre ^c	Children 1–6 years (5,6,8,9,10)	(4.13)	(15)	109	145
Nutrini low energy multi fibre ^c	Children 1–6 years (5,6,8,9,10)	(2.06)	(10)	146	194
Isosource junior ^g	Children 1–6 years (5,6,8,9,10)	(2.7)	(12)	133	178
<i>Product name</i>	<i>Age indications (conditions)</i>	<i>Protein g/100g powder (or 100 ml)</i>	<i>Iodine µg per 100g powder (or 100 ml)</i>	<i>Iodine intake (10 kg infant at 1 year) µg per day</i>	<i>Iodine intake (32 kg child at 10 years) µg per day</i>
Frebini original & original fibre ^d	Children 1–10 years (5,6,8,9,10)	(2.5)	(10)	120	256
Frebini energy & energy fibre ^d	Children 1–10 years (5,6,8,9,10)	(3.8)	(15)	118	253
Paediasure & fibre ^e	Children 1–10 years (5,6,8,9,10)	(2.8)	(10)	107	229
Paediasure Plus ^e	Children 1–10 years (5,6,8,9,10)	(4.2)	(15)	107	229
Clinutren Junior ^f	Children 1–10 years (5,6,8,9,10)	13.9	37	80	170
Neocate Advance ^a	Children 1–10 year (4,5,6)	10	28	84	179

Table 41.12 (Continued)

<i>Product name</i>	<i>Age indications (conditions)</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (10kg infant at 1 year) µg per day</i>	<i>Iodine intake (32kg child at 10 years) µg per day</i>
MCT Peptide 1+ ^a	Children 1–10 year (5,6)	13.8	38.7	84	179
Generaid Plus ^a	Children 1–10 year (18)	11	49	134	285
Peptide 1+ ^a	Children 1–10 year (5,6,16)	13.8	38.7	84	179
Resource Junior ^d	Children 1–10 year (5,6,8,9,10)	(3.0)	(12.5)	125	267
Elemental 028 ^a	Children 1–10 years (5,6,8,9,16)	10	33.3	100	213
Elemental 028 ^a Extra	Children 1–10 years (5,6,8,9)	12.5	33.3	80	170

<i>Product name</i>	<i>Age indications (conditions)</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (23kg infant at 7 year) µg per day</i>	<i>Iodine intake (40kg child at 12 years) µg per day</i>
Tentrini & multi fibre ^c	Children 7–12 years (5,6,8,9,10)	(3.3)	(11.5)	160	209
Tentrini energy & multi fibre ^c	Children 7–12 years (5,6,8,9,10)	(4.9)	(17.3)	162	212

<i>Product name</i>	<i>Age indications (conditions)</i>	<i>Protein g/100g powder (or 100ml)</i>	<i>Iodine µg per 100g powder (or 100ml)</i>	<i>Iodine intake (20kg child at 6 year) µg per day</i>	<i>Iodine intake (60kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
Fresubin original & original fibre ^b	Children >5 years ^j (5,6,8,9,10)	(3.8)	(13.3)	140	210
Fresubin energy & energy fibre ^b	Children >5 years ^j (5,6,8,9,10)	(5.6)	(30)	214	321
Fresubin 1000 complete ^b	Children > 5 years ⁱ (5,6,8,9,10)	(5.5)	(20)	145	218
Fresubin 1200 complete ^b	Children >5 years ^j (5,6,8,9,10)	(6.0)	(22)	147	220
Emsogen ^a	Children >5 years ^j (6)	12.5	33.3	107	160
Jevity ^e	Children > 5 years ⁱ (5,6,8,9,10)	(4.0)	(13)	130	195
Ensure ^e	Children 5 years ^j (5,6,8,9,10)	(4.0)	(11)	110	165
Osmolite ^e	Children >5 years ^j (5,6,8,9,10)	(4.0)	(11)	110	165
Enrich ^e	Children >5 years ^j (5,6,8,9,10)	(3.76)	(10)	106	160
Peptamen ^f	Children >5 years ^j (5,6,8,9,10)	(4.0)	(10)	100	150
Modulen IBD ^f	Children >5 years ^j (16)	18	49	109	163
Isosource standard ^g	Children >5 years ^j (5,6,8,9,10)	(4.0)	(12)	120	180
Isosource energy ^g	Children >5 years ^j (5,6,8,9,10)	(5.7)	(12)	84	126
Isosource fibre ^g	Children >5 years ^j (5,6,8,9,10)	(3.8)	(12)	126	189
Isosource energy fibre ^g	Children >5 years ^j (5,6,8,9,10)	(4.9)	(13)	106	159
Novosource GI Forte ^g	Children >5 years ^j (5,6,8,9,10)	(6.0)	(15)	100	150
Novosource GI Control ^g	Children >5 years ^j (5,6,8,9,10)	(4.1)	(12)	117	176

(continued)

Table 41.12 (Continued)

<i>Product name</i>	<i>Age indications (conditions)</i>	<i>Protein g/100g powder (or 100 ml)</i>	<i>Iodine µg per 100g powder (or 100 ml)</i>	<i>Iodine intake (23 kg child at 7 year) µg per day</i>	<i>Iodine intake (60 kg non-pregnant, non-lactating woman at 20 years) µg per day</i>
Nutrison standard & multi fibre ^c	Children >6 years ^j (5,6,8,9,10)	(4.0)	(13)	150	195
Nutrison energy & multi fibre ^c	Children >6 years ^j (5,6,3,8,9)	(6.0)	(20)	153	200
Nutrison 1000 & 1200 complete multi fibre ^c	Children >6 years ^j (5,6,8,9,11)	(5.5)	(20)	167	218
Nutrison soya ^c	Children >6 years ^j (5,6,8,9,10,13)	(4.0)	(13)	150	195
Nutrison MCT ^c	Children >6 years ^j (5,6,8,9,10)	(5.0)	(13)	120	156
Peptisorb ^c	Children >6 years ^j (5,6,8,9,10)	(4.0)	(13)	150	195
Clinutren 1.5 & 1.5 Fibre ^f	Children >6 years ^j (5,6,8,9,10)	(5.6)	(15)	123	161
Clinutren ISO ^f	Children >6 years ^j (5,6,8,9,10)	(3.8)	(10)	121	158
Fortisip multi fibre & Fortifresh ^c	Children >6 years ^j (5,6,8,9,10)	(6.0)	(20)	153	200

<i>Product name</i>	<i>Age indications (conditions)</i>	<i>Protein g/100g powder (or 100 ml)</i>	<i>Iodine µg per 100g powder (or 100 ml)</i>	<i>Iodine intake (35 kg child at 11 years) µg per day</i>	<i>Iodine intake (60 kg nonpregnant, nonlactating woman at 20 years) µg per day</i>
Jevity Plus & Jevity Promote ^e	Children >10 years ^j (5,6,8,9,10)	(5.6)	(15)	141	161
Jevity 1.5 ^e	Children >10 years ^j (5,6,8,9,10)	(6.4)	(15)	123	141
Osmolite Plus ^e	Children >10 years ^j (5,6,8,9,10)	(5.6)	(15)	141	161

Note: 1, galactosaemia; 2, galactokinase deficiency; 3, lactose intolerance; 4, whole protein intolerance; 5, short bowel syndrome; 6, intractable malabsorption; 7, glucose-galactose intolerance; 8, inflammatory bowel disease; 9, bowel fistulas; 10, disease-related malnutrition; 11, fluid restriction; 12, growth failure; 13, cows' milk protein intolerance; 14, soya intolerance; 15, calcium and vitamin D restricted; 16, Crohn's disease; 17, disordered metabolism of long-chain fatty acids; 18, hepatic disease; 19, very long-chain fatty acid oxidation defects.

^aSHS International Ltd., Liverpool, UK.

^bFresenius Kabi, Cheshire, UK.

^cNutricia Ltd., Wiltshire, UK.

^dMead Johnson, Middlesex, UK.

^eAbbott UK, Maidenhead, UK.

^fNestle UK Ltd., Surrey, UK.

^gNovartis Surrey, UK.

^hSMA Nutrition, Berkshire, UK.

ⁱHJ Heinz Ltd, Middlesex, UK.

^jIncluding adults and pregnant women.

prescribed (e.g., Phlexy-Vits or other vitamin and mineral mixtures) for nutritional completeness.

Specialized Feeds for Tyrosinaemia

Hereditary tyrosinaemia type I is a rare genetic disease caused by mutations in the gene for the enzyme fumarylacetoacetase, which results in severe liver failure and early death.

The clinical manifestations stem from the cytotoxicity of tyrosine metabolites accumulating proximal to the metabolic defect. Nitisinone 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione acts on tyrosine metabolism upstream of the defect to prevent the production of these metabolites, and is used in combination with a tyrosine- and phenylalanine-restricted diet. If the patient is unresponsive to Nitisinone, a tyrosine-, phenylalanine- and methionine-restricted diet is used.

Hereditary tyrosinaemia type II is caused by a deficiency of tyrosine aminotransferase, leading to eye lesions, skin lesions and neurological complications. The aim of dietary management is to prevent the accumulation of tyrosine and phenylalanine by a low-protein diet. The protein requirements are met by supplementing the diet with a tyrosine- and phenylalanine-free amino acid mixture.

Hereditary tyrosinaemia type III is a very rare form of tyrosinaemia resulting in convulsions, ataxia and mental retardation. The aim of dietary management is to prevent the accumulation of tyrosine and phenylalanine by a low-protein diet. The protein requirements are met by supplementing the diet with a tyrosine- and phenylalanine-free amino acid mixture.

The phenylalanine- and tyrosine-free infant formula, XPHEN TYR Analog, and phenylalanine-, tyrosine- and methionine-free infant formula XPTM Analog, available for the first year of life are identical in protein, amino acid and iodine content. At a minimal daily amino acid requirement of 3 g amino acid per kg bodyweight, a newborn term infant of average birth weight of 3.5 kg would have an iodine intake of 38 µg/day; the infant at 1 year would have an iodine intake of 108 µg/day. The principles of supplementation with natural protein using breast milk, or standard infant formula, and subsequent weaning regimens with low-protein foods are outlined in the section dealing with phenylketonuria, also apply for tyrosinaemia. The current recommendations for neonates and infants of 90 µg/day of iodine may be difficult to achieve given the dietary limitations of this condition.

The phenylalanine- and tyrosine-free powdered drink or gel mixes XPHEN TYR Maxamaid, TYROFLEX and TYR gel, and the phenylalanine-, tyrosine- and methionine-free drink mix XPTM Maxamaid are suitable for children in the age range of 1–10 years' and contain essential and nonessential amino acids (as well as carbohydrate, vitamins, minerals and, in TYROFLEX, added fats) to meet the requirements of this age group (Table 41.5). Children aged 1 year with an assumed weight of 10 kg, on a 3 g amino acid per kg bodyweight diet, and with no other sources of iodine other than their prescribed drinks or gels, have intakes of around 86–120 µg/day of iodine. Currently, the recommended iodine intake for infants is 90 µg/day. These recommendations for iodine intake may be exceeded if all daily amino acids are sourced only from proprietary phenylalanine-free drinks or gels; for example, a 10-year-old child weighing 32 kg could have iodine intakes of 183–221 µg/day, with current recommendations of only 120 µg/day.

XPHEN TYR Maxamum and TYR express are proprietary tyrosine- and phenylalanine-free powdered drink mixes suitable for children over 8 years, teenagers and adults, including pregnant women (Table 41.5). For a 25 kg child around 8 years requiring 2 g protein/kg/day and with no source of protein or iodide other than tyrosine- and phenylalanine-free drinks, the daily iodide intakes are around 137 µg/day of iodine, which are higher than current

recommendations for children (120 µg/day of iodine). Similarly, for teenagers and adults iodine intake is marginally higher than the recommended amount (150 µg/day); for example, a 16-year-old weighing 60 kg requiring 1 g protein/kg/day has an iodine intake of around 165 µg/day, with no other source of protein or iodide other than tyrosine- and phenylalanine-free drinks. In contrast, the recommended iodine intake of the pregnant or lactating mother (200 µg/day) would not be met by these products, assuming a non-pregnant weight of 60 kg and an early pregnancy intake of 1 g protein/kg/day.

The phenylalanine- and tyrosine-free infant drink mix XPHEN TYR Tyrosidon, and the phenylalanine-, tyrosine- and methionine-free infant drink mix XPTM Tyrosidon are suitable for infants, children, adolescents and adults; but, unlike other proprietary preparations suitable for the management of tyrosinemia, they are carbohydrate, vitamin and mineral free, which need to be added to the diet. This may allow more appropriate age-specific supplementation, including iodine.

Specialized Feeds for Maple Syrup Urine Disease

Maple syrup urine disease is a rare genetic disorder affecting the metabolism of leucine, isoleucine and valine. Accumulation of these branched-chain amino acids results in severe neurological damage and death. The aim of dietary management is to prevent the accumulation of these amino acids, by the use of a low-protein diet and leucine-, isoleucine- and valine-free amino acid preparations. Natural protein supplies the necessary leucine, isoleucine and valine, based on a leucine exchange system with additional isoleucine and/or valine available as single amino acid supplements.

The leucine-, isoleucine- and valine-free infant formula MSUD Analog is suitable for the first year of life. At a minimal daily amino acid requirement of 3 g amino acid per kg bodyweight, a newborn term infant of average birth weight of 3.5 kg would have an iodine intake of 38 µg/day; the infant at 1 year would have an iodine intake of 108 µg/day. This iodine intake is identical to that supplied by the phenylalanine-restricted formula XPAnalog, and the principles of supplementation with natural protein using breast milk, or standard infant formula, and the subsequent weaning regimens with low-protein foods are outlined in the section dealing with phenylketonuria. Current recommendations for neonates and infants are an intake of 90 µg/day iodine, which may be difficult to achieve given the protein restriction necessary and the iodine content of available foods.

The leucine-, isoleucine- and valine-free powdered drink or gel mixes MSUD Maxamaid, Mapflex, and MSUD gel are suitable for children in the age range 1–10 years and contain essential and nonessential amino acids (as well as carbohydrate, vitamins, minerals and, in addition, fats

in Mapleflex) to meet the requirements of this age group (Table 41.6). Children aged 1 year with an assumed weight of 10 kg, put on a 3 g amino acid per kg bodyweight diet, and with no other sources of iodine other than their prescribed drinks or gels, have intakes of around 86–120 µg/day of iodine. Currently, the recommended iodine intake for children is 90 µg/day. These recommendations for iodine intake may be exceeded if all daily amino acids are sourced only from proprietary leucine-, isoleucine- and valine-free drinks or gels; for example, a 10-year-old child weighing 32 kg could have iodine intakes of 183–221 µg/day.

MSUD Maxamum and MSUD express are proprietary leucine-, isoleucine- and valine-free powdered drink mixes suitable for children over 8 years, teenagers and adults, including pregnant women (Table 41.6). MSUD express and MSUD cooler are two interchangeable products; the former is a drink mix powder, the latter a ready-made liquid drink. The daily iodide intake for a 25 kg child of 8 years requiring 2 g protein/kg/day, and with no other source of protein or iodide other than from their prescribed preparations, is around 138 µg/day of iodine, which is higher than the current recommendations for children (120 µg/day of iodine). Similarly for teenagers and adults, iodine intake is higher than the recommended amount (150 µg/day); for example, a 16-year-old weighing 60 kg requiring 1 g protein/kg/day, and with no other source of protein or iodide other than the prescribed preparations, has an iodine intake around 165 µg/day. In contrast, the recommended iodine intake of the pregnant or lactating mother (200 µg/day), would not be met by these products, assuming a nonpregnant weight of 60 kg and an early pregnancy intake of 1 g protein/kg/day.

The leucine-, isoleucine- and valine-free drink mix MSUD Aid III is suitable for infants, children, adolescents and adults; but, unlike other proprietary preparations suitable for the management of Maple syrup urine disease, it is carbohydrate, fat, vitamin and mineral free, which need to be added to the diet. This may allow more appropriate age-specific supplementation including iodine.

Specialized Feeds for Methylmalonic Acidemia and Propionic Acidemia

Methylmalonic acidemia and propionic acidemia are disorders of intermediate metabolism involving the metabolism of three-carbon-unit metabolites. The clinical features vary from mild involvement to severe neurological impairment, coma and death. The dietary management is to restrict the availability of methionine, threonine, valine and isoleucine, by means of a low-protein diet with protein requirements met by methionine-, threonine-, valine- and isoleucine-free amino acid mixtures.

The methionine-, threonine-, valine- and isoleucine-free infant formula XMTVI Analog is suitable for the

first year of life. At a minimal daily amino acid requirement of 3 g amino acid per kg bodyweight, a newborn term infant of average birth weight of 3.5 kg would have an iodine intake of 38 µg/day. This iodine intake is identical to that supplied by the phenylalanine-only restricted formula XPAnalog, and the principles of supplementation with natural protein using breast milk, or standard infant formula, and the subsequent weaning regimens with low-protein foods are outlined in the section dealing with phenylketonuria. Current recommendations for neonates and infants are an intake of 90 µg/day of iodine, which may be difficult to achieve given the protein restriction necessary and the iodine content of available foods.

The methionine-, threonine- valine- and isoleucine-free powdered drink or gel mixes XMTVI Maxamaid, and MMA/PA gel are suitable for children in the age range of 1–10 years and contain essential and nonessential amino acids (as well as carbohydrate, vitamins, minerals) to meet the requirements of this age group (Table 41.7). Children aged 1 year with an assumed weight of 10 kg, on a 3 g amino acid per kg bodyweight diet, and with no other sources of iodine other than their prescribed drinks or gels, have intakes of around 86–120 µg/day of iodine. Currently, the recommended iodine intake for children is 90 µg/day. These recommendations for iodine intake may be exceeded if all daily amino acids are sourced only from proprietary methionine-, threonine-, valine- and isoleucine-free drink or gels; for example a 10-year-old child weighing 32 kg could have iodine intakes of 183–200 µg/day.

XMTVI Maxamum and MMA/PA express are proprietary methionine-, threonine-, valine- and isoleucine-free powdered drink mixes suitable for children over 8 years of age, teenagers and adults, including pregnant women (Table 41.7). For a 25 kg child of 8 years requiring 2 g protein/kg/day and with no source of protein or iodide other than the prescribed preparations, the daily iodide intake is around 137 µg/day of iodine, which is higher than the current recommendation for children (120 µg/day of iodine). Similarly for teenagers and adults, iodine intake is marginally higher than the recommended amount (150 µg/day); for example, a 16-year-old weighing 60 kg requiring 1 g protein/kg/day, with no source of protein or iodide other than the prescribed preparations, has an iodine intake around 165 µg iodine/day. In contrast, the recommended iodine intake of the pregnant or lactating mother (200 µg/day iodine) would not be met by these products, assuming a nonpregnant weight of 60 kg and an early pregnancy intake of 1 g protein/kg/day.

The methionine-, threonine-, valine- and isoleucine-free drink mix XMTVI Asadon is suitable for infants, children, adolescents and adults; but, unlike other proprietary preparations, it is carbohydrate, fat, vitamin and mineral free, which need to be added to the diet. This may allow more appropriate age-specific supplementation including iodine.

Specialized Feeds for Glutaric Aciduria Type I

Glutaric aciduria type I is a rare inherited disorder caused by the deficiency of glutaryl-CoA dehydrogenase, resulting in the accumulation of lysine and tryptophan causing progressive neurological deterioration. Dietary management aims to prevent the accumulation of lysine and tryptophan by means of a low-protein diet and supplementing the diet with lysine-free and low-tryptophan amino acid mixtures.

The lysine-free and low-tryptophan infant formula, XLYS, LOW TRY Analog, is suitable for the first year of life. At a minimal daily amino acid requirement of 3 g amino acid per kg of bodyweight a newborn term infant of average birth weight of 3.5 kg would have an iodine intake of 38 µg/day; the infant at 1 year would have an iodine intake of 108 µg/day. The principles of supplementation with natural protein using breast milk, or standard infant formula, and the subsequent weaning regimens with low-protein foods are outlined in the section dealing with phenylketonuria. Current recommendation for neonates and infants is an intake of 90 µg/day iodine, which may be difficult to achieve given the protein restriction necessary and the iodine content of available foods.

The lysine-free and low-tryptophan powdered drink, XLYS, LOW TRY Maxamaid is suitable for children in the age range 1–8 years. Children aged 1 year with an assumed weight of 10 kg, on a 3 g amino acid per kg bodyweight diet, and with no other sources of iodine, have an intake of 120 µg/day. Currently, the recommended iodine intake for children is 90 µg/day. An 8-year-old child weighing 25 kg would have iodine intake of 200 µg/day, which is above the recommended level of 120 µg/day.

XLYS, LOW TRY Maxamum is suitable for children over 8 years, teenagers and adults, including pregnant women (Table 41.8). For a 25 kg child at 8 years of age requiring 2 g protein/kg/day and with no source of protein or iodide other than the prescribed preparations, the daily iodide intakes will be 137 µg/day iodine, which is higher than the current recommendations for children (120 µg/day iodine). Similarly, for teenagers and adults iodine intake is higher than the recommended amount (150 µg/day iodine); for example, a 16-year-old weighing 60 kg requiring 1 g protein/kg/day has an iodine intake of 165 µg/day iodine with no source of protein or iodide other than the phenylalanine-free drinks, which is above the recommended daily intake. The recommended iodine intake of the pregnant or lactating mother (200 µg/day iodine) would not be met by this product, assuming a nonpregnant weight of 60 kg and an early pregnancy intake of 1 g protein/kg/day.

The XLYS, LOW TRY Glutaridon preparation is suitable for infants, children, adolescents and adults with glutaric aciduria type I; but, unlike other proprietary preparations suitable for the management of glutaric aciduria

type I, it is mineral free, which needs to be added to the diet. This may allow more appropriate age-specific supplementation including iodine.

Specialized Feeds for Isovaleric Acidemia and Disorders of Leucine Metabolism

Isovaleric acidemia is a rare disorder of leucine metabolism caused by the deficiency of isovaleryl-CoA dehydrogenase: this leads to mild neurological impairment and, at its severest, to coma and death. Dietary management aims to limit leucine intake by means of a low-protein diet, and by supplementing the diet with a leucine-free amino acid mixture if necessary.

The leucine-free infant formula XLEU Analog is suitable for the first year of life. At a minimal daily amino acid requirement of 3 g amino acid per kg bodyweight, a newborn term infant of average birth weight of 3.5 kg would have an iodine intake of 38 µg/day; the infant at 1 year would have an iodine intake of 108 µg/day. The principles of supplementation with natural protein using breast milk, or standard infant formula, and the subsequent weaning regimens with low-protein foods are outlined in the section dealing with phenylketonuria. Current recommendation for neonates and infants is an intake of 90 µg/day of iodine, which may be difficult to achieve given the protein restriction necessary and the iodine content of available foods.

The leucine-free powdered drink, XLEU Maxamaid, is suitable for children in the age range 1–8 years. Children aged 1 year with an assumed weight of 10 kg, on a 3 g amino acid per kg bodyweight diet, and with no other sources of iodine, have an intake of 120 µg/day iodine. Currently, the recommended iodine intake for children is 90 µg/day iodine. An 8-year-old child weighing 25 kg would have iodine intake of 200 µg/day, which is above the recommended level of 120 µg/day.

XLEU Maxamum is suitable for children over 8 years, teenagers and adults, including pregnant women (Table 41.9). For a 25 kg child of 8 years requiring 2 g protein/kg/day and with no source of protein or iodide other than the prescribed preparations, the daily iodide intake is around 137 µg/day of iodine, which is higher than the current recommendation for children (120 µg/day of iodine). Similarly, for teenagers and adults iodine intake is higher than the recommended amount (150 µg/day); for example, a 16-year-old weighing 60 kg requiring 1 g protein/kg/day, with no source of protein or iodide other than the prescribed preparations, has an iodine intake of around 165 µg/day. In contrast, the recommended iodine intake for the pregnant or lactating mother (200 µg/day iodine) would not be met by this product, assuming a nonpregnant weight of 60 kg and an early pregnancy intake of 1 g protein/kg/day.

The XLEU Faladon preparation is suitable for infants, children, adolescents and adults; but, unlike other proprietary preparations suitable for the management of isovaleric academia, it is mineral free, which needs to be added to the diet. This may allow more appropriate age-specific supplementation including iodine.

Specialized Feeds for Homocystinuria

Classical homocystinuria is a rare inherited disorder, caused by the deficiency of cystathione beta-synthase resulting in accumulation of methionine; it can cause mental retardation, eye problems and thrombosis. Dietary management aims to prevent accumulation of methionine by means of a low-methionine diet and by supplementing the diet with a methionine amino acid mixture.

The methionine-free infant formula, XMET Analog, is suitable for the first year of life. At a minimal daily amino acid requirement of 3 g amino acid per kg bodyweight a newborn term infant of average birth weight of 3.5 kg and the infant at 1 year would have an iodine intake of 38 µg/day and 108 µg/day, respectively. The principles of supplementation with natural protein using breast milk, or standard infant formula, and the subsequent weaning regimens with low-protein foods are outlined in the section dealing with phenylketonuria. Current recommendation for neonates and infants is an intake of 90 µg/day iodine, which may be difficult to achieve given the protein restriction necessary and the iodine content of available foods.

The methionine-free powdered drink, XMET Maxamaid, is suitable for children in the age range 1–8 years. Children aged 1 year with an assumed weight of 10 kg, on a 3 g amino acid per kg bodyweight diet and with no other sources of iodine, have an intake of 120 µg/day iodine. Currently, the recommended iodine intake for children is 90 µg/day iodine. An 8-year-old child weighing 25 kg would have an iodine intake of 200 µg/day, which is above the recommended level of 120 µg/day.

The methionine-free HCU gel is suitable for children in the age range of 1–10 years. Children aged 1 year with an assumed weight of 10 kg, on a 3 g amino acid per kg bodyweight diet and with no other sources of iodine, have an intake of 86 µg/day iodine. Currently, the recommended iodine intake for children is 90 µg/day. A 10-year-old child weighing 32 kg would have an iodine intake of 183 µg/day, which is above the recommended level of 120 µg/day.

XMET Maxamum, HCU LV, and HCU Express are suitable for children over 8 years, teenagers and adults, including pregnant women (Table 41.10). For a 25 kg child aged 8 years requiring 2 g protein/kg/day and with no source of protein or iodide other than the prescribed preparations, the daily iodide intakes could be between 117 and 138 µg/day iodine. Two preparations are higher than the current recommendation for children (120 µg/day of

iodine). Similarly, for teenagers and adults, iodine intake is higher than the recommended amount (150 µg/day); for example, a 16-year-old weighing 60 kg requiring 1 g protein/kg/day, with no source of protein or iodide other than the prescribed preparations, has an iodine intake between 140 and 165 µg/day. One preparation is below the recommended daily intake. The recommended iodine intake of the pregnant or lactating mother (200 µg/day) would not be met by any product, assuming a nonpregnant weight of 60 kg and an early pregnancy intake of 1 g protein/kg/day.

The XMET Homidon preparation is suitable for infants, children, adolescents and adults; but, unlike other proprietary preparations suitable for the management of homocystinuria, it is mineral free, which needs to be added to the diet. This may allow more appropriate age-specific supplementation including iodine.

Specialized Feeds for Miscellaneous Disorders of Amino Acid Metabolism

Preparations for the treatment of miscellaneous disorders of amino acid metabolism are available for sulfite oxidase deficiency, hyperlysinemia and nonketotic hyperglycinemia (Table 41.11). There are formulas suitable for the first year of life. At a minimal daily amino acid requirement of 3 g amino acid per kg bodyweight, a newborn term infant of average birth weight of 3.5 kg, and the infant at 1 year would have an iodine intake of 38 µg/day and 108 µg/day, respectively.

XMET XCYS Maxamaid and XLYS Maxamaid are suitable for children in the age range of 1–8 years. Children aged 1 year with an assumed weight of 10 kg, on a 3 g amino acid per kg bodyweight diet and with no other sources of iodine, have an intake of 120 µg/day iodine. Currently, the recommended iodine intake for children is 90 µg iodine/day. An 8-year-old child weighing 25 kg would have an iodine intake of 200 µg/day, which is above the recommended level of 120 µg/day.

Special Foods for Conditions of Intolerance

There is a wide range of conditions with intolerance to specific dietary components, for example, galactosemia where enzyme deficiency results in the accumulation of toxic levels of galactose and its derivatives. Dietary management consists of exclusion of sources of galactose and lactose (including breast and cows' milk sources) and replacement of a galactose-free alternative. In addition, there are enteral feeds for the treatment of malnutrition, growth failure and malabsorption from a variety of causes (Table 41.12).

There are 21 formulas suitable for the first year of life. At a minimal daily amino acid requirement of 3 g amino

acid per kg bodyweight a newborn term infant of average birth weight of 3.5 kg would have an iodine intake of between 36 and 75 $\mu\text{g}/\text{day}$; none of these preparations meet the iodine requirement of 90 $\mu\text{g}/\text{day}$ iodine. The infant at 1 year would have an iodine intake of between 102 and 215 $\mu\text{g}/\text{day}$; thus, all preparations, if used as the sole source of nutrition, provide higher amounts than the recommended 90 $\mu\text{g}/\text{day}$ iodine.

There are nine enteral preparations suitable for 1–6 years. At a minimal daily amino acid requirement of 3 g amino acid per kg bodyweight, an infant aged 1 year with a weight of 10 kg and an infant at 6 years would have an iodine intake of 109–146 $\mu\text{g}/\text{day}$ and 145–194 $\mu\text{g}/\text{day}$, respectively. The iodine content of all these preparations is higher than the iodine requirement of 90 $\mu\text{g}/\text{day}$, if used as a sole source of nutrition.

There are 15 enteral preparations suitable for 1–10 years. At a minimal daily amino acid requirement of 3 g amino acid per kg bodyweight, an infant at 1 year with a weight of 10 kg would have an iodine intake of between 80 and 134 $\mu\text{g}/\text{day}$; five preparations do not meet the daily requirement of 90 $\mu\text{g}/\text{day}$ iodine. The child at 10 years would have an iodine intake of between 170 and 285 $\mu\text{g}/\text{day}$; thus all preparations, if used as the sole source of nutrition, provide higher amounts than the recommended 120 $\mu\text{g}/\text{day}$ iodine.

There are two enteral preparations suitable for 7–12 years. At a minimal daily amino acid requirement of 2 g amino acid/kg bodyweight, a child at 7 years with a weight of 23 kg would have an iodine intake of around 160 $\mu\text{g}/\text{day}$; which is more than the daily requirement of 120 $\mu\text{g}/\text{day}$ of iodine. The child at 12 years would have an iodine intake of around 210 $\mu\text{g}/\text{day}$; thus both preparations, if used as the sole source of nutrition, provide higher amounts than the recommended 120 $\mu\text{g}/\text{day}$ of iodine.

There are 19 enteral preparations suitable for children over 5 years of age and adults (including pregnant and lactating women). At a minimal daily amino acid requirement of 2 g amino acid per kg bodyweight, a child at 6 years with a weight of 20 kg could have an iodine intake of 84–214 $\mu\text{g}/\text{day}$; 10 preparations provide lower than the daily requirement of 120 $\mu\text{g}/\text{day}$ of iodine. For adults (nonpregnant, nonlactating, 60 kg), these preparations would provide an iodine intake of 126–321 $\mu\text{g}/\text{day}$; thus, one preparation, if used as the sole source of nutrition, provides lower amounts than the recommended 150 $\mu\text{g}/\text{day}$ of iodine. Pregnant and lactating women have a recommended iodine intake of 200 $\mu\text{g}/\text{day}$, and only three of these preparations would meet this requirement if used as the sole source of nutrition.

There are 14 enteral preparations suitable for children aged over 6 years and adults (including pregnant and lactating women). At a minimal daily amino acid requirement of 2 g amino acid per kg bodyweight a child aged 7 years with a weight of 23 kg could have an iodine intake of

120–167 $\mu\text{g}/\text{day}$; all preparations provide the minimum daily requirement of 120 $\mu\text{g}/\text{day}$ iodine. For adults (nonpregnant, nonlactating, 60 kg) these preparations would provide an iodine intake of 156–218 $\mu\text{g}/\text{day}$; thus, all preparations, if used as the sole source of nutrition, meet the recommended dose of 150 $\mu\text{g}/\text{day}$ iodine. Pregnant and lactating women have a recommended intake of 200 $\mu\text{g}/\text{day}$ of iodine, and only three of these preparations would meet this requirement if used as the sole source of nutrition.

There are four enteral preparations suitable for children over 10 years and adults (including pregnant and lactating women). At a minimal daily amino acid requirement of 2 g amino acid per kg bodyweight, a child at 11 years with a weight of 35 kg could have an iodine intake of 123–141 $\mu\text{g}/\text{day}$; all preparations provide the minimum daily requirement of 120 $\mu\text{g}/\text{day}$ of iodine. For adults (nonpregnant, nonlactating, 60 kg) these preparations would provide an iodine intake of 141–161 $\mu\text{g}/\text{day}$; thus all preparations, if used as the sole source of nutrition, meet the recommended dose of 150 $\mu\text{g}/\text{day}$ iodine. Pregnant and lactating women have a recommended iodine intake of 200 $\mu\text{g}/\text{day}$ iodine; none of these preparations would meet this requirement if used as the sole source of nutrition.

Summary Points

- No standard formula for term infants provides the recommended iodine intake of 90 $\mu\text{g}/\text{day}$ at birth. Recommendations are met at 2–15 weeks, and thereafter are exceeded by 19–100%.
- Some formulas for preterm infants meet the criteria of an iodine intake of 30 $\mu\text{g}/\text{kg}/\text{day}$ at birth and subsequently in the postnatal period.
- Estimating requirements on a per kilo basis for a recommended daily intake as a preterm infant progresses toward term and beyond has not been recognized.
- There is appreciable variability in the iodine contents of both infant formula and specialized enteral preparations, with some providing well below and others well above the recommended daily allowances.
- Because of the variability of iodine content in specialized enteral preparations, the iodine status of individuals should be monitored with prolonged use of these products and regular assessment of goiter, urinary iodine content, and thyroid function tested. Such monitoring is also appropriate if specialized enteral preparations are only part of the total nutritional intake, particularly if protein- and iodine-rich foods are restricted.
- Because vitamins and minerals (such as iodine) are incorporated into most specialized enteral preparations, their use across a wide age range often results in inappropriate iodine intakes.
- In a minority of specialized enteral preparations, where the mineral and vitamin supplements are not incorporated by the manufacturer and are prescribed separately,

excellent compliance with the recommend daily allowances can be achieved. Currently, very few preparations are available and are limited to specific age groups and conditions, e.g. younger age groups and pregnant women.

- Pregnant and lactating women are vulnerable to iodine deficiency if specialized enteral preparations are their sole source of nutrition. Only 9 out of 44 specialized enteral preparations for adults provide the current requirement of 200 µg/day. If the WHO technical consultation presently under review is accepted, then only two preparations will meet the proposed allowance of 250 µg/day. This has particular consequences for the brain development of the fetus and infant if the mother has an iodine deficiency during pregnancy and if she breast-feeds.
- Generally, standard infant formula are recommended for use by the manufacturers for up to 1 year, but as infants are weaned from approximately 6 months, estimation of iodine intakes can only be approximate as the mixed diet progresses.

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Section 2.4

Iodine Metabolism

Trimester-Specific Changes in Maternal Thyroid Hormones: Implications for Iodine Nutrition

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Abstract

During the first trimester of pregnancy, the demand for thyroid hormone (TH) increases by approximately 50%. Since the normal ranges of thyroid function tests change across pregnancy, gestation-specific reference intervals are important in providing normal ranges for comparison and facilitating clinical management of thyroid disease in pregnancy. As a result, treatment and dietary iodine supplementation can be adjusted to provide appropriate intake, in order to keep up with the increasing demands by the maternal and fetal thyroids for adequate TH synthesis. This chapter will focus on the implications for iodine nutrition due to trimester-specific changes in maternal TH concentrations.

Abbreviations

CAP PT	College of American Pathologists Proficiency Testing
FT4	Free thyroxine
GM	Geometric Mean
GW	Gestation week
hCG	Human chorionic gonadotropins
IA	Immunoassay
IDD	Iodine deficiency disorders
ID	Iodine deficiency
IOM	The US Institute of Medicine
LC/MS/MS	Liquid chromatography tandem mass spectrometry
RNI	Recommended nutrient intake
TBG	Thyroxine-binding globulin
Tg	Thyroglobulin
TH	Thyroid hormone
T3	Triiodothyronine
T4	Thyroxine, tetraiodothyronine

TSH	Thyroid-stimulating hormone
TT3	Total T3
TT4	Total T4
µg/L	Micrograms per liter
UI	Urinary iodide
UI/Cr	Urinary iodine per gram creatinine ratio
WHO	World Health Organization

Introduction

Thyroid hormones in pregnancy

Euthyroid women experience dramatic changes in the demand for thyroid hormone (TH) production as early as the first trimester of pregnancy. Hormonal changes and metabolic demands during pregnancy result in profound alterations in the biochemical parameters of thyroid function, resulting in an increase in TH synthesis. Iodine, an essential constituent of the THs, comprises 65% of thyroxine (T4) and 58% of triiodothyronine (T3) by weight (Table 42.1). All of T4 and about 20% of T3 are synthesized in the thyroid gland (approximately 100 µg T4 and 30 µg T3 are synthesized daily in nonpregnant adults),

Table 42.1 Daily minimal iodine requirement in normal iodine-sufficient adults

Hormone	Thyroidal production rate in nonpregnant adult	Iodine amount needed
T4	100 µg/day	Iodine 65% by weight
T3	30 µg/day	Iodine 58% by weight

Note: The production rate of T4 is 100 µg/day and that of T3 is 30 µg/day; therefore, an intake of at least 80 µg of iodine is needed daily.

while extrathyroidal deiodination of T₄ in the tissues provides the remaining part of T₃. Therefore, an adequate dietary iodine intake is crucial for normal TH production and maintenance of a euthyroid state across these changing demands throughout pregnancy.

Thyroid hormone and iodine requirements in pregnancy

Dietary iodine is found in food, iodized salt, milk and drinking water in the form of iodide or iodate of potassium, calcium, or sodium (Venkatesh and Dunn, 1995). Certain diets are naturally iodine-rich, while others contain very little iodine. Furthermore, it is known that certain geographical regions are iodine-poor (e.g., mountainous areas), and lower economic status may afford less variable food products, resulting in limited access to iodine-containing or iodine-enriched foods.

When iodine requirements are not met, TH synthesis is reduced, resulting in enlargement of the thyroid gland to compensate for this reduction. In adults, mild iodine deficiency (ID) is associated with nontoxic nodular goiter and, less often, with toxic nodular goiter, due to increases in the constitutive (thyrotropin-independent) growth and functional potential of some clones of thyroid cells. In pregnancy, this can result in fetal neurodevelopmental deficits and mental retardation.

Indication of Iodine Status in Pregnancy Daily urinary excretion of iodide closely reflects iodine intake, as only a small fraction is excreted in feces. Urinary iodide (UI) excretion of about 100 µg/l represents an approximate daily intake of 150 µg iodine, with an excretion range of 100–199 µg/l considered adequate (WHO/UNICEF/ICCIDD, 1994). These values correspond to 70–80% of the daily intake. UI excretion in a 24 h urine specimen is a good tool to indicate daily iodine intake (Hetzel and Dunn, 1989; Dunn *et al.*, 1993). However, 24 h urine samples are difficult and impractical to collect, especially in large field studies. Nonfasting casual urine samples are usually obtained. Instead, in population studies, it is the median UI excretion level that serves as a monitoring tool for evaluation of the adequacy of iodine nutrition of that given population.

The use of thyroid-stimulating hormone (TSH) and T₄ in pregnancy as screening tools for epidemiological studies have not proven adequate, and no relationship has been shown between UI and TSH or T₄ levels (Soldin *et al.*, 2005). However, as aforementioned, casual urine samples are not suitable for estimating the iodide excretion of individuals, even when expressed as the iodide per gram creatinine ratio (µg/Cr) (Nicolau *et al.*, 1989).

For individuals, UI is not a valid tool for the estimation of the adequacy of iodine nutrition, since it may only represent the most recent dietary intake (especially in cases where UI is high). A more reliable indicator of iodine

Table 42.2 Thyroid parameters indicating deficient iodine intake in pregnancy

Indicator	Insufficiency iodine intake
Serum FT ₄	Lower
Serum TT ₃ /TT ₄ ^a	Higher
Serum TSH	Higher
Serum Tg	Higher
Thyroid volume	Increased

Note: The thyroid function tests change in iodine-deficient women during pregnancy.

^aTotal molar T₃/T₄ ratio. Glinoe, (1997).

nutrition adequacy for individuals would be the estimation of stored iodine within the thyroid gland. However, it is not feasible to measure this parameter in humans. Instead, measurements of serum-free thyroxine (FT₄), total T₄ (TT₄), total T₃ (TT₃), serum TSH, thyroid volume and serum thyroglobulin (Tg) can facilitate the clinical management of thyroid dysfunction. It is here that trimester-specific reference intervals of euthyroid hormone concentrations are important instrumental, in determining whether there is a decrease or increase from expected values. For example, a reliable measurement of FT₄ indicating lower than expected serum FT₄ level can indicate inadequacy in iodine intake (Table 42.2). There is, of course, greater value for using two or more indicators of iodine status. The trimester-specific reference intervals are important for clinical thyroid disease management. For example, for the management of hypothyroidism, clinicians rely on an accurate assessment of both serum FT₄ and TSH concentrations.

Physiological Changes in Thyroidal Activity during Pregnancy As soon as pregnancy is established, dramatic physiological changes occur that lead to increasing demands in thyroidal activity in order to accommodate the pregnant mother and the developing fetus. During normal pregnancy, the increase in plasma volume and plasma concentration of thyroxine-binding globulin (TBG) results in a several-fold increase in the total T₄ pool (summarized in Table 42.3). In addition, the stimulatory effect of human chorionic gonadotropins (hCG) on the thyroid induces a partial TSH suppression below the normal range at the end of the first trimester (Glinoe *et al.*, 1995; Levy *et al.*, 1980; Soldin, 2006). During the second and third trimesters in iodine-sufficient areas, serum TSH returns to the normal levels seen before pregnancy. Increases in renal blood flow and glomerular filtration lead to an increased iodide clearance, resulting in decreased plasma iodide and an increased requirement for iodide intake (Burrow, 1993; Glinoe, 1997).

Women who are iodine-sufficient at conception have natural intrathyroidal iodine reserves that should remain ample (Lieberman *et al.*, 1998), unless iodine intake decreases and the rate of increase in iodine loss exceeds the

Table 42.3 Regulation of thyroid function in normal pregnancy

	Thyroidal implications	Trimester		
		1	2	3
<i>Pregnancy-related changes in iodine-sufficient women</i>				
Renal blood flow and glomerular filtration increase	Increase in iodide plasma clearance resulting in: (1) a decreased plasma iodide; (2) increased iodide excretion; and (3) increased requirement for dietary iodide intake. Dworkin et al., 1966 ; Burrow, 1993 ; Glinoe, 1997 .	1	2	3
Intrathyroidal iodine stores are plentiful at the time of conception	Iodine stored remain unaltered throughout gestation. Lieberman et al., (1998) .	1	2	3
Plasma volume increase (until delivery)	Increased total T4 and T3	1	2	3
hCG concentrations increase (first trimester)(partial structural homology between hCG and TSH)	A small transient increase in FT4 and FT3 (at end of first trimester) leading to a partial TSH suppression	1		
	20% of euthyroid women may have TSH transiently below the normal range during first trimester and increases in FT4 and T3	1		
Placental inner ring deiodination of T4 and T3	Acceleration in T4 and T3 degradation			
Increased plasma concentration of thyroxine-binding globulin (TBG) mainly by GW 10	A several-fold increase in the total T4 pool: (1) T4 degradation decreased during early pregnancy; and (2) increased T4 production throughout gestation. There is a 30–50% increase in T4 production during gestation	1	2	3
Transplacental passage of maternal T4	Decrease in T4	1	2	3
<i>Physiological changes in pregnancy in iodine-deficient women</i>				
Renal blood flow and glomerular filtration increase	UI concentrations may show an early increase, but steadily decrease from first to third trimester of gestation	1	2	3
	Intrathyroidal iodine stores are low at the time of conception and deplete throughout gestation. Lieberman et al., (1998) .	1	2	3
LT4 requirements are markedly enhanced during pregnancy in hypothyroid women	ID becomes significant during pregnancy when the iodine intake falls below 100 µg/day	1	2	3
Reduced iodine intake during pregnancy	Chronically enhanced thyroidal stimulation through the pituitary–thyroid feedback mechanism and is frequently accompanied by thyroidal alterations, mainly relative hypothyroxinemia and goitrogenesis	1	2	3

Note: The table describes the consequences on the thyroid that result from the physiological changes during pregnancy. Source: Adapted from [Glinoe \(2003, 2006b\)](#).

intake. It is therefore imperative to maintain appropriate iodine dietary supplementation throughout pregnancy.

Clinically, enhanced glandular stimulation associated with iodine restriction can be assessed using biochemical parameters, such as relative hypothyroxinemia (decreased serum FT4) ([Elnagar et al., 1998](#)), changes in serum TSH (usually remaining within the normal range) frequently doubling the initial TSH concentrations near term ([Vermiglio et al., 1999](#)), and changes in Tg concentrations ([Glinoe et al., 1990](#)). Comparison of these thyroid function parameters with the normal gestation-specific reference intervals during normal pregnancy is imperative ([Glinoe, 2004b](#)).

Delivery leads to a rapid reversal of these pregnancy-related changes, and serum TBG, T4 and T3 concentrations return to pregestational levels within 4–6 weeks. In order to detect abnormalities in TH concentrations during

the progression of pregnancy, it is necessary to determine their normal ranges throughout pregnancy by defining reference intervals for each of the trimesters.

The Consequences of Insufficient Iodine Supplementation in Pregnancy A systematic review by the Cochrane group demonstrated that iodine supplementation during pregnancy decreased neonatal mortality [RR 0.71 (0.56–0.9)], and decreased the incidence of cretinism in children under 4 years [RR 0.27 (0.12–0.6)] ([Ibrahim et al., 2006](#)). Furthermore, mild ID can result in learning disabilities, poor growth and diffuse goiter in school-age children. Even maternal hypothyroxinemia (defined as low levels for gestational-age maternal T4, with normal levels of TSH) in areas of severe ID causes the birth of neurological cretins and other mental deficits ([Choufoer et al., 1965](#)). It is important to note that, while fetal motor and

cognitive impairments can be associated with maternal hypothyroxinemia, the mothers are not necessarily clinically hypothyroid (Morreale de Escobar *et al.*, 2000).

Increased Production of Thyroid Hormone in Pregnancy Depends on Adequate Dietary Iodine Intake During pregnancy, while physiological adaptations provide the necessary increases in TH production when the iodine intake is adequate, this is replaced by pathologic alterations when iodine intake is insufficient. Thus, underlying deficits are typically revealed in pregnancy. Severe ID may be associated with impairment in the psycho–neuro–intellectual outcome in the progeny, because both mother and offspring are exposed to ID during gestation (and the postnatal period).

The western hemisphere has made great progress toward correcting ID through mandated salt iodization programs and increased awareness, though pockets of deficiency still remain. Because the consequences of ID are severe and the risks of excess treatment with modest supplements are minimal, iodine supplementation during pregnancy has been recommended. In most public health programs concerned with iodine deficiency disorders (IDD), iodized salt has been the preferred strategy to provide iodine supplements to households. Iodized salt, however, is not the ideal method of iodine supplementation for pregnant or lactating women, because of the necessity to limit salt intake. Since the early 1990s, iodine supplementation has been systematically given to pregnant and lactating women in Europe by administering multivitamin tablets containing iodine supplements in order to achieve the ideal recommended dietary allowance of 220–290 µg iodine/day.

The recommendations of the Institute of Medicine (IOM) for daily iodine intake during pregnancy are similar to the recommended nutrient intake (RNI) for iodine during pregnancy established by the WHO (Institute of Medicine, 2002; World Health Organization, 2005). The technical consultant's panel meeting at the WHO (Geneva, 2005) recommended an increase in the iodine intake of pregnant women to 250 µg/day (range 200–300 µg/day) (World Health Organization, 2005). Most individuals can tolerate fairly high intakes of iodine without problems (Glinioer, 2004a). There are experts in thyroidology who propose (not without controversy) that iodine intake in pregnancy should be increased in most countries, even in countries with marginal ID (Glinioer, 2006a), so that iodine intake increases to at least 300–400 µg daily (Utiger, 2006).

Trimester-Specific Changes in Maternal Thyroid Hormones

Trimester-specific reference intervals for thyroid functions are especially important, since thyroid insufficiency may

be associated with fetal neurodevelopmental deficits and adverse obstetric outcomes. The availability of the normal ranges, according to changes in TH concentrations throughout pregnancy, allows individualized thyroxine therapy, as well as iodine supplementation when necessary.

It is important to recognize that gestation-specific normal ranges can change with the method of analysis, and that local “normal” ranges may be totally unacceptable clinically, due to ID in the population. In general, immunoassays (IAs) are notoriously unreliable, with increasing evidence in the published literature supporting their lack of specificity (Klee, 2000; Sapin and d’Herbomez, 2003; d’Herbomez *et al.*, 2003). In addition, the presence of circulating iodothyronine-binding autoantibodies that interfere with total T4 and T3 IAs is a known phenomenon (Beck-Peccoz *et al.*, 1984; Despres and Grant, 1998; Martel *et al.*, 2000; Sakata *et al.*, 1985). These autoantibodies may give false high or low values of TH measurements, depending on the assay separation method used, and are often not in accordance with the clinical features. Direct serum FT4 and FT3 measurements are technically difficult to determine, since they are measured in the picomole range and must be free from interference by the much higher total hormone concentrations to be valid (Sapin and d’Herbomez, 2003; d’Herbomez *et al.*, 2003). It is therefore easier to measure total TH concentrations at nanomolar levels.

Serum TT4 and TT3 concentrations are most commonly measured by IA methods. In proficiency testing of samples for different methods of measurement of both TT4 and TT3, the College of American Pathologists Proficiency Testing (CAP PT) Program reported that the difference in specificity of the various antibodies used in IAs can vary by a factor of two. In addition to method differences, under certain conditions such as pregnancy, estrogen therapy or genetic abnormalities in protein binding have also reportedly made IA methods for T4 and T3 diagnostically unreliable.

Trimester-specific reference intervals for healthy, iodine-sufficient women by means of liquid chromatography tandem mass spectrometry (LC/MS/MS) using an isotopically labeled internal standard and IA are available (Soukhova *et al.*, 2004; Thienpont *et al.*, 1999) (Table 42.4). In several studies, the measurement of TT3 and TT4 by IAs were compared with measurements using LC/MS/MS in a longitudinal study of Swedish women during the first, second and third trimesters of pregnancy and 1-year postpartum (Table 42.4) (Soldin *et al.*, 2004a, c). Across the pregnancy, TT3, FT4, TSH and Tg concentrations were significantly different between the first and third trimesters (all $p \leq 0.05$); the second and third trimester levels were not significantly different for FT4, TSH and Tg (all $p > 0.25$), although TT3 was significantly higher in the third, relative to the second, trimester (Soldin *et al.*, 2004a, b, c; Soldin, 2006). T4 was not significantly different at any trimester

Table 42.4 Trimester-specific reference intervals for thyroid function tests in pregnancy

Analyte	N	Mean (\pm SE)		Median		Reference intervals				
		ng/dl	nmol/l	ng/dl	nmol/l	2.5th %tile		97.5th %tile		
TT3 (by MS/MS)										
Tri 1 ^a	47	174.0 \pm 6.0	2.7 \pm 0.09	164.0	2.5	94.2	1.45	269.6	4.1	
Tri 2	46	182.6 \pm 6.3	2.8 \pm 0.1	181.0	2.8	106.4	1.6	274.5	4.2	
Tri 3	46	208.2 \pm 7.2	3.2 \pm 0.1	208.0	3.2	103.0	1.6	296.8	4.6	
1-Year postpartum	47	126.0 \pm 4.2	1.9 \pm 0.06	122.0	1.9	78.6	1.2	217.4	3.3	
TT4 (by MS/MS)										
Tri 1	47	10.0 \pm 0.3	127.7 \pm 3.6	10.0	127.7	5.8	74.6	14.4	184.3	
Tri 2	46	10.0 \pm 0.3	127.7 \pm 3.6	10.0	127.7	6.2	79.4	14.7	188.2	
Tri 3	46	10.1 \pm 0.3	129.3 \pm 3.6	10.2	130.6	5.8	74.2	14.2	181.8	
1-Year postpartum	47	7.0 \pm 0.2	89.2 \pm 2.9	6.7	85.8	4.7	60.2	12.0	153.6	
FT4 (by IA)										
Tri 1	46	0.96 \pm 0.03	12.3 \pm 0.4	0.96	12.3	0.26	3.3	1.92	24.6	
Tri 2	46	0.82 \pm 0.02	10.5 \pm 0.3	0.79	10.1	0.59	7.5	1.56	20.0	
Tri 3	45	0.82 \pm 0.02	10.5 \pm 0.2	0.78	10.0	0.65	8.3	1.25	16.0	
1-Year postpartum	46	1.07 \pm 0.04	13.7 \pm 0.5	1.04	13.3	0.77	9.9	2.26	28.9	
TSH (mIU/l)			G. mean (\pm SE) ^b	Median						
Tri 1	46		0.89 \pm 0.08	0.98		0.24		2.99		
Tri 2	45		1.17 \pm 0.08	1.20		0.46		2.95		
Tri 3	45		1.16 \pm 0.08	1.09		0.43		2.78		
1-Year postpartum	47		1.06 \pm 0.07	0.99		0.28		2.94		
Thyroglobulin (ng/ml)			Mean	Median						
Tri 1	47		15.48 \pm 1.96	12.60		1.17		67.10		
Tri 2	46		14.92 \pm 2.05	11.20		1.36		62.27		
Tri 3	46		18.55 \pm 2.84	14.65		3.52		94.15		
1-Year postpartum	46		13.95 \pm 1.6	14.45		2.69		51.70		

Note: All women in this longitudinal study were Swedish, iodine-sufficient and antithyroid-antibodies negative (both TPOAb negative and TgAb negative) throughout the study period. The data analysis excludes women who tested antithyroid-antibodies positive only at 1-year postpartum. These trimester-specific reference intervals represent normal intervals during gestation for T4, T3, FT4, TSH and Tg.

Source: Based on unpublished data and Soldin *et al.*, (2004a, b), Soldin (2006).

^aTri 1 = first trimester (GW = 12); Tri 2 = second trimester (GW = 22); Tri 3 = third trimester (GW = 32); 1-year postpartum = approximately 1-year postpartum.

^bTSH values are geometric means \pm SE.

(all $p > 0.80$) (Soldin *et al.*, 2004a), undoubtedly due to the fact that the first trimester samples were drawn at gestation week (GW) 12, after serum TT4 concentrations had increased. With two exceptions, analyte concentrations tended not to be correlated at each trimester and at 1-year postpartum. One exception was that TT3 and TT4 tended to be associated (all $p < 0.05$) at all time points except the third trimester ($\rho = 0.239$, $p > 0.05$). T4 and FT4 concentrations tended to correlate positively during pregnancy ($\rho = 0.361$ – 0.382 , all $p < 0.05$), but not postpartum ($\rho = 0.179$, $p > 0.05$) (Soldin *et al.*, 2004a, b, c; Soldin, 2006). These trends suggest that trimester-specific measurements of TT3, FT4, Tg and possibly TSH are warranted.

Trimester-Specific Changes in Maternal Thyroid Hormones: Implications for Iodine Nutrition

National surveys to determine iodine sufficiency using UI can be misleadingly reassuring, since the median is usually skewed toward the higher end of the distribution curve of UI excretion. For example, in the United States, a country considered to be iodine-sufficient (median UI was 168 μ g/l in 2001–2002), as many as 15% of the women of childbearing age and almost 7% of pregnant women had iodine excretion levels below 50 μ g/l (Caldwell *et al.*, 2005) (also see Chapter 115 “Iodine Status Reflected by Urinary Concentrations: Comparison with USA and

Other Countries” in this book). In other countries with a known ID, despite national efforts to implement the mandatory use of iodized salt, (e.g., in Switzerland, Italy and Spain), mild-to-moderate ID is still present in certain counties or geographical areas (Als *et al.*, 2000; Caron *et al.*, 1997). In addition, iodine intake may vary unexpectedly, because of significant variations in the natural iodine content of local food and water, and because of the variability in response to supplementation (Nohr *et al.*, 1993; Nohr and Laurberg, 2000). Therefore, a close monitoring of trimester-specific thyroid functions is recommended.

The WHO and the IOM recommended that 220–250 µg/day of iodine is the ideal daily supplement for pregnant women (World Health Organization *et al.*, 2005; Institute of Medicine, 2002). To prevent gestational goitrogenesis, it is recommended that women should ideally be supplemented with an adequate iodine intake of at least 150 µg/day before becoming pregnant. It is reaching a long-term steady state of intrathyroidal iodine stores prior to gestation that prevents the triggering of the thyroid by pregnancy.

Summary

Physiological changes associated with pregnancy require a 40–100% increase in TH synthesis to meet maternal and fetal needs during gestation. Pregnancy has an effect on other thyroid functions, with significant changes in iodine metabolism and clearance. This is particularly relevant in areas of ID. For the maternal thyroid gland to meet the demands of pregnancy, it must have a sufficient supply of iodine and be disease-free.

The fetus is totally dependent on maternal iodine supply throughout gestation, and on thyroxine supply during the first trimester of pregnancy for normal neurological development and nervous system maturation. It is therefore imperative that TH synthesis is adequate and is met with the appropriate iodine intake. Accordingly, it is important to know the trimester-specific reference intervals for THs and other thyroid functions in pregnancy.

In addition to the increased need for iodine intake for TH synthesis, increased iodide clearance results in decreased plasma iodide and an increased requirement for iodide intake. Women who are iodine-sufficient at conception have natural intrathyroidal iodine reserves that should remain euthyroid, unless iodine intake decreases thereby having the rate of increase in iodine loss exceed intake. There is therefore, a need to maintain appropriate iodine dietary supplementation throughout pregnancy. The situation is different in the case of pregnant women who are iodine-deficient. Even though their UI concentrations may increase during early gestation, due to a pregnancy-associated underlying tendency toward ID, a steady decrease in

UI concentration will follow throughout their pregnancy. Trimester-specific data determined by IA and tandem mass spectrometry are now available and can facilitate comparisons across different laboratories and populations. The availability of the normal ranges of TH concentrations throughout pregnancy allows individualized thyroxine therapy, as well as iodine supplementation when necessary.

Summary Points

- Physiological changes associated with pregnancy require a 40–100% increase in TH synthesis to meet maternal and fetal needs during gestation.
- Pregnancy has an effect on other thyroid functions, with significant changes in iodine metabolism and clearance. This is particularly relevant in areas deficient in iodine.
- For the maternal thyroid gland to meet the demands of pregnancy, it must have a sufficient supply of iodine. When increased iodine requirements are not met, TH synthesis is reduced.
- Trimester-specific reference intervals for thyroid functions are especially important, since thyroid insufficiency may be associated with fetal neurodevelopmental deficits and adverse obstetric outcomes.
- The availability of normal ranges of TH concentrations throughout pregnancy allows individualized thyroxine therapy, as well as iodine supplementation when necessary.
- Normal trimester-specific intervals can change with the method of analysis.

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The Relationship between Population Iodine Statistics and Iodine Status of Individuals: A Possible Approach for More Comprehensive Characterization of Iodine Nutritional Status

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Abstract

Monitoring and adjustment of iodine intake is an essential part in preventing thyroid diseases. Therefore, the WHO founded a global micronutrient deficiency information system. The United States is iodine sufficient whereas Europe, especially Eastern Europe, is still below the threshold of iodine sufficiency. The monitoring systems for IDD defined and recommended by the WHO include external monitoring by governments, internal monitoring by producers and distributors, and monitoring at the household level.

Abbreviations

ANOVA	Analysis of variance
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDD	Iodine deficiency disease
TGP	Total goiter prevalence
UI	Urinary iodine excretion
UNICEF	United Nations Children's Fund
WHO	World Health Organization

Previous studies demonstrated an improvement in the general iodine supply in different areas and different age groups in Europe. The underlying reasons for this improvement, and its consequences for thyroid pathologies, are not always obvious.

An adequate long-term monitoring of iodine intake/iodine status and thyroid diseases could be achieved by a program including regular assessments of the population's current iodine status, by performing epidemiological surveys and investigating thyroid disorders in areas with different levels of iodine intake, as well as monitoring the effects of increasing iodine status in subjects.

Urinary iodine was, especially, a suitable method to assess iodine status in a large cohort. Urinary iodine

excretion (UI) is specific and sensitive, and has the advantage of being more accurate than intake estimations derived from dietary questionnaires. However, an overall UI > 100 µg/l (µg/g) did not ensure individual iodine sufficiency in all probands. It was associated with moderate-to-severe iodine deficiency [UI < 50 µg/l (µg/g)] in 21% of the probands. Moreover, our data suggest that it might not be possible to achieve both criteria for iodine sufficiency, e.g., UI between 100 and 200 µg/l (µg/g) and <20% of the study population with UI < 50 µg/l (µg/g), in all investigated cohorts. Therefore, the World Health Organization (WHO) criteria for iodine sufficiency [UI > 100 µg/l (µg/g)] applies to populations and not to individuals. It should be discussed whether it is acceptable to label a population as iodine-sufficient according to the WHO criteria, although up to 20% of the population can be severely iodine deficient. Since all subjects with regular iodine supplementation through iodine tablets showed sufficient UIs according to the WHO criteria, iodine tablets probably remain the most efficient means to ensure adequate iodine intake in Europe, especially in subgroups at increased risk for iodine deficiency, such as adolescents and pregnant or nursing women.

Government programs should gather quantitative and qualitative information on iodine deficiency disease (IDD) indicators, such as goiter prevalence and UI, in all age groups and subgroups with increased risk for iodine deficiency. Assessments could be based on food production surveillance and prospective clinical studies measuring UI, iodine content in daily diets and goiter prevalence. There is a lack of relevant long-term outcome data for dosage and best supply of iodine supplementation in different populations, subpopulations and settings.

Monitoring Iodine Status

More than one-quarter of the world's population suffers from some level of IDD, and especially one-third of the

world's school-age child population have UI levels below $100\mu\text{g/l}$ (Andersson *et al.*, 2005). Clinically significant goiter is common among both young and elderly subjects in iodine-deficient areas (Laurberg, 1994; Laurberg *et al.*, 2000). Since goiter and nodules as the long-term consequences of iodine deficiency, and also the increased incidence of thyroid autonomy due to iodine deficiency, are considerable health problems, it is important to continuously assess a population's iodine status (Laurberg, 1994; Laurberg *et al.*, 2000).

The monitoring systems for IDD defined and recommended by the WHO (2001) include external monitoring by governments, internal monitoring by producers and distributors, and monitoring at the household level. Basic steps to assess iodine intake at the household level are cross-sectional surveys and community-based monitoring (e.g., qualitative rapid test kits used by nutrition officers and others).

Most European countries generally have weak or no governmental programs for monitoring iodine nutrition (Delange *et al.*, 2002; Laurberg *et al.*, 2006). In the United States, iodine has been supplemented in 70% of the table salt sold ($15\text{--}70\mu\text{g}$ iodine/g salt, Lightowler and Davis, 1998; WHO, 2001; Delange, 2002). Additional sources of dietary iodine in the United States are egg yolks, milk and milk products, because of iodine supplementation in chicken feed and the treatment of milk cows and cattle with supplemental dietary iodine to prevent hoof rot and to increase fertility (WHO, 2001). Ninety percent of Latin Americans have access to iodinated salt at the household level (Delange, 2002). However, only 28% of the Europeans had access to iodinated salt in 1999 (Delange, 2002).

Normal dietary iodine intake is $150\mu\text{g/day}$ for adults, as recommended by the WHO (2001).

The assessment of population, as well as individual iodine status, is most often based on three different procedures/indicators (Andersson *et al.*, 2005; WHO, 2001):

- UI.
- Questionnaires estimating iodine uptake.
- Total goiter prevalence (TGP).

UI concentration is a good marker of recent dietary iodine intake, since about 90% of ingested iodine is excreted in the urine, and variances from day-to-day tend to even out among populations (Delange, 1994).

Indicators of lower impact are:

- TGP (not sensitive to recent changes in iodine nutrition).
- Thyroid-stimulating hormone and thyroglobulin (difficulties in obtaining blood samples, difficult to interpret, high cost).

As benign thyroid disease can create high costs for public health systems, the benefit of preventing iodine

deficiency is apparently underestimated by the authorities. According to cost-effectiveness analyses, annual costs for therapies of iodine-deficiency-related benign thyroid disorders amount to 1 bn € a year in Germany (Kahaly and Dietlein, 2002; Dietlein *et al.*, 2003).

The WHO recommendation (WHO, 2001) for monitoring a population's iodine status at the country level is to record salt iodine content and measure UI, thyroid size (palpation/ultrasonography, if available) and neonatal TSH, if available.

Epidemiological Criteria for Assessing the Severity of IDD

Median/mean UI

The assessment of iodine intake is mostly based on urinary excretion, as given in Table 43.1.

Goiter prevalence

The assessment of iodine-deficiency-related diseases is often based on the prevalence of goiter in school-aged children, as given in Table 43.2.

A cut-off point of 5% for nonexistent IDD allows some marginal error in goiter assessment, and is attributed to the fact that goiter can be caused by other agents such as autoimmune thyroid diseases or goitrogens (WHO, 2001).

Survey Methods Monitoring IDD

Periodic prevalence surveys

Large-scale, epidemiologic cross-sectional trials are too cost-intensive to be used as an instrument for regular monitoring of IDD. Instead of cross-sectional trials, periodic prevalence surveys are an adequate method to evaluate changes in goiter prevalence/UI over time (WHO, 2001).

Target groups for surveillance are all age groups, and especially school-aged children, neonates and women of child-bearing age.

Controversies in Determining UI and Confounder for Assessing UI as a Risk Factor for Thyroid Diseases

UI excretion variations

UI concentrations vary within a day and from day-to-day, and seem to be independent of age, gender and season (Soldin, 2002). Considerable variations were demonstrated in all iodine deficiency stages (Soldin, 2002). UI/creatinine ratios reflect iodine status better than UI concentrations

Table 43.1 Assessment of iodine intake based on urinary excretion

Urinary iodine excretion ($\mu\text{g/l}$ or μg iodine iodine/g creatinine) ^a deficiency stage		Assessment of populations iodine intake/nutrition status
>300	–	Excessive/risk of adverse health consequences such as iodine-induced hyperthyroidism, autoimmune thyroid disease
200–299	–	More than adequate/risk of iodine-induced hyperthyroidism within 5–10 years in susceptible groups
100–199	0	Adequate/optimal
50–99	I	Insufficient/mild iodine deficiency
25–49	II	Insufficient/moderate iodine deficiency
<25	III	Insufficient/severe iodine deficiency

Note: The table was modified according to the WHO (2001) and summarizes the assessment of iodine intake based on urinary iodine excretion.

^aIf there is a normal distribution of the data, conversion of the WHO values (mg/l) into micrograms iodine per gram of creatinine leads to equivalent iodine deficiency stages I (II, III, Brauer *et al.*, 2005). This fact gained importance, since determination of iodine/creatinine ratios in spot urine samples has been suggested as the gold standard for the assessment of iodine status of a population (Hetzel, 1993; WHO, 2001) and the correlation to creatinine allows a 24 h estimation.

Table 43.2 Assessment of iodine deficiency-related diseases based on the prevalence of goiter in school-aged children

Iodine deficiency stage based on	None	Mild	Moderate	Severe
Median urine iodine ($\mu\text{g/l}$)	>100	50–99	20–49	<20
Goiter prevalence	<5%	5–20%	20–30%	>30%

Note: The table was modified according to the WHO (2001). The assumption of goiter prevalence is based on urinary iodine excretion in school-aged children.

alone. UI/creatinine ratios are highly reliable within 24 h (Soldin, 2002). Spot UI/creatinine ratios are of value to describe a population’s iodine status when used in cross-sectional studies of appropriate sample size (Brauer *et al.*, 2005; Soldin, 2002).

Age and physical activity

UI, as determined by the iodine/creatinine ratio, is influenced by muscle mass and physical activity (Manz *et al.*, 2002; Nohr *et al.*, 1993), and may be underestimated in younger and overestimated in older study populations, since creatinine excretion is negatively correlated with age.

Other goitrogens

Although iodine status is the major environmental factor that determines goiter incidence and prevalence, other goitrogens have been identified. Tobacco smoking, especially due to thiocyanate inhalation (Brauer *et al.*, 2006a), seems to increase thyroid volume, depending on the underlying iodine status (Knudsen *et al.*, 2002). Voelzke *et al.* (2005) found an association between current smoking and goiter in both

genders. Knudsen *et al.* (2002) suggested that reversibility in smoking-induced thyroid volume increases particularly in young subjects. Neither long-term nor intermittent, high exposure to perchlorate seems to induce hypothyroidism or goiter in adults (Braverman *et al.*, 2005).

Alcohol consumption is associated with a lower prevalence of goiter and thyroid nodules (Knudsen *et al.*, 2002). The mechanism behind this association remains unclear. Probably, selenium does not significantly influence thyroid volume in at least borderline iodine-sufficient populations (Brauer *et al.*, 2006b). Moreover, gender, age and parity can play a role in the pathogenesis of goiter (Knudsen *et al.*, 2002), as discussed later in this article.

Statistical Considerations

Description of an overall population iodine status

Investigators aim to give an exact measure of a population’s iodine status by the statistical central tendency of UI, because iodine deficiency is considered to be a major health problem in countries in which median/mean UI is below 100 ($\mu\text{g/l}$). Therefore, it is of great value to define the iodine status as exactly as possible, especially in borderline-deficient/sufficient settings. As mentioned by Brauer *et al.* (2005), Manz (2002) and Gunton *et al.* (1999), the exact value of a population’s (>500 subjects) iodine status is better described by using mean values than median values, as mean values better describe metric variables than median values. The underlying statistical assumption for using means instead of medians is a symmetric distribution of the data. Median values containing considerable outliers should not be pooled and used in meta-analyses, since this could lead to false results (Andersson *et al.*, 2005).

Current Global Iodine Population Status

Monitoring and adjustment of iodine intake is an essential leasure in preventing thyroid diseases (Andersson *et al.*, 2005; Laurberg *et al.*, 2006). Therefore, the WHO founded a global micronutrient deficiency information system. The database on iodine deficiency (available at: http://www3.who.int/whosis/mn/mn_iodine/mn_iodine_database.cfm?path=whosis,mn,mn_iodine,mn_iodine_data,mn_iodine_data_database&language=english) summarizes data by country on goiter prevalence and/or urinary iodine. The US is iodine sufficient whereas Europe, especially Eastern Europe, is still below the overall threshold of iodine sufficiency.

Iodine Status in Different Age Groups

Current status in Germany is representative of the borderline iodine-deficient Central Europe Region

Several authors readdressed the issue of iodine supply in different age groups. Gaertner *et al.* (2001) investigated UI in selected risk groups for iodine deficiency. The mean daily iodine excretion was 134 µg/day in 278 men aged 50–70 years, 117 µg/day in 288 women aged 50–70 years, 125 µg/day in 772 conscripts and 74 µg/day in 53 breast-feeding women. Rendl *et al.* (2001) have investigated a group of schoolchildren in Würzburg and found that the proportion of samples with concentrations below 100 µg/l or below 50 µg/l was 15.4 and 4.3%, respectively. Liesenkotter *et al.* (1997) have screened a cohort of children aged 3–15 years from Berlin and found a mean iodine concentration of 115.8 µg iodine/g creatinine. There has been progress toward overcoming previous iodine deficiency (UI ≤ 100 µg/l, Hampel *et al.*, 1995), since the iodine status of the more recent cohorts (Gaertner *et al.*, 2001; Rendl *et al.*, 2001; Liesenkotter *et al.*, 1997) was above the defined cut-off level by the WHO (UI ≥ 100 µg/l, WHO, 2001, Table 43.1). However, continued efforts are needed to monitor risk groups, since even overall iodine sufficiency (UI = 183 µg/l, Rendl *et al.*, 2001) implies that >15% of the investigated subjects are still iodine deficient (Rendl *et al.*, 2001). Also, Voelzke *et al.* found moderate-to-severe iodine deficiency (UI < 50 µg/l) in 11% and mild iodine deficiency (UI < 100 µg/l) in at least 37% of the subjects, although the overall UI was 124 µg/l (Voelzke *et al.*, 2003). Long-term monitoring studies would allow action against iodine deficiency in an appropriate and timely manner in specific subgroups at risk for iodine deficiency. Moreover, even in mild iodine deficiency there is an increased risk for development of goiter and hyperthyroidism, especially in middle-aged and elderly subjects (Laurberg *et al.*, 2001). Five percent of the general population showed clinical or

subclinical thyroid dysfunction in a Danish region with borderline iodine sufficiency (UI = 103 µg/day; Knudsen *et al.*, 1999). Pregnant women in regions with mild iodine deficiency, without further iodine supplementation during pregnancy, are an important subgroup at risk. Mild iodine deficiency during pregnancy can lead to mild thyroid hormone deficiency and impaired intellectual development of the child (Antonangeli *et al.*, 2002; Laurberg *et al.*, 2001, 2006; Morreale de Escobar and Escobar del Rey, 2006). This risk is even higher in pregnant women with postpartum thyroiditis after a preceding pregnancy that will often decrease the thyroid reserve, and will thus often require, thyroid hormone treatment during pregnancy additional (Antonangeli *et al.*, 2002; Laurberg *et al.*, 2006; Morreale de Escobar and Escobar del Rey, 2006).

A Possible Approach for Full Characterization of Iodine Nutrition Status

How to achieve the WHO requirements?

Previous studies (as mentioned above) demonstrated an improvement in the general iodine supply in different areas and different age groups. The underlying reasons for this improvement (e.g., altered nutrition, use of iodinated salt) and its consequences for thyroid pathologies are not always obvious. To clearly assess a population's iodine status, the WHO recommended monitoring the iodine content in salt at the production level, measurement of urinary iodine concentration and carrying out surveys in large cohorts under local circumstances as the most suitable method for iodine status assessment (WHO, 2001).

Which goals should be achieved?

An extensive monitoring of iodine intake/iodine status and thyroid diseases could be achieved by a government program in Denmark (Laurberg *et al.*, 2006). This program includes regular assessments of the population's current iodine status, by performing epidemiological surveys and investigating thyroid disorders in areas with different levels of iodine intake, as well as monitoring the effects of increasing iodine status in subjects. Moreover, the Danish program also provides means to clarify the influence of various environmental factors, such as selenium, on IDD (Laurberg *et al.*, 2006).

Periodic prevalence surveys for goiter are an adequate method to evaluate changes.

To assess a local population's iodine status, according to the WHO requirements, by measurement of UI and estimation of nutritional iodine intake and TGP, we used a comprehensive scheme to evaluate iodine status (Brauer *et al.*, 2005) in Leipzig, a previously iodine-deficient area (Gruning *et al.*, 2001; Hampel *et al.*, 1995). We combined the investigation of different sources of iodine and different

approaches to get a more comprehensive picture and to assess the limitations of each approach to evaluate the iodine status by:

- Description of the influence of clearly characterized iodine-containing food on UI.
- Description of the influence of iodine-containing tablets on UI.
- Use of high-resolution thyroid ultrasound to evaluate the prevalence of thyroid nodules and goiter.
- Additional assessment of sociodemographic characteristics to define risk factors for goiter, as well as to estimate other sources of iodine nutrition (iodine-rich foods).
- Determination of the impact of regularly consumed canteen meals and quantification of their iodine content.
- Evaluation of iodine status in a clearly described (age, gender, smoking, medical history, concomitant medication), large sample size population ($n > 800$) in a cross-sectional survey.

Thus, we screened a cohort of 805 employees and students of the University of Leipzig, an East German city in

a former iodine-deficient area (Meng *et al.*, 1986; Gruning *et al.*, 2001; Hampel *et al.*, 1995) for the prevalence of nodular thyroid disease. In this cohort, we analyzed UI *per se*, as well as in relation to nutritional iodine supply by university canteen meals. The actual iodine content of representative canteen meals was determined. Moreover, we assessed the consumption of foods with low or high iodine content by a special food questionnaire. In addition, we inquired about regular intake of iodine tablets as an important source of iodine. Previous studies did not assess the use of iodine tablets in their study subjects (Heinisch *et al.*, 2002; Knudsen *et al.*, 2002; Voelzke *et al.*, 2003).

Results of a Comprehensive Prospective Assessment of Iodine Status in Leipzig

Urinary iodine

Mean overall UI was $109 \pm 81 \mu\text{g}$ iodine/g creatinine ($\mu\text{g/g}$). It differed significantly for males ($79 \pm 44 \mu\text{g/g}$) and

Table 43.3 Iodine excretion, intake and thyroid disorders of the study population

	Males	Females	All subjects
Population ^a	156 (19.4%)	649 (80.6%)	805 (100%)
Age ^b (years)	31.33 ± 12.07	35.42 ± 12.44	34.63 ± 12.46
Thyroid volume (ml) ^b	15.42 ± 10.28	12.12 ± 7.13	12.76 ± 7.94
Goiter ^a	11 (7%)	81 (12.5%)	92 (11.4%)
Thyroid nodules ^a	30 (19.9%)	201 (31.9%)	231 (29.6%)
Spot urine samples available ^a	153 (99.3%)	639 (98.5%)	792 (98.4%)
Overall iodine excretion (μg iodine/g creatinine) ^b	79 ± 44	116 ± 86 ^c	109 ± 81
Excessive iodine intake ^a (urinary excretion $> 300 \mu\text{g}$ iodine/g creatinine)	0	26 (4%)	26 (3%)
More than adequate iodine intake ^a (urinary excretion 200–299 μg iodine/g creatinine)	2 (1%)	67 (11%)	69 (9%)
Adequate iodine intake ^a (urinary excretion 100–199 μg iodine/g creatinine)	43 (28%)	201 (31%)	244 (31%)
Mild iodine deficiency ^a (urinary excretion 50–99 μg iodine/g creatinine)	64 (42%)	219 (34%)	283 (36%)
Moderate-to-severe iodine deficiency ^a (urinary excretion $< 50 \mu\text{g}$ iodine/g creatinine)	44 (29%)	125 (20%)	169 (21%)
Daily iodine tablet ^a	4 (2.6%)	66 (10%)	70 (9%)
History of iodine-containing contrast media ^a	18 (11.5%)	86 (13.3%)	104 (13%)
Radioiodine therapy ^a	1 (0.6%)	3 (0.5%)	4 (0.5%)
Other iodine-containing medication ^a	1 (0.6%)	12 (1.8%)	13 (1.6%)
Thyroidectomy ^a	2 (1.3%)	14 (2.2%)	16 (2%)
Pregnancy/lactation ^a		27 (4%)	

Note: The table was modified according to Brauer *et al.*, (2005).

^aNumber of patients.

^bMean ± standard deviation.

^cOne female with history of iodine-containing X-ray after 01.01.02 and urinary iodine excretion $> 2000 \mu\text{g}$ iodine/g creatinine excluded.

Table 43.4 Subgroup analysis of iodine excretion at different ages

Years	Males	Females	All subjects
Age <25			
Spot urine samples available ^a	63	176	239
Iodine excretion (μg iodine/g creatinine) ^b	78.3 \pm 42.15	101.96 \pm 70.66	95.73 \pm 65.14
Age 25–35 years			
Spot urine samples available ^a	35	128	163
Iodine excretion (μg iodine/g creatinine) ^b	63.10 \pm 43.15	113.10 \pm 91.33	102.36 \pm 85.76
Age 35–45 years			
Spot urine samples available ^a	13	117	130
Iodine excretion (μg iodine/g creatinine) ^b	107.19 \pm 65.27	103.1 \pm 67.96	103.5 \pm 67.46
Age 45–55 years			
Spot urine samples available ^a	13	91	104
Iodine excretion (μg iodine/g creatinine) ^b	73.39 \pm 41.70	125.75 \pm 94.08	119.21 \pm 90.77
Age 55–65 years			
Spot urine samples available ^a	11	59	70
Iodine excretion (μg iodine/g creatinine) ^b	83.34 \pm 24.63	156.83 \pm 93.21	145.29 \pm 90.08

^aNumber of patients.^bMean \pm standard deviation (Brauer *et al.*, 2005).

females (116 \pm 86 $\mu\text{g}/\text{g}$) (*t*-test, $p < 0.001$). The proportion of subjects within the ranges of iodine deficiency stages, defined according to the WHO, are given in Table 43.3.

In spite of a mean UI within the range of iodine sufficiency, 36% of the study subjects had mild iodine deficiency with UIs between 50 and 99 $\mu\text{g}/\text{g}$. Moreover, 21% of the study population had moderate-to-severe iodine deficiency [UI < 50 $\mu\text{g}/\text{l}$ ($\mu\text{g}/\text{g}$)]. It should, therefore, be discussed urgently whether it is acceptable to label a population as iodine sufficient according to the WHO criteria even though up to 20% of the population can be severely iodine deficient and 36% can be mildly iodine deficient.

UI ($\mu\text{g}/\text{g}$) correlated positively with age ($p < 0.01$, Spearman $\rho = 0.151$). As expected, the creatinine excretion correlated negatively with age of subjects ($p < 0.01$, Spearman $\rho = -0.232$).

The UI in different age groups is shown in Table 43.4. UI in females rises with increasing age.

Iodine content in meals

One hundred and four (28 males and 76 females) of the 805 subjects in our cohort had regular lunches, defined as ≥ 3 times per week, at the university canteen since it opened in 1997.

Analysis of the iodine content in representative meals prepared with iodinated salt at the university canteen showed a mean iodine content of 72 $\mu\text{g}/\text{meal}$, corresponding to nearly half of the WHO recommended daily iodine intake for adults (150 $\mu\text{g}/\text{day}$) (WHO, 2001). Of note, iodine content in the individual menus differed considerably, ranging from 18 to 136.5 $\mu\text{g}/\text{meal}$ (Table 43.5).

Comparison of UI between subjects having regular lunches at the university canteen (95 \pm 64 $\mu\text{g}/\text{g}$) and a matched (age, gender, history of iodine exposure and intake of iodine tablets) control group of 400 subjects

(103 \pm 62 $\mu\text{g}/\text{g}$) who did not eat at the university canteen did not attain statistically significant differences.

Influence of nutrition as assessed by a questionnaire on UI

The analysis of the food questionnaire did not reveal a statistically significant influence of a single nutritional determinant (milk, meat, fish and cereal products) on the UI. According to our experience, the discriminatory power of a questionnaire is low. For example, 89% of all 805 subjects reported the use of iodinated salt at home (≥ 3 times per week) and consumption of fish (≥ 1 times per week) was reported by 98% of the participants. This is very unlikely, based on previous investigations in Germany (Metges *et al.*, 1996).

Influence of iodine tablets and smoking as confounders on UI

An important point was that daily intake of iodine tablets was reported by 70 subjects ($n = 66$ females, $n = 4$ males) in the study population. The underlying reasons for iodine medication were a history of goiter ($n = 39$) and/or thyroid nodules ($n = 31$) in 68 participants. The UI was significantly higher in urine samples of these subjects (169 \pm 130 $\mu\text{g}/\text{g}$), compared to 722 controls without intake of iodine tablets (103 \pm 87 $\mu\text{g}/\text{g}$, *t*-test, $p < 0.05$). Of note, iodine tablet intake in only 8% of the study population led to an increase of the mean overall UI from 103 \pm 87 $\mu\text{g}/\text{g}$ to 109 \pm 81 μg iodine/g creatinine ($\mu\text{g}/\text{g}$).

Mean thyroid volume was higher in smokers (13.9 ml) than in nonsmoking subjects (12.5 ml), without reaching statistical significance (*t*-test, $p = 0.085$, 2-tailed). Thyroid volume correlated positively with the reported pack/years of the smokers [Pearson correlation coefficient 0.124 significant at the 0.01 level (2-tailed)].

Table 43.5 Iodine content in 14 representative menus from the university canteen

No.	Menu	Weight (g)	I/100g menu (μg)	I/menu (μg)
I	Two eggs, potatoes, mustard sauce and broth	553	12	66.4
II	Meatball, potatoes and French beans	457	13.7	62.6
III	Pork chop, mashed potatoes, sauerkraut and yoghurt	670	14.1	94.5
IV	Herbal curd, butter, potatoes, leek soup and yoghurt	520	16.9	88.1
V	Deep fried pork, mashed potatoes and peas/carrots	379	18.5	70.2
VI	Fricassee with rice	327	15.4	50.4
VII	Russian soup with sausage and chocolate-pudding	635	18.4	117
VIII	Fish-fillet, mashed potatoes and sauce	410	22.5	92.1
IX	Roast, potatoes, sauerkraut, gravy and kiwifruit	533	8.3	44
X	Vegetarian cutlet, cauliflower and pasta	485	3.7	18
XI	Pasta, soup and chocolate-pudding	639	9.6	61.2
XII	Rib (pork), potatoes and French beans	442	11.8	52.2
XIII	Stew and rice with peas/carrots and kiwifruit	427	13.9	59.2
XIV	Soufflé (potatoes, tomatoes, cheese, sausage, and haricot beans)	665	20.5	136.5
^a	Juice	200	0.3	0.6

Notes: Weight \equiv overall weight of the menu and weights of the meal constituents; I/100g menu \equiv iodine content in 100g of the menu (average); I/menu \equiv overall iodine content of the selected menu.

^aSupplementary to every menu. Brauer *et al.*, (2005).

Prediction of thyroid volume

We evaluated whether the parameters age, smoking, gender, pregnancy or nursing period and urinary iodine can predict variable thyroid volume. Linear regression analysis (analysis of variance, ANOVA) revealed a prediction of thyroid volume by age ($p < 0.001$), gender ($p < 0.001$) and smoking ($p < 0.05$).

Despite borderline iodine sufficiency in the overall Leipzig population ($n = 805$), goiter was present in 92 (11.4%) subjects (7% males, 12.5% females). Thyroid nodules were found in 231 (29.6%) subjects ($n = 156$, single nodule; $n = 75$, >1 nodule), including 68 subjects (6%) with known thyroid disease.

As compared to our recent findings (Brauer *et al.*, 2005) with 21% of subjects with UI below $50 \mu\text{g}/\text{l}$, previous investigators in Germany reported a lower frequency. Voelzke *et al.* (2003) reported a frequency of 11% of subjects with UI below $50 \mu\text{g}/\text{l}$, and Gruning *et al.* (2001) reported a frequency of 12% of males and 8% of females with moderate-to-severe iodine deficiency in an adult population in Saxony in 1996. Rendl *et al.* (2001) found an overall frequency of 4% of moderate-to-severe iodine deficiency in a younger population in Bavaria. Comparing previous data for goiter prevalence of 32% in 1996 and 25% in 1997 (Gruning *et al.*, 2001) with our recent findings of 11%, there is a substantial decrease in goiter prevalence in Germany since 1996 (Gruning *et al.*, 2001).

UI is widely used and accepted as an indicator of nutritional iodine intake (Rasmussen *et al.*, 2001; Knudsen *et al.*, 2002; Voelzke *et al.*, 2003; Laurberg *et al.*, 2006). The mean UI in our cohort was within the lower range of adequate iodine intake (Brauer *et al.*, 2005; WHO, 2001).

The WHO (2001) criteria for adequate iodine status of a population are:

- UI between 100 and $200 \mu\text{g}/\text{l}$ ($\mu\text{g}/\text{g}$); and
- $<20\%$ of the study population with $\text{UI} < 50 \mu\text{g}/\text{l}$ ($\mu\text{g}/\text{g}$).

Applying these criteria, we found that only a small proportion (31%) of our study group reached an optimal iodine intake ($100\text{--}200 \mu\text{g}/\text{g}$), even when overall iodine excretion was sufficient (Brauer *et al.*, 2005). In our study population, an unexpectedly high proportion (21%) had UIs within the ranges of moderate-to-severe iodine deficiency ($<50 \mu\text{g}/\text{g}$, Table 43.1, Brauer *et al.*, 2005). Therefore, in the Leipzig cohort, iodine sufficiency of the overall study population according to the WHO criteria (WHO, 2001) allows moderate-to-severe iodine deficiency ($<50 \mu\text{g}/\text{g}$, Table 43.1) of a considerable part of the cohort (Brauer *et al.*, 2005). Consequently, the iodine status of the cohort cannot be decided according to the WHO criteria for adequate iodine status, e.g., UI between 100 and $200 \mu\text{g}/\text{l}$ ($\mu\text{g}/\text{g}$) and $<20\%$ of the study population with $\text{UI} < 50 \mu\text{g}/\text{l}$ ($\mu\text{g}/\text{g}$). Therefore, the lower threshold for iodine sufficiency of a population with $100 \mu\text{g}/\text{g}$ (Table 43.1) needs to be questioned, especially if there are further environmental or genetic goiter risks in a population (Laurberg *et al.*, 2001; Brauer *et al.*, 2005).

Several authors (Rasmussen *et al.*, 2001; Knudsen *et al.*, 2002; Voelzke *et al.*, 2003) stated that food questionnaires are reliable instruments to assess a population's iodine intake. However, there are indications that self-estimations concerning iodine intake recorded on a diary card are unreliable when compared with iodine intakes obtained by direct analysis of real diets (Lightowler and Davis, 2002).

Accordingly, investigators should estimate iodine intake through defined sources of this trace element (i.e., meals prepared with iodized salt, iodine tablets, or exposure to contrast medium), since at least 20% of European study populations are exposed to such sources of iodine intake (Brauer *et al.*, 2005).

The consumption of (canteen) meals prepared with iodized salt, is theoretically able to introduce a stable source of iodine intake. However, even regular consumption of canteen meals, for which an average iodine content of 50% of the daily recommended intake was determined, did not result in higher UI in 104 subjects, compared to 400 matched participants who did not eat at the canteen (Brauer *et al.*, 2005). This is also reflected by the results of a special food questionnaire, since no single nutritional determinant contributing to adequate UI could be identified (Voelzke *et al.*, 2005; Brauer *et al.*, 2005). This indicates a lack of discrimination by food questionnaires for the identification of nutritional habits of possible importance for UI. This also appears to be the case for the use of iodinated salt in food production, which is not under investigation. Moreover, it has been established that household usage of iodinated salt with an average iodine content of 20 µg iodine/1 g salt is not sufficient to ensure adequate intake at the individual level (Manz *et al.*, 2002). This assumption is based on calculations that the iodine status of a population's subgroup, especially males younger than 35 years of age and older than 45 years of age, was below the WHO recommendations with an iodine intake deficit of 30% (Table 43.4, Brauer *et al.*, 2005; Manz *et al.*, 2002). Iodized salt is indeed effective in increasing the iodine status of a population, but no conclusions can be drawn about improvements in other, more patient-oriented outcomes, such as physical and mental development in children and mortality (Wu *et al.*, 2002).

Regular intake of iodine tablets significantly raises iodine excretion, in comparison to subjects who do not consume iodine tablets (Brauer *et al.*, 2005). Also, Gaertner *et al.* (2001) reported higher levels of iodine excretion in 17 lactating women (114 µg/g) using iodine tablets, compared to a control group of 53 women (74 µg/g), who were not using iodine tablets. Since UI was significantly influenced by intake of iodine tablets, this may lead to a bias of the statistical analysis in a study population, if the percentage of subject with regular iodine tablet intake is not known or not given, as it was the case in many previous studies (Gruning *et al.*, 2001; Heinisch *et al.*, 2002; Voelzke *et al.*, 2005).

Iodine intake level (based on UI) affects the type of benign thyroid disease (Laurberg *et al.*, 2001). A number of studies (Laurberg *et al.*, 2001; Suzuki *et al.*, 1975; Stanbury *et al.*, 1998) indicated the following specific associations: a high iodine intake causes endemic goiter (Suzuki *et al.*, 1975). A switch from low to sufficient iodine

intake raises the incidence of hyperthyroidism, depending on the severity of the pre-existing iodine deficiency (Stanbury *et al.*, 1998). Severe iodine deficiency causes the development of brain disorders, as well as goiter, and has to be corrected immediately (Laurberg *et al.*, 2001). Regular monitoring and adjustment of iodine intake in the population is important, since small differences in the intake level have profound effects on the prevalence of thyroid diseases (Laurberg *et al.*, 2006; Brauer *et al.*, 2005). Up to now it is unclear how goiter, thyroid nodules and autoimmune thyroiditis change during noncoordinated supplementation programs, since iodine-containing salt supply is not under investigation in some European countries (Voelzke *et al.*, 2003; Brauer *et al.*, 2005). Development of iodine-induced hyperthyroidism can easily be overlooked if symptoms only are assessed (Wu *et al.*, 2002).

Brauer *et al.* (2005) and Reiners *et al.* (2004) found a frequency of goiter (11%) below the ranges of previous investigations in Germany dating from 1995 to 2001 (>30%, >30%, and 36%) using the same cut-off values for defining goiter as in the previous investigations (Hampel *et al.*, 1995, Gruning *et al.*, 2001; Voelzke *et al.*, 2003). One can speculate that the decrease in goiter prevalence in Germany and the Central Europe Region (Vella, 2005) resulted from an improvement in the general iodine supply over the past years (Barker and Phillips, 1984; Manz *et al.*, 2002; Laurberg *et al.*, 2000; Vella, 2005). However, this contrasts with considerable increases in current goiter prevalence (Table 43.6) in 192 member states, in comparison to estimates from 1993 reported by the WHO for Africa, the Eastern Mediterranean and Eastern Europe (Andersson *et al.*, 2005). The increase in goiter prevalence in Europe is mostly based on data from Eastern Europe and overestimation in large countries, such as Spain (Andersson *et al.*, 2005). Moreover, there is a bias in the data (Table 43.6),

Table 43.6 Change in total goiter prevalence between 1993 and 2003 by WHO region

WHO region based on 192 member states	General population goiter prevalence (%)		Percentage change
	1993	2003	
Africa	15.6	28.3	+81.4
Americas	8.7	4.7	-46.0
Eastern Mediterranean	22.9	37.3	+62.9
Europe	11.4	20.6	+80.7
South-East Asia	13.0	15.4	+18.5
Western Pacific	9.0	6.1	-32.2
Total	12.0	15.8	+31.7

Note: The illustration of changes in total goiter prevalence between 1993 and 2003 by WHO region was modified according to Andersson *et al.*, (2005).

since sensitivity and specificity are low, as TGP was assessed by palpation only.

What is the Meaning of Population Iodine Statistics (UI) for Individual Iodine Status?

UI that indicates adequate iodine intake at the population level

The WHO recommends limiting UI $< 50 \mu\text{g/l}$ to $< 20\%$ of individuals in a population. Some authors (Delange *et al.*, 2002) stated that a threshold of $100 \mu\text{g/l}$ (UI) would achieve this goal. This statement (Delange *et al.*, 2002) is based on a retrospective setting using a questionnaire on frequency distribution of urinary iodine in iodine-replete populations. However, the Leipzig prospective clinical trial investigating UI and the prevalence of thyroid disorders found UI $< 50 \mu\text{g/l}$ in 21% of subjects, moderate-to-severe iodine deficiency [UI $< 50 \mu\text{g/l}$ ($\mu\text{g/g}$)] in 21%, and mild iodine deficiency (urinary excretion $50\text{--}99 \mu\text{g}$ iodine/g creatinine) in 36% of the subjects, although it met the overall WHO criteria UI $100\text{--}200 \mu\text{g/l}$ ($\mu\text{g/g}$) for adequate/optimal iodine intake with a mean value of $109 \pm 81 \mu\text{g}$ iodine/g creatinine. This mean value only ensured individual iodine sufficiency in 43% of the subjects (Brauer *et al.*, 2005). The most likely reasons for this observation are that using iodinated salt at a household level with an iodination level between 15 and $25 \mu\text{g}$ iodine/g salt, such as in Germany (Federal Ministry for Nutrition and Agriculture, http://www.bmelv.de/eln_045/nn_749972/sid_D993051251EFC5D6585366AF691019A4/DE/03-Ernaehrung/01-Aufklaerung/Jodversorgung.html__nnn=true), is not enough to ensure adequate iodine supplementation. The application of iodine tablets did lead to iodine sufficiency for all treated individuals (Brauer *et al.*, 2005). Similar data were reported for the Dan Thy Study. A median UI of $101 \mu\text{g/l}$ was associated with $< 50 \mu\text{g/l}$ in 20% of the study population, and iodine-containing supplements were associated with significant differences in urinary iodine excretion (Rasmussen *et al.*, 2008). Also, Soldin *et al.* (2003) showed wide ranges for urinary iodine, from 30 to $> 500 \mu\text{g}$ iodine/g creatinine in individuals without thyroid diseases in iodine sufficiency in the United States. Therefore, the WHO criteria for iodine sufficiency [UI $> 100 \mu\text{g/l}$ ($\mu\text{g/g}$)] apply to populations and not to individuals. It should be urgently discussed whether it is acceptable to label a population as iodine sufficient according to these WHO criteria even though up to 21% of the population can be severely iodine deficient and 36% can be mildly iodine deficient. Moreover, our data suggest that it might not be possible to achieve both criteria for iodine sufficiency, e.g., UI between 100 and $200 \mu\text{g/l}$ ($\mu\text{g/g}$) and $< 20\%$ of the study population with UI $< 50 \mu\text{g/l}$ ($\mu\text{g/g}$) in all investigated cohorts.

Summary Points

- Urinary iodine is a suitable method to assess the iodine status in a large cohort. UI is specific and sensitive, and has the advantage to be more accurate than intake estimations derived from dietary surveys (Knudsen *et al.*, 2002; Brauer *et al.*, 2005; Laurberg *et al.*, 2006; Ovesen and Boeing, 2002).
- Despite widely available information on the effective prevention of iodine-deficiency-related thyroid diseases, there is still borderline deficiency in the Central Europe Region at the population level. Iodine supply through salt/usage in the food industry with an iodination level between 15 and $25 \mu\text{g}$ iodine/g salt, such as in Germany, is associated with moderate-to-severe iodine deficiency (urinary excretion $< 50 \mu\text{g}$ iodine/g creatinine) in 21% of the investigated population and is not sufficient to eradicate iodine deficiency in all individuals.
- Since subjects with regular iodine supplementation through iodine tablets show sufficient UIs according to the WHO criteria (Brauer *et al.*, 2005), iodine tablets probably remain the most efficient means to ensure adequate iodine intake in Europe, especially in subgroups at increased risk for iodine deficiency, such as adolescents and pregnant or nursing women (Gaertner *et al.*, 2001; Nohr *et al.*, 1993).
- Governmental programs should gather quantitative and qualitative information on IDD indicators, such as goiter prevalence and UI, in all age groups and subgroups with increased risk for iodine deficiency, to further adjust iodination programs.
- Iodine status assessments should also be based on food production surveillance and prospective clinical studies measuring UI, iodine content in daily diets and goiter prevalence.
- There is a lack of relevant long-term outcome data for the optimal dosage and best supply of iodine supplementation in different populations, subpopulations and settings.

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Variations in Iodine Excretion in Healthy Individuals

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Abstract

Variation in urinary iodine excretion is important because it influences the study of iodine nutrition. Urinary iodine excretion exhibits large variations. The components of variation include preanalytical, analytical and biological variations. Biological variation consists of between- and within-individual variations, and can be broken down into chronobiological variation, i.e., diurnal and seasonal variations, and variations related to dilution, dietary peculiarities and supplement use. This is important for the evaluation and planning of studies of iodine nutrition, and it can be calculated that 500 urine samples depict population iodine nutrition level within $\pm 5\%$, while 125 urine samples are required for a value of $\pm 10\%$. Estimating 24 h urinary iodine excretion lowers variation, and consequently the number of urine samples needed is reduced by around 20%. Similarly, it can be calculated that less than 10–15 urine samples from an individual may be misleading.

Abbreviations

ANOVA	Analysis of variance
CI	Confidence interval
CV	Coefficient of variation
IQR	Interquartile range
n	Number of sample
TSH	Thyroid-stimulating hormone
x	A measured value
μ	Mean value
σ	Variance

Introduction

Iodine excretion varies markedly within individuals and within groups. The variation in biological measures consists of analytical and biological variations (Harris *et al.*, 1970; Fraser and Harris, 1989; Andersen *et al.*, 2003). Biological variation is divided into two components: variation within an individual and variation between individuals. The former is characterized

by rhythmic aberrations of multiple frequencies, and the latter is caused by different set points around which each individual varies (Harris *et al.*, 1970; Andersen *et al.*, 2003).

This chapter aims to provide a description of these variations in urinary iodine excretion, some components and determinants of this variation, and the importance of variation for the interpretation of measurements of iodine excretion used to describe iodine nutrition in groups and in individuals.

The following questions are to be answered:

- What is variation in an analyte?
- What is the variation in iodine excretion?
- What lessons can be learned from variation in iodine excretion?

Components of Variation

Variation in biological measures is described by a number of factors. The components included in the measurement of an analyte can be described by the following equation.

$$x = \mu_{\text{group}} + \sigma_{\text{preanalytical}} + \sigma_{\text{analytical}} + \sigma_{\text{between-individual}} + \sigma_{\text{within-individual}} + \sigma_{\text{others}}$$

where x is a measured value of urinary iodine content, μ_{group} the overall group mean value, $\sigma_{\text{preanalytical}}$ the variance due to preanalytical conditions, $\sigma_{\text{analytical}}$ the variance due to analytical errors, $\sigma_{\text{between-individual}}$ the inter-individual variance, $\sigma_{\text{within-individual}}$ the intra-individual variance, and σ_{others} the random variation not accounted for. Biological variation consists of $\sigma_{\text{between-individual}} + \sigma_{\text{within-individual}}$ (Andersen *et al.*, 2003, with permission).

The measured value, x , is determined by an overall group mean, μ_{group} . Deviation from this grand mean value is caused by the sum of components of variation (Andersen *et al.*, 2003; Fraser and Harris, 1989). Minimizing each of these components improves the precision of the measured value, x .

Preanalytical variation ($\sigma_{\text{preanalytical}}$)

Recommendations are that preanalytical conditions should be standardized, in order to minimize preanalytical variation

Table 44.1 The analytical goals (CV%) calculated from data on biological variation in a routine laboratory setting (calculated from Andersen *et al.*, 2001)

Content in urine	Analytical goals	
	CV% \leq^a	CV% \leq^b
Iodine	24.0	27.8
TSH	11.6	21.9
TT3	6.8	10.2
TT4	5.1	9.7

^aCalculated from $CV_{\text{analytical}} \leq 1/2CV_{\text{intra-individual}}$ Fraser, (1983).

^bCalculated from $CV_{\text{analytical}} \leq 1/2CV_{\text{intra-individual+inter-individual}}$ Browning *et al.*, (1986).

in the study of biological variation (Fraser and Harris, 1989): samples should be collected at a specific hour, by the same individual, or from a fasting, rested individual. However, highly standardized preanalytical conditions hamper the external validity, and the use of such data is limited. Hence, a study of variation in iodine excretion to be used for evaluating methods in clinical practice will portray iodine excretion in unstandardized spot urine collection, i.e., urine collected during laboratory opening hours or usual working hours (Andersen *et al.*, 2003).

Analytical variation ($\sigma_{\text{analytical}}$)

Analytical variation adds imprecision to biological variation. Conversely, if biological variation is high, analytical variation becomes relatively less important and the requirement for analytical precision diminishes (Fraser, 1983). The goal set-up is that variance due to analytical error should not exceed 20% of the variation (Fraser, 1983). It can be shown that this is acceptable when $CV_{\text{analytical}} \leq 1/2CV_{\text{intra-individual}}$ or $\leq 1/2CV_{\text{biological}}$ (Fraser and Harris, 1989).

Table 44.1 shows the analytical goals for urinary iodine excretion, and includes analytical goals for thyroid function tests for comparison, calculated from variation in a study using a routine laboratory setting (Andersen *et al.*, 2001). The analytical variation is less important for urinary iodine, because the biological variation is high in this set-up.

Variation will be lower with standardized preanalytical conditions, as found for urinary iodine in a slightly standardized study (Busnardo *et al.*, 2006) compared with the variation in nonstandardized urine collections (Rasmussen *et al.*, 1999; Andersen *et al.*, 2001).

Within-individual variation ($\sigma_{\text{within-individual}}$)

Repeated measurements of an analyte in one individual scatter around an individual mean value. This individual variation in urinary iodine content may differ widely between subjects (Andersen *et al.*, 2001), even when corrected for mean values, as illustrated in Figure 44.1

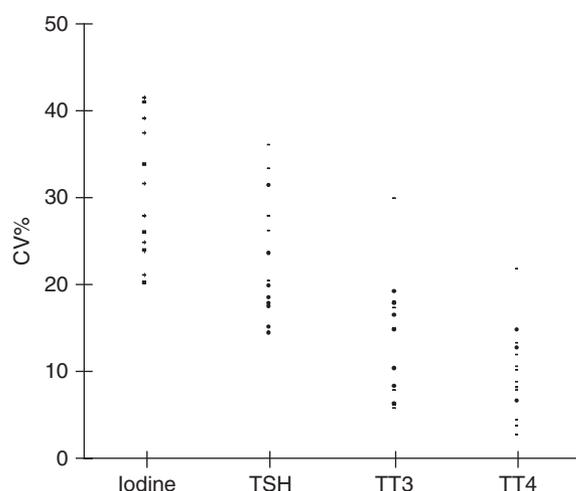


Figure 44.1 Within-individual variance is illustrated by a scatter plot of individual CV% for urinary iodine content, calculated from 12 consecutive monthly samples from 15 healthy men. From Andersen *et al.*, (2001), reproduced with permission. Similar estimates for TSH and thyroid hormones are included for comparison. From Andersen *et al.*, (2001, 2003), reproduced with permission. Each dot represents the CV% for an individual.

(Bartlett's test, $p < 0.01$). The variation in urinary iodine is even wider than the variation in thyroid-stimulating hormone (TSH), renowned for its huge variation compared with other biochemical analytes (Andersen *et al.*, 2002b, 2003).

The variation around the individual mean value is described by a probability distribution. The larger the difference between the measured value and the mean value, the less likely it is for this difference to be caused by random variation. Thus, a confidence interval (CI) in an individual can be calculated for each individual from the data on variation.

Between-individual variation ($\sigma_{\text{between-individual}}$)

Figure 44.2 is based on the same data as (Andersen *et al.*, 2001), and illustrates that individual mean values vary (Andersen *et al.*, 2003). The difference between individual mean urinary iodine concentrations is highly significant (Kruskal–Wallis test; $p < 0.001$ for all variables), compared to that of TSH in serum (Andersen *et al.*, 2001). This is consistent with other findings of marked differences in urinary iodine excretion between individuals (Rasmussen *et al.*, 1999; Busnardo *et al.*, 2006).

Variation in Urinary Iodine Excretion

Urinary iodine excretion displays very wide variations compared with most other biological analytes.

A longitudinal depiction of the iodine content of 12 consecutive monthly spot urine samples collected from

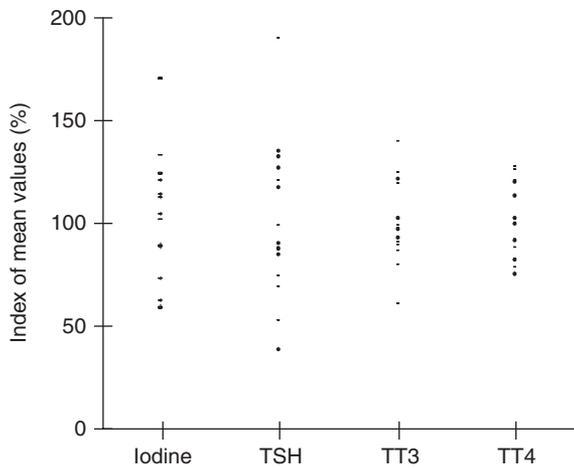


Figure 44.2 Between-individual variance is illustrated by a scatter plot of the individual mean iodine content of 12 consecutive monthly urine samples collected from 15 healthy men living in the same area. From Andersen *et al.*, (2001), reproduced with permission. Similar estimates for TSH and thyroid hormones are included for comparison. From Andersen *et al.*, (2001, 2002b, 2003), reproduced with permission.

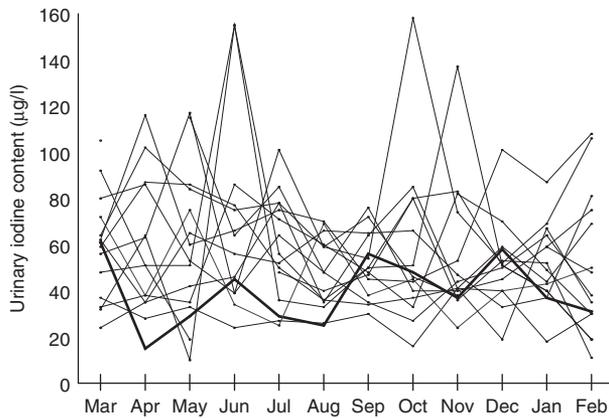


Figure 44.3 Iodine content of 12 consecutive monthly spot urine samples in each of the 15 healthy free-living men in an area with mild-to-moderate iodine deficiency. From Andersen *et al.*, (2001, 2003), reproduced with permission.

each of 15 healthy, free-living men is shown in Figure 44.3 (Andersen *et al.*, 2001).

These men had mild-to-moderate iodine deficiency, with median urinary iodine content of 50.0 µg/l. Inspection of Figure 44.3 reveals substantial and random variations in the iodine content of spot urine samples in each of the 15 subjects. No systematic difference between individuals is apparent.

An additional understanding of individual and group variations is gained if the individual coefficients of variation (CVs) for urinary iodine from Figure 44.1 are added to the individual mean values of urinary iodine content from Figure 44.2, spread out side-by-side. This produces Figure 44.4, in which the iodine contents of the 12 monthly spot urine samples are

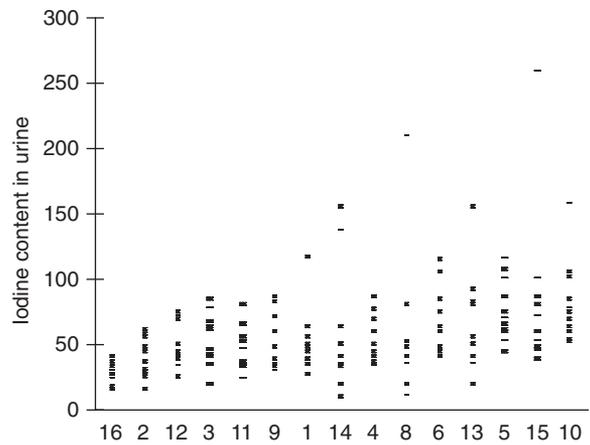


Figure 44.4 Iodine content of 12 consecutive monthly spot urine samples from each of 15 healthy free-living men in an area with mild-to-moderate iodine deficiency. From Andersen *et al.*, (2001, 2003), reproduced with permission. Each dot represents one monthly iodine measurement, and participants are sorted by increasing mean.

represented by 12 dots for each participant, sorted by increasing mean iodine concentration values (Andersen *et al.*, 2001).

Figure 44.4 gives an impression of the two components of biological variation in urinary iodine excretion: within-individual variation (vertical) and between-individual variation (horizontal).

The overall CV of 57% was in keeping with the CVs of 47% and 52% for men and women, respectively, in a study where spot urine samples were collected at random over a 2-year period in an area with a higher average iodine excretion of 118 µg/l (Als *et al.*, 2000).

In addition to large variations, both between individuals and within each individual, the individual variations differed widely between individuals with individual CVs, ranging from 20% to 70% (Andersen *et al.*, 2001). These were similar to the findings of a short-term study of 10 men and women with a higher mean iodine excretion (139 µg/24 h), where the CV% varied between 14% and 78% (Rasmussen *et al.*, 1999).

Sample iodine concentration varies markedly more than the iodine status of an individual evaluated from average iodine excretion over a period. This adds variation (Figure 44.2) to the between-individual variance (Figure 44.1), i.e., individual variation added to the variation caused by different set points. In the study by Andersen *et al.* (2001), the iodine concentration in individual urine samples varied between 10 and 260 µg/l, while the average annual urinary iodine concentration in the 15 men was between 29 and 81 µg/l. Thus, the variation in iodine status evaluated from a single spot urine concentration was five times wider than the range of variation evaluated from the average urinary iodine concentration over a period. This was halved to 2.5 times if iodine in urine was corrected for dilution (to be further discussed later).

Table 44.2 Variation in urinary iodine concentrations and estimated 24h urinary iodine excretion in individual samples and in average annual values over 1 year

	CV%	Interquartile range	Range
Average annual urinary iodine concentration (n = 15)	23.6	11.7 µg/l	52 µg/l
Urinary iodine concentration in individual samples (n = 180)	57.3	32.0 µg/l	250 µg/l
Average annual estimated 24 h urinary iodine excretion (n = 15)	27.0	13.8 µg/24 h	53 µg/24 h
Estimated 24 h urinary iodine excretion in individual samples (n = 180)	45.0	23.7 µg/24 h	123 µg/24 h

Notes: For comparison, values are expressed as CV%, interquartile range and total range. Reproduced from Andersen *et al.*, (2001), with permission.

Table 44.2 shows measures of dispersion of urinary iodine concentration, and includes estimated 24 h urinary iodine excretion both for individual samples and for the average of 12 monthly samples.

It can be observed from Table 44.2 that the variation in iodine content is markedly higher among individual urine samples than the variation in average annual urinary iodine concentration. In comparison, CV% is 2.4 times larger for spot urine iodine concentration than for the average annual iodine content. This difference is 2.7 for interquartile range (IQR) (Andersen *et al.*, 2001).

This is a rather simple model to describe the components of variation that can be obtained from most statistical programmes using analysis of variance (ANOVA) facilities, and the interpretation here is that the biological variation in urinary iodine excretion is around 2.5 times larger than the variation between individuals.

Urinary iodine excretion reflects dietary iodine intake over a limited period prior to urine sampling, and generally 90% of the ingested iodine is excreted in the urine (Keating and Albert, 1949; Dworkin *et al.*, 1966; Pallardo *et al.*, 1979; Hays, 1993; Hurrell, 1997; Jahreis *et al.*, 2001).

The iodine content of different foods varies widely, and variations in food choice are important for variations in iodine excretion. Thus, a meal of cod may supply 400 times more iodine than a meal of meat from a terrestrial animal (Andersen *et al.*, 2002a), and iodine excretion may vary by a factor of 4 within the same society, depending on the composition of meals (Andersen *et al.*, 2005). Variable bioavailability may add to the variation in iodine excretion

(Katamine *et al.*, 1987; Aquaron *et al.*, 2002; Andersen *et al.*, 2007b). Furthermore, variations in iodine excretion may be amplified by differences in cooking procedures (Goindi *et al.*, 1995; Pedersen *et al.*, 1999), drinking-water iodine (Hales *et al.*, 1969; Pedersen *et al.*, 1999; Felgentäger *et al.*, 1983; Fordyce *et al.*, 2000; Chandra *et al.*, 2005), and the use of dairy milk (Nelson *et al.*, 1987; Rasmussen *et al.*, 2002; Als *et al.*, 2003; Dahl *et al.*, 2004). Additional factors, such as feeding of dairy herds and maternal smoking habits, may influence iodine excretion in dairy milk and human milk to variable extents (Papas *et al.*, 1979; Hermansen *et al.*, 1995; Laurberg *et al.*, 2002, 2004), i.e., by diverting part of the iodine from thyroidal uptake to renal excretion to varying extents (Papas *et al.*, 1979; Laurberg *et al.*, 2004).

Finally, unusual high iodine content has been seen in some foods (Chilean Iodine Educational Bureau, 1952; London *et al.*, 1965; Wenlock and Buss, 1982; Andersen *et al.*, 2002a) and may be caused by enrichment with iodine-containing additives or contamination with iodine-containing chemicals used by the food industry (London *et al.*, 1965; Phillips, 1997). Such single unusual values will skew distributions, and this variation will favor the use of median, rather than mean, values when describing population iodine excretion. This is in keeping with the nutritional aspects, as rare, but exceedingly high, urinary iodine values will have a limited impact on the actual iodine excretion level of a population.

Variation in Urinary Iodine Excretion and Studies of Iodine Nutrition

Study of iodine in samples, not subjects

Populations are evaluated in the study of iodine nutrition. Population iodine excretion is described by two variables: mean and variation. When describing iodine excretion in populations, a common approach is to compare mean or median values. However, this value may be identical, even though the populations differ markedly in distribution (Andersen *et al.*, 2004), and thus differ despite similar mean values. Variance is thus another parameter to consider in the description of iodine excretion, and comparison of variances may differ from comparison of mean values.

Variation in population urinary iodine excretion is often described as the number of individuals with urinary iodine excretion below a certain value. Such a number is, however, a parameter in the description of the variance characteristic to the population, and not a fraction of the population with low urinary iodine, as interpreted in quite a number of studies.

The distinction between iodine content of urine samples and the estimated urinary iodine excretion in participating individuals is important for the interpretation of study results. Table 44.2 illustrates this difference, and in the study by Andersen *et al.* (2001), more than 10% of

the urine samples had an iodine content below the lowest average annual iodine content in any of the participants. The correct interpretation of the study results is that 10% of the low results of urinary iodine measurements were due to variation in iodine excretion, and not an indicator of severe iodine deficiency. Similarly, 13% of the urine samples had an iodine content above the highest average urinary iodine excretion in any of the participants of the study. These 13% high values were caused by variance, and were not an indicator of iodine excess.

Estimating 24 h urinary iodine excretion

Variable fluid intake causes variable volumes of urine. Around 90% of dietary iodine is excreted in the urine, and variable urine volumes cause variable dilution of the iodine excreted in urine, and thus in the concentration of iodine in urine. If the variation in dilution is corrected, the variation in urinary iodine content is lowered. This can be achieved by correcting for an analyte that is excreted in parallel with urine volume.

Adjusting urinary iodine for creatinine excretion was suggested more than 40 years ago, to reduce the variation in iodine excretion caused by dilution (Vought *et al.*, 1963; Dworkin *et al.*, 1965; Jolin and Rey, 1965). The reliability of the simple ratio of iodine to creatinine was questioned on the basis of investigations of African children (Furnée *et al.*, 1994) with a creatinine excretion of 0.4 g/day, compared with adults from Europe (Bourdoux *et al.*, 1985) with a markedly higher creatinine excretion of 0.9–1.7 g/day. Thus, adjusting for creatinine should be stratified, i.e., for gender (Vought *et al.*, 1963; Dworkin *et al.*, 1965; Jolin and Rey, 1965), age (Kampmann *et al.*, 1974; Kesteloot and Joossens, 1996; Knudsen *et al.*, 2000), and probably also ethnicity (Haddow *et al.*, 2007).

In the study of variation in urinary iodine excretion, an estimated 24 h urinary iodine excretion was calculated from age-, gender- and ethnic-specific creatinine excretions (Kampmann *et al.*, 1974; Kesteloot and Joossens, 1996). The reduction in variation in iodine excretion is seen in Figure 44.5.

The variation in estimated 24 h urinary iodine excretion is clearly lower than the variation in iodine concentration in spot urine samples. This improves the accuracy of calculations based on variation in iodine excretion (Andersen *et al.*, 2007a).

Timing of spot urine sampling

Urinary iodine excretion reflects short-term iodine intake, and part of the ingested iodine appears in the urine with a delay of around 4–5 h (Keating and Albert, 1949; Dworkin *et al.*, 1966; Pallardo *et al.*, 1979; Hays, 1993; Hurrell, 1997; Als *et al.*, 2000a). Thus, the morning sample of spot urine iodine is clearly lower than 24 h urine iodine excretions

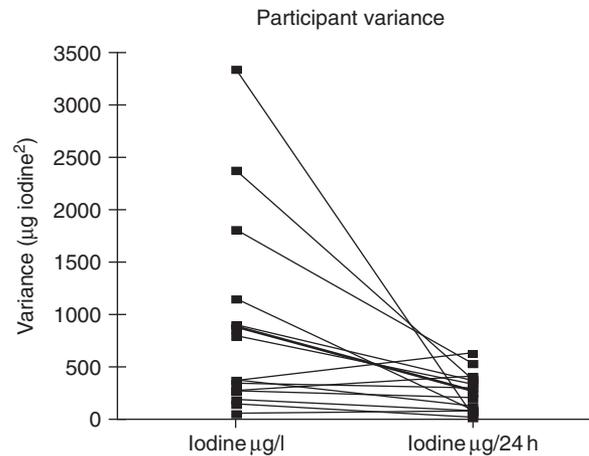


Figure 44.5 Variation in urinary iodine excretion expressed as crude urinary iodine content (µg/l) and as 24 h urinary iodine excretion estimated from creatinine excretion in an age- and gender-matched group (µg/24 h).

(Rasmussen *et al.*, 1999; Als *et al.*, 2003). Consequently, morning urine samples are rarely representative of the average iodine intake in an individual or a population, and increasing the number of urine samples does not correct this error.

Collecting morning urine samples only for iodine measurements suggests lower intra-individual variation in iodine excretion (Busnardo *et al.*, 2006) than is found in studies with urine sampling also including afternoon sampling (Rasmussen *et al.*, 1999; Als *et al.*, 2000a; Andersen *et al.*, 2001). Caution should be taken when applying such data to studies of iodine nutrition and estimations of the usefulness of single urine samples (Ovesen *et al.*, 2002; Andersen *et al.*, 2007a).

Urinary iodine excretion in an individual

The number of samples needed to assess individual iodine excretion level can be calculated as described in detail by Fraser and Harris (1989). The statistics are relatively simple and relate to z -statistics. A rearrangement of the formula gives us the following equation, from which the number of urine samples required can be calculated.

$$n = \left(\frac{Z \times CV_{\text{analytical} + \text{intra-individual}}}{D} \right)^2$$

where n is the number of urine samples needed to describe the iodine excretion within a precision range, D , and a CI of Z (Fraser and Harris, 1989).

To apply this we have to consider two things. First, how far from the actual iodine excretion can we accept our result to lie? This deviation of measured values from the

true value (measured iodine – mean iodine value) is called the precision range, usually denominated *D*.

Secondly, we have to consider the certainty of the outer limits set by the precision range, i.e., the confidence we have in these limits. To apply this CI, denominated *Z*, we may use a standard deviation of 1.96 in the calculation for a 95% CI. This tells us that the results will be within the precision range 95 times if we perform the scheduled collection 100 times.

The CV% for the individual was available in the study by Andersen *et al.* (2001), and we only need to fill in the numbers. Doing so we get the number of samples shown in Table 44.3 (Andersen *et al.*, 2007a).

If we want to be 95% confident of the results, and we accept that the true iodine excretion is likely to lie within $\pm 5\%$ of the value obtained, we need around 200 urine samples from an individual. However, if the measured

Table 44.3 Number of samples needed to be 95% confident of the iodine excretion level in an individual with a specified precision

Precision range	Number of spot urine samples	Number of estimated 24 h urine samples
$\pm 10\%$	122	96
$\pm 5\%$	489	383

Notes: Calculated from $n = [z \times (CV^2_{\text{analytical}} + CV^2_{\text{intra-individual}})] / (1/2) / D]^2$, where *D* is % closeness to the homeostatic set point, *Z* the number of standard deviations required for a confidence level (1.96 for 95%), and *n* the number of specimens.

Source: Adapted from Andersen *et al.* (2007a), with permission.

Table 44.4 Number of spot urine samples needed to be 95% confident of being within a specified range for crude urinary iodine concentration and for estimated 24 hour urinary iodine excretion calculated from the variation in iodine excretion among healthy men undertaking daily lives (Andersen *et al.*, 2007b)

Precision Range ^e	Number of spot urines needed to sample for estimation of urinary iodine excretion ^a with a specified Precision Range					
	Urinary iodine concentration ($\mu\text{g/L}$)			Estimated urinary iodine excretion ($\mu\text{g}/24\text{h}$) ^b		
	In a population ^c	In an individual ^d		In a population ^c	In an individual ^d	
<i>n</i>	Median variation	Lowest – highest variation	<i>n</i>	Median variation	Lowest – highest variation	
+/-1%	12,218	5,471	1,827–28,338	9,575	4,587	1,587–19,307
+/-2%	3,054	1,368	457–7,084	2,394	1,147	397–4,827
+/-5%	489	219	73–1,134	383	183	63–772
+/-10%	122	55	18–283	96	46	16–193
+/-20 %	31	14	5–71	24	11	4–48
+/-30%	14	6	2–31	11	5	2–21
+/-40%	8	3	1–18	6	3	1–12
+/-50%	5	2	1–11	4	2	1–8

^aCalculated from $n = (Z \cdot CV/D)^2$ where: *Z* = 1.96 for 95% Confidence Interval; *CV* = Coefficient of Variation; *D* = Precision Range.

^bCorrected for gender and age specific creatinine excretions (18, 19) as recommended (8, 13, 20, 21).

^cNumber of individuals needed to produce one urine sample was calculated based on the variation in the population.

^dVariation differs between individuals. Number of samples needed to sample in an individual are given for individuals with median, lowest and highest variation.

^eCalculated with a Confidence Interval of 95% (*Z* = 1.96).

iodine excretion is $100 \mu\text{g/l}$, we really need to know this by $\pm 5 \mu\text{g/l}$, equal to $95\text{--}105 \mu\text{g/l}$ for the individual. We might accept a wider range of, say, $80\text{--}120 \mu\text{g/l}$, equal to a precision range of $\pm 20\%$. This would require approximately 10–15 samples from an individual (Andersen *et al.*, 2007a).

An additional aspect of estimating iodine excretion in the individual is that the thyroid gland has the capacity to store considerable amounts of iodine in thyroglobulin (Silva, 1985; Delange, 1994). Thus, a short period of low iodine intake is not necessarily an indicator of iodine deficiency in a subject.

Reliability of iodine nutrition studies

The variation in urinary iodine excretion affects the reliability of estimates of population iodine nutrition. Low urinary iodine is seen in iodine-replete individuals due to random variation (Andersen *et al.*, 2001). However, a high number of samples increases the reliability of the estimates of iodine excretion in a population, but what is the reliability of a study including a certain number of spot urine samples from a population?

This reliability of studies of iodine nutrition can be estimated using the same statistics as discussed in the previous section (Fraser and Harris, 1989). Now we know the number of samples, *n*, while the precision range, *D*, is the question to be answered from Table 44.4.

Seek out the column for population in Table 44.4 and find the number of samples. If you have 12 000 spot urine samples from a population, the iodine excretion level of

that population is determined within a precision range of $\pm 1\%$. This means that, if the average iodine excretion in that study was $100\mu\text{g}$, the population iodine excretion is likely to lie between 99 and $101\mu\text{g}$. However, if the study includes only 120 individuals from that population producing one spot urine sample each, the iodine excretion level is described within $\pm 10\%$. If the measured average iodine excretion again is $100\mu\text{g}$, the iodine excretion level of the population lies between 90 and $110\mu\text{g}$ with 95% confidence.

If the study provides estimated 24h urinary iodine excretion, the precision range, or reliability of estimates, is approximately 20% higher, as can be read from [Table 44.4](#).

Number of spot urine samples needed

When planning a study of iodine nutrition, the number of urine samples needed to describe the iodine excretion level in a population can be read from [Table 44.4](#) ([Andersen et al., 2007a](#)). If a precision range of $\pm 10\%$ is aimed for, still with 95% confidence, about 125 spot urine samples are needed. Using the estimated 24h urine iodine excretion reduced the number of samples needed by 20%, i.e., to 100 samples. A precision range of $\pm 5\%$ required around 500 spot urine samples, while estimating 24h urinary iodine excretion reduced the required number of spot urine samples to around 400, as can be read from [Table 44.4](#) ([Andersen et al., 2007a](#)).

Again, if the average iodine excretion is $100\mu\text{g}$ in 500 samples from a population, the true iodine excretion of that population is likely to be between 95 and $105\mu\text{g}$ with 95% confidence. Given 125 spot urine samples, this will be between 90 and $110\mu\text{g}$, equal to widening the precision range to $\pm 10\%$. This is parallel to the validity of subgroup analysis.

The limitation of the data is that the study population from which the variance measure was derived was rather homogeneous, and the estimates were minimum requirements ([Andersen et al., 2007a](#)).

Variation study: a way to describe thyroid iodine deficiency

In the study of variation in more than one variable, variations can be correlated. Such associations may be analyzed ([Feldt-Rasmussen et al., 1989](#)). The association between iodine excretion and serum TSH was studied in 15 healthy men in an area with mild-to-moderate iodine deficiency ([Andersen et al., 2001](#)). The association differed between individuals, and when individuals were grouped according to urinary iodine excretion levels, a negative correlation was found only in individuals with an iodine excretion below $50\mu\text{g}/24\text{h}$ ([Figure 44.6](#)).

This indicated substrate-dependent insufficient thyroid hormone synthesis when the average daily urinary iodine excretion was less than $50\mu\text{g}$, and provided a different

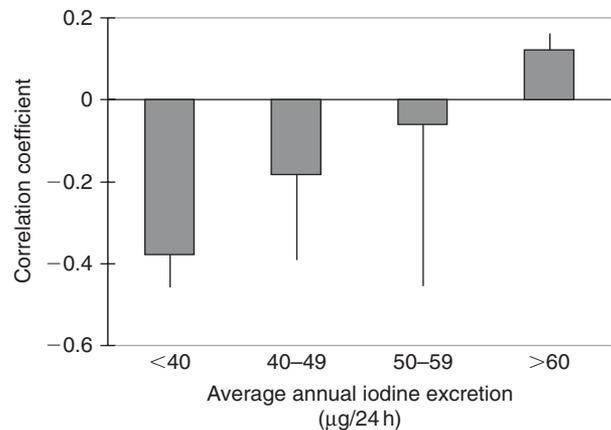


Figure 44.6 Average correlation coefficients between estimated 24h urinary iodine excretion and serum TSH in 15 healthy men grouped according to average annual estimated 24h urinary iodine excretion. Pearson's correlation coefficients on ln-transformed data were used. From [Andersen et al., \(2001\)](#), reproduced with permission.

approach to the delineation of moderate and mild iodine deficiency ([Andersen et al., 2001](#)).

Conclusions

Variations in urinary iodine portray variations in iodine intake. However, huge variations in sample iodine content do not reflect similar variations in the iodine nutrition in that population. This is important for risk estimation in the evaluation of population iodine intake, and has important implications for studies of iodine nutrition. Furthermore, the reliability of studies of iodine nutrition and the number of urine samples needed to assess iodine excretion level can be calculated.

Summary Points

- A considerable fraction of samples have low or high values due to variation in urinary iodine.
- This does not indicate a number of individuals in that range, but is rather delineation of a likelihood of a fraction of individuals in a population.
- Adjusting urinary iodine excretion for age-, gender- and ethnic-specific creatinine excretions markedly reduces variation in urinary iodine.
- Refrain from fasting- and morning-urine samples for estimation of iodine status.
- Assessing individual iodine excretion level requires at least 10–15 urine samples.
- Five hundred spot urine samples describe the iodine excretion level within $\pm 5\%$.
- One hundred and twenty-five spot urine samples describe the iodine excretion level within $\pm 10\%$.
- Estimating 24h urinary iodine excretion reduced the number of samples needed by 20%.

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Assessment of Iodine Intake and Iodine Status in Vegans

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Abstract

The iodine concentration in foods of all types varies considerably due to a number of factors, such as soil composition, animal breeding, climate, and other environmental variables. Moreover, the amount of iodine from each food depends on the source, preparation, processing and volume consumed. The iodine value for a food represents an average and does not consider the wide variations in the iodine content of the food; hence some values, such as those from food composition tables, used in the evaluation of iodine intakes are likely to be subject to error. In addition, the use of food composition tables may be considered inappropriate to reasonably estimate iodine intake in groups of individuals consuming unconventional foods not listed, or inconsistently listed, in such tables.

Abbreviations

COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
FFQ	Food frequency questionnaire
FT4	Free thyroxine
MAFF	Ministry of Agriculture, Fisheries and Food
NAS	National Academy of Sciences
SCF	Scientific Committee on Food
T3	Triiodothyronine
T4	Total thyroxine
TSH	Thyroid-stimulating hormone
UK	United Kingdom

Introduction

Iodine concentrations in foods and total diets are extremely variable depending on geochemical, soil and cultural conditions that influence the iodine uptake of staple crops and foods of animal origin. The iodine content of food ultimately depends on the concentration of iodine in the soil of the region in which the food is produced. Thus, in

developing countries, particularly rural areas, where local food is the major source of iodine, poor iodine content in the water and soil will be reflected in locally-grown foods. In addition, seasonal variations in the iodine content of foods are particularly evident in milk and milk products.

The assessment of iodine intake in vegans consuming their habitual diet may be difficult. The weighed record method is considered to be the most accurate measure of dietary intake (Bingham *et al*, 1995). However, as the final calculation of nutrient intake relies on the data from food tables, a variation in the iodine content within foods means that it is difficult to assess iodine intake accurately using this method. Furthermore, vegans consume a variety of unconventional foods that are not found in such food tables. Direct chemical analysis of foods or diets, in particular the duplicate portion technique, has been considered the best method for the assessment of trace element intake (Abdulla *et al*, 1989; West and van Staveren, 1997), although the process of duplicate food collection may underestimate habitual intake (Stockley, 1985; Isaksson, 1993). This chapter explores the major sources of iodine in the vegan diet, and the assessment of iodine intake and status in vegans using different dietary and biochemical techniques.

Sources of Iodine in the Diet

The major natural food source of iodine is fish. The iodine content of fish reflects that of the water they inhabit; thus, high levels of iodine are present in marine fish and shellfish compared to freshwater fish (Figure 45.1). Milk and dairy products contain relatively high amounts of iodine and are considered to be the most important sources of iodine in developed countries. The iodine content of milk is largely influenced by the use of iodophors as teat sterilants and equipment sanitizers in dairy husbandry, and the supplementation of animal feeds due to iodine deficiency in dairy cattle, exacerbated by the inclusion of goitrogenic feeds in the diet (COT, 2003). In addition, seasonal variations in the iodine content of foods are particularly evident in cows' milk, with greater iodine

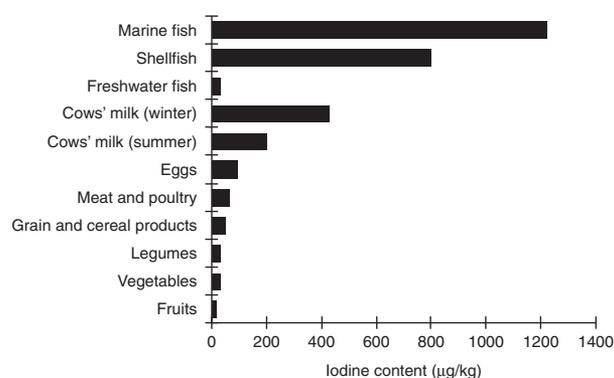


Figure 45.1 Mean iodine content of selected food groups. MAFF, (2000); SCF, (2002).

Table 45.1 Characteristics of vegetarian diets

Type of diet	Excluded foods	Included foods
Lacto-ovo-vegetarian	Meat, poultry, fish	Eggs, dairy products, grains, legumes, vegetables, fruits, nuts, seeds
Lacto-vegetarian	Meat, poultry, fish, eggs	Dairy products, grains, legumes, vegetables, fruits, nuts, seeds
Vegan	Any animal products	Grains, legumes, vegetables, fruits, nuts, seeds

content in winter milk than in summer milk, reflecting a higher intake of manufactured feed in dairy cattle in the winter (MAFF, 2000; Pearce *et al.*, 2004).

The levels of iodine in processed foods depend on naturally-occurring iodine and the use of iodized salt and additives. The iodine content of some manufactured foods (e.g., meat products, biscuits, cakes and fruit products) is mainly due the food colorant “erythrosine” (58% iodine by weight).

Sources of iodine in the vegan diet

As the major sources of iodine in the diet are animal based, it is reasonable to suggest that vegans (a strict vegetarian diet of plant foods only) (Table 45.1) are likely to have a low dietary intake of iodine. So where can vegans obtain their dietary iodine?

Possible dietary sources of iodine for vegans include iodized salt and seaweed (Lightowler and Davies, 1996). In many developing countries, iodized salt is considered a good medium for iodine prophylaxis. However, although some iodized salt contains 1527–2557 µg/100g (equivalent to 76–128 µg/teaspoon), encouragement to use this

Table 45.2 Iodine content of edible seaweeds

Common name	Total iodine (µg/g)
Arame	586
Dulse	72
Hijiki	629
Kelp (whole)	1820
Kelp (capsule/powdered/granules)	3283
Kombu	1350
Nori	16
Wakame	58

Source: Teas *et al.*, (2004).

commodity as a vehicle for increasing iodine intakes in vegans may be less acceptable in the interest of recent reports on dietary sodium and health outcomes (Cook *et al.*, 2007).

As grains do not derive much iodine from the soil, the iodine content of cereal products is mainly due to the addition of potassium and calcium iodate used as stabilizers in baking. Moreover, the addition of iodate to bread in some countries is specifically for the prevention of iodine deficiency (Dunn, 1993).

Seaweed Edible seaweeds, common in eastern diets, are becoming increasingly common foods and supplements in western diets. Seaweed is a rich natural source of iodine; however, the iodine content varies, both between and within species (Table 45.2). Teas *et al.* (2004), in particular, found that the iodine content of kelp (in capsule, powder, or granular form) varied from 1259 to 8165 µg/g. The high variability in the iodine content of edible seaweeds is affected by a number of factors, including geographic location, season, preparation and storage conditions.

Although the iodine concentration of edible seaweeds is high, the amount of seaweed consumed in terms of food portions is relatively small. There is also a misconception that seaweed products are a rich source of iodine, although these foods may contribute little to the iodine intake, as they are normally eaten in small amounts and may contain variable quantities of iodine. However, it is possible that the iodine content of some seaweed dishes may exceed the tolerable upper limits (1100 µg/day) for adults (NAS, 2001). Excess iodine intake has well-established effects on thyroid status and can result in functional impairment and tissue damage to the thyroid gland; moreover, it has been suggested that an excessive iodine intake from seaweed used as a staple food can result in endemic goiter (Lamberg, 1991).

The influence of seaweed on iodine intake may be considered adverse in some situations, as the intake in vegans who eat the food item may approach or exceed the tolerable upper intake level for iodine (Lightowler and Davies, 1998). However, although edible seaweed contains very high levels of iodine, the proportion of vegans who

consume seaweed, the actual amount consumed and the frequency of consumption are low (Lightowler and Davies, 1996). Thus, subclinical effects of high but infrequent iodine intake in vegans needs to be addressed. Although compensatory mechanisms are known to exist to protect the thyroid against excessive intake of iodine, the effect of high levels of iodine on the thyroid depends on the current and previous iodine status of the individual, and their current and previous thyroid dysfunction.

Dietary Supplements Dietary supplements are an important source of iodine in the vegan diet; however, the use of these may also have adverse effects. The use of kelp tablets, if taken according to the manufacturers' recommended daily dose, can often exceed the tolerable upper intake level for iodine. In addition, kelp is subject to natural variability, making control of the iodine content of such supplements difficult. Moreover, iodine content in some supplements may be much higher than the level indicated by the manufacturing company (Lee *et al.*, 1994); thus, individuals may be ingesting more iodine than they are aware of. Other iodine supplements, such as multi-mineral and multi-nutrient tablets, contain a lower concentration of iodine and generally have an iodine concentration (per dose) below the recommended intake. However, the iodine content may also depend on the length of time that has elapsed between the manufacture and the purchase of the supplement (Norman *et al.*, 1987).

Assessment of Iodine Intake in Vegans

Previous dietary studies have shown that, while vegan diets provide adequate intakes of many nutrients, intakes of others may fall below the recommended levels. Various potential nutrient deficiencies associated with the vegan diet have been thoroughly investigated, but limited research has been undertaken to assess the iodine intake of vegans. As the major sources of iodine in the diet are animal-based, it is reasonable to suggest that vegans are likely to have a low dietary intake of iodine, unless they regularly consume seaweed or dietary supplements containing iodine. Therefore, vegans may be considered a group "at risk" for iodine deficiency. Indeed, previous research not only confirms that the iodine content of vegan diets is often below the recommended levels, but also indicates that vegans need not be iodine deficient. Vegans who avoid iodized salt and iodine supplements (including seaweed preparations) are at increased risk of iodine deficiency, while those who use such supplements may risk excessive intakes of iodine. Thus, iodine deficiency seems to be a concern for vegans, and the risk of deficiency may depend on their appropriate use of seaweed, iodized salt, or dietary supplements.

Establishing links between actual dietary iodine intake and iodine deficiency disorders relies on the availability of

precise techniques to enable accurate estimation of nutritional intake. We know that the evaluation of iodine intake is difficult, due to the wide variations in iodine content of food. Thus, the techniques used in the assessment of iodine intake are indicative of the reliability of the results, although dietary analysis, used in conjunction with biochemical measurements, may reduce the margin of error.

The use of food tables and nutrient databases to determine individual nutrient intake from food can be a major source of inaccuracy, as they may not contain comprehensive information on the nutritional content of all foods likely to be consumed by a study population. This is exacerbated in vegans who consume many unconventional foods not listed, or inconsistently listed, in such tables and databases. Moreover, micronutrients, which are found only in a small number of foods and in small quantities, are often poorly represented in nutrient databases.

It has been suggested that conventional dietary assessment techniques, in conjunction with food tables, do not provide realistic estimates of micronutrient intakes and that accurate data on dietary intake of such nutrients can only be obtained by direct chemical analysis of foods or diets (Abdulla *et al.*, 1989; Bro *et al.*, 1990). There are three different methods of collecting data for direct chemical analysis (duplicate portion technique, aliquot sampling technique and equivalent composite technique); however, the most precise method of direct chemical analysis is the duplicate portion technique, as it directly measures actual nutrient intake (West and van Staveren, 1997).

Previous studies investigating dietary intake of micronutrients have reported that the duplicate portion technique offers the most accurate estimation. A study comparing dietary assessment methods to measure selenium intake concluded that diet record assessment was not adequate for predicting selenium intakes of individuals, and that duplicate diet analysis remains the recommended measure for research purposes (Duffield and Thomson, 1999). Furthermore, Koutras *et al.* (1970) state that if iodine intake is to be measured, the best method is the duplicate portion technique. However, the duplicate portion technique is labor intensive and requires a significant amount of subject commitment; therefore, its use is usually restricted to small groups and data are usually collected over a short period of time.

Studies assessing the iodine intake in vegans

A number of investigations have studied the iodine intake of vegans (Table 45.3). The accuracy of the results from the five studies will ultimately depend on the dietary assessment employed. Two of these studies assessed iodine intake using the duplicate portion technique, while two estimated intake from food records, and the fifth study estimated iodine intake using a semi-quantitative food frequency questionnaire (FFQ).

Table 45.3 Daily iodine intake in vegans using different dietary assessment methods (mean values \pm standard deviation)

Study	Location	Dietary assessment method	Duration (days)	Number of subjects (M:F)	Iodine intake ($\mu\text{g/day}$)	
					Male	Female
Abdulla <i>et al.</i> , (1981)	Sweden	Duplicate portions	4	6 (3:3)	82 \pm 29	58 \pm 12
Draper <i>et al.</i> , (1993)	UK	Weighed food record	3	38 (18:20)	98 \pm 42	66 \pm 22
Lightowler and Davies (1998, 2002)	UK	Duplicate portions	4	30 (11:19)	137 \pm 149	187 \pm 346
		Weighed food record	4	26 (11:15)	42 \pm 46	1448 \pm 3878
Rauma <i>et al.</i> , (1994)	Finland	Food records	7	9	29 \pm 13	
Waldmann <i>et al.</i> , (2003)	Germany	Food frequency questionnaires	14	98 (48:50)	88 \pm 31	82 \pm 34

Note: M, male; F, female.

The iodine content of foods varies between countries and even within countries, and these factors may likely have a bearing on the iodine intake of the vegan subjects. The Abdulla *et al.* (1981), Draper *et al.* (1993) and Lightowler and Davies (1998, 2002) studies restricted the geographical location to a specific area, whereas the Waldmann study investigated a nation-wide sample providing a greater representation of the population. Subjects in two of the investigations consumed unusual diets. The vegans in the Abdulla study followed a modified diet to which they were unaccustomed; thus, their iodine intake would not be a true reflection of their habitual diet. Furthermore, Rauma *et al.* (1994) investigated a group of strict vegans, known as “living food eaters,” who consumed only uncooked food; therefore, the iodine intake would not be representative of the vegan population in Finland.

Studies by Draper *et al.* (1993) and Rauma *et al.* (1994) used food records to estimate the iodine intake in vegans. In the Draper investigation, the nutrient intake of the subjects was determined using a weighed dietary intake method for three consecutive days. Similarly, the subjects participating in the Rauma study completed a 7-day food record, although it was not clear whether food intakes were estimated or weighed. The results from the methods employed in these studies ultimately depend on the quality of the food composition tables to estimate iodine content. Furthermore, as food composition tables frequently omit many foods found in a vegan diet and provide insufficient iodine information for foods, the iodine intakes in these studies may be incomplete.

FFQs, as a direct semi-quantitative method of dietary assessment, are often easier to handle and can result in higher subject compliance, compared to weighed food records and the duplicate portion technique. However, the use of FFQs, as used in the study performed by Waldmann *et al.* (2003), may result in more sources of error in the estimation of iodine intake compared to other dietary assessment techniques, due to the choice of food portion

sizes, frequency of intake and the values used for iodine content in food. Moreover, while similar FFQs have shown good validity with weighed records for energy and macronutrient intakes, intakes of vitamins and minerals are usually overestimated.

Two studies (Abdulla *et al.*, 1981; Lightowler and Davies, 1998) used the duplicate portion technique to assess the iodine content of the vegans' diets. Usual restraints and influences identified with this technique, such as the possible influence on dietary habits of collecting the food samples and gaining adequate cooperation from the subjects, might have been reduced in the Abdulla study that was undertaken in a controlled environment. These individuals consumed a modified diet to which they were unaccustomed, their iodine intakes would therefore not be a true reflection of their habitual diet.

In a separate study, Lightowler and Davies (2002) compared the iodine intake in vegans using two different dietary assessment methods – the duplicate portion technique and weighed diet records. Both techniques showed a wide variation in iodine intake, and differences in estimation of iodine intake between the two methods were seen. These differences were attributed to the use of food composition tables to estimate iodine intake in the weighed diet records. First, low iodine intakes estimated from the diet records in some individuals were due to the absence of vegan foods and incomplete iodine content of other foods in the tables. Secondly, the variability in iodine content of seaweed and, in particular, the higher iodine content of seaweed reported in food tables compared with that analyzed in the duplicate diets, resulted in an overestimation of iodine intake in vegans consuming seaweed during the 4-day study period.

It has been questioned whether the assessment of micronutrient intake over 4 days is representative of habitual diets; however, increasing the recording period from 4 to 7 days is associated with only a marginal increase in the precision of estimated habitual nutrient intakes (Gay,

2000). An increase in precision may be achieved by increasing the number of subjects, although more involved dietary assessment methods, such as the duplicate portion technique, are labor intensive and require a significant amount of subject commitment; therefore, their use is usually restricted to small groups and data are collected over a short period of time (Petersen and Barra, 1996). However, short time periods will miss any seasonal differences in the iodine content of foods that may occur. Seasonal variations were minimized in the Waldmann study, as 7-day FFQs were completed in both the autumn and spring seasons.

Factors affecting the assessment of iodine intake in vegans

The findings from previous studies highlight the need to assess the iodine content of the vegan diet accurately. There are a number of factors that can influence the accurate assessment of iodine intake in vegans, including incomplete duplicate diet collections, the use of dietary supplements and the intake of dietary goitrogens.

Duplicate Portion Collections The completeness of duplicate portion collections is important for an accurate assessment of iodine intake. However, due to the process of duplicate food collection, subjects may modify their dietary behavior, resulting in an underestimation of their habitual iodine intake. In addition, there is evidence that unweighed duplicate collections of food are incomplete (James *et al.*, 1981; Stockley, 1985); thus, if replicates are estimated by eye rather than weighed, an underestimation of total food intake may occur.

To help overcome possible limitations of the duplicate portion technique Lightowler and Davies (2002), in their study on the iodine intake of vegans, used weighed dietary records in conjunction with the duplicate portion technique. However, differences in weights of food and beverages between the two methods were still evident in some subjects. Although the mean weight estimated from weighed food records and that provided in duplicate diets were similar in male subjects, in female subjects the weight of the duplicate diets was lower than that estimated from the food records, highlighting the reluctance of some subjects to provide duplicate diet collections.

In the study by Abdulla *et al.* (1981), subjects were asked to provide duplicate food samples by visual measurement rather than weighed records, thus increasing the likelihood of underestimation of iodine intake. However, in addition, the urinary excretion of sodium, potassium and nitrogen was compared with the corresponding intake values to test the validity of the duplicate portion collections. Results from their analysis indicated that, as in the Lightowler and Davies study, females, but not males, underestimated their duplicate food collections.

Both studies highlight the difficulty of obtaining complete and representative duplicate diet collections for accurate assessment of iodine intake. Asking individuals to collect a duplicate diet does affect their dietary habits, and this must be taken into consideration in the assessment of iodine intake. Ways of alleviating the limitations of the duplication portion technique should be addressed, such as reimbursement for food and beverage costs of the duplicate diet collection.

Dietary Supplements As dietary supplements are an important source of iodine in the vegan diet, the accurate assessment of iodine intake from this source is important. The use of dietary supplements by vegans is a crucial factor in determining their iodine intake and assessing whether supplement users could be at risk of excessive intakes of iodine. However, dietary supplements may be a confounding factor in the assessment of iodine intake, as there is uncertainty and variability in their iodine content (Lee *et al.*, 1994). Furthermore, an excessive intake of iodine through some dietary supplements is not difficult because of their concentrated formulation and manufacturers' recommendations about the number of tablets to be taken, which may further exacerbate excessive intake.

Previous studies assessing iodine intake in vegans have estimated intake from dietary supplements based on manufacturers' declarations. Therefore, such intakes may be considered an approximation, as analysis has shown that the iodine content may differ from that stated on the packaging of the supplements and may also vary from tablet to tablet.

Draper *et al.* (1993) found that dietary supplements contributed to total iodine intake, providing approximately 11% of the recommended intake. Similarly, Lightowler and Davies (1998) observed that iodine-containing supplements significantly increased iodine intake in vegans, and estimated that such supplements provided, on average, 54 µg iodine per day or 39% of the recommended intake of 140 µg.

In contrast, Rauma *et al.* (1994) found that, although the daily consumption of kelp tablets ensured a sufficient intake of iodine, the use of such supplements may lead to excessive iodine intake. In addition, Key *et al.* (1992) observed that vegans who regularly took kelp supplements had markedly raised concentrations of thyroid-stimulating hormone (TSH) as a result of excessive iodine intake. Thus, it has been suggested that the high iodine content of kelp tablets may cause hyperthyroidism in susceptible individuals.

High Intake of Dietary Goitrogens Another confounding factor in the assessment of iodine intake may be environmental goitrogens, substances that interfere with thyroid hormone production or utilization (Gaitan, 1990). Vegetables of the genus *Brassica* have been considered to

Table 45.4 Thyroid function tests in vegans (mean values unless otherwise stated)

Study	Location	Number of subjects (M:F)	T ₃ (nmol/l)	T ₄ (nmol/l)	FT ₄ (pmol/l)	TSH (mU/l)
Abdulla <i>et al.</i> , (1981)	Sweden	6 (3:3)	2.3 (1.5–3.0)	97 (57–134)	–	3.2 (<10)
Key <i>et al.</i> , (1992)	UK	48 (48:0)	–	91 ^a (70–140)	–	2.4 (<5)
Rauma <i>et al.</i> , (1994)	Finland	9	–	–	16.1 (11–24)	2.1 (0.2–4.0)

Note: M, male; F, female. T₃, triiodothyronine; T₄, total thyroxine; FT₄, free thyroxine; TSH, thyroid-stimulating hormone. Laboratory reference values in *parentheses*.

^aMean of five subjects with TSH concentrations >5mU/l.

possess goitrogenic properties, due to a simultaneous effect of thiocyanate, isothiocyanate and thiooxazolidones. Moreover, a potent antithyroid compound, “goitrin,” is found in some *Brassica* seeds, and seaweeds of the genus *Laminaria* (such as kombu) have a high content of the potent antithyroid compound, phloroglucinol and other polyhydroxyphenols. Thus, sustained seaweed ingestion may play an additional role to that of iodine excess in the development of goiter.

Environmental goitrogens may normally be ineffective when in low concentration, and most are not of major clinical importance unless there is coexisting iodine deficiency. However, a vegan diet is likely to include more foods possessing goitrogenic properties, such as brassicas and seaweed, compared with an omnivorous diet, and previous research has shown that vegans are at increased risk of iodine deficiency. Thus, excess intake of these substances may affect iodine metabolism in vegans.

Assessment of Iodine Status in Vegans

The validity of the duplicate portion technique may be problematic as the completeness of duplicate portions is often difficult to assess. However, the use of biochemical markers, such as plasma, serum and urine, may be incorporated into nutritional assessment studies to validate dietary surveys or confirm nutritional status. There are a number of different methods that can be used to assess iodine status and, in particular, for the determination of the severity of iodine deficiency. However, two main methods used for the assessment of iodine status are measurement of urinary iodine excretion and thyroid function tests (an indirect method of iodine sufficiency).

As over 90% of the body's iodine is excreted in urine, urinary excretion of iodine is currently the most commonly-used biochemical marker of iodine intake. The determination of urinary iodine excretion in 24-h urine specimens is considered to be the most reliable method (Dunn, 1993) for testing urinary iodine levels. However, the accuracy and validity of the results will depend on the completeness of the 24-h collections; hence, measures to assess the completeness of such collections, e.g., urinary creatinine excretion or para-aminobenzoic acid (Gibson, 2005), should be considered.

Table 45.5 Urinary iodine excretion in vegans (mean values unless otherwise stated)

Study	Location	Number of subjects (M:F)	Urinary iodine
Krajčovičová-Kudláčková <i>et al.</i> , (2003)	Slovakia	15 (6:9)	78 μg/l
Lightowler and Davies (1998)	UK	30 (11:19)	34 μg/l (M)
Rauma <i>et al.</i> , (1994)	Finland	9	24 μg/l (F) <200–1700 μg/day

Note: M, male; F, female.

The determination of the levels of thyroxine (T₄) and TSH in serum may be used to indicate thyroid function, thus providing an indirect measurement of iodine sufficiency. However, although serum T₄ and TSH levels can be accurately and precisely measured, the tests tend to be costly and technically time-consuming. In comparison, measurements of urinary iodine excretion are cheaper and technically simpler, with no requirement to take a blood sample.

Five studies have used biochemical markers to assess the iodine status of the vegan population (Tables 45.4 and 45.5). Three studies included measurements of thyroid function, while three measured the urinary iodine excretion of vegan subjects.

The iodine intake of vegans participating in the Rauma investigation was extremely low, despite the consumption of seaweed, although the levels of serum T₄ and TSH were within the normal range (Table 45.4), indicating normal thyroid function, and the urinary iodine excretion level was high (Table 45.5). The difference in dietary iodine intake and biochemical measurements was attributed to the use of seaweed and seaweed products with unknown iodine content.

Although Abdulla *et al.* (1981) found that the iodine intake in their vegans did not meet the recommended daily amount, the serum levels of T₄ and TSH did not indicate any signs of iodine deficiency (Table 45.4). The differing results of low dietary iodine intakes, but adequate biochemical measurements, may be attributed to the length of time the body may take to adapt to a specific diet, as

Table 45.6 Mean iodine intake and urinary iodine in vegans

Subjects	Iodine intake ($\mu\text{g}/\text{day}$)	Urinary iodine ($\mu\text{g}/\text{day}$)
Seaweed ($n = 3$)	866	88
Iodine supplements ($n = 5$)	151	170
Others ($n = 22$)	87	45

Source: Lightowler (1997).

the vegans participating in the Abdulla study were within a controlled environment consuming a diet to which they were unaccustomed.

As illustrated in the assessment of iodine intake, Lightowler and Davies (1998) anticipated that the daily urinary iodine excretion would be variable and inconsistent, due to the wide variation in the iodine content of food and iodine intake. They reported that the urinary iodine level in vegans was low, with a moderate probability of iodine deficiency in the group as a whole, although the range of individual values was wide. As expected, daily urinary iodine excretion was greatest in subjects who took iodine supplements and those who consumed seaweed. The probability of iodine deficiency in vegans who did not consume seaweed or take iodine supplements was defined as severe. Similarly, Krajčovičová-Kudláčková *et al.* (2003) reported that iodine deficiency (urinary iodine concentration $<100\mu\text{g}/\text{l}$) was present in 80% of vegans, with severe deficiency (urinary iodine concentration $<50\mu\text{g}/\text{l}$) occurring in 27% of vegans.

However, Lightowler and Davies (1998) found that urinary iodine excretion was not commensurate with iodine intake (Table 45.6), and attributed this to a number of different factors. First, obtaining urinary iodine that truly reflects iodine intake is difficult. Malvaux *et al.* (1969) suggest that iodine output only equals iodine input over a relatively long period of time; therefore, the study period in the Krajčovičová-Kudláčková (24 h), Lightowler (4 days) and Rauma (24 h) studies may have been too short, although the feasibility of collecting urine for a longer period of time is questionable. Secondly, previous research indicates that some seaweeds contain much indigestible iodine (Katamine *et al.*, 1987); therefore, iodine excretion in vegans who consume seaweed may not reflect iodine intake.

These studies highlight that the measurement of urinary iodine excretion may be used as an indicator of iodine intake and deficiency. The specific aim of the research, *i.e.*, estimation of iodine intake in an individual or a population, will influence the sampling method to be used. Quantitative estimates of iodine intake from casual urinary samples are sufficient to give an estimate of intake in a population; however, if the aim is to obtain an accurate estimation of habitual iodine intake in an individual,

several 24-h urine collections may be required (Ovesen and Boeing, 2002).

Conclusion

Various potential nutrient deficiencies associated with the vegan diet have been thoroughly investigated, but limited research has been undertaken to assess the iodine intake of vegans. It has been shown that, although the iodine content of vegan diets is often low, vegans need not be iodine deficient, depending on their use of seaweed, iodized salt, or dietary supplements.

Establishing links between actual dietary iodine intake and iodine deficiency disorders relies on the availability of precise techniques to enable accurate estimation of nutritional intake. However, the evaluation of iodine intake is difficult, due to wide variations in the iodine content of food; hence, obtaining an accurate assessment is not possible with food composition tables. This is particularly pertinent to vegans who consume a variety of unconventional foods not listed, or inconsistently listed, in such tables.

Previous research has highlighted the importance that the choice of dietary assessment technique has on the estimation of iodine intake in vegans and the necessity of using an appropriate dietary survey technique in the determination of iodine intake to ensure accurate and reliable results. Differences in iodine intakes in vegans between studies may be attributed, in part, to inconsistencies in food tables. This poses a serious concern, as it is not always practical to determine iodine intake using the duplicate portion technique. Moreover, difficulties with the duplicate portion technique, such as modification of habitual diets, incomplete duplicate diet collections, and the expense of providing duplicate portions and the subsequent analysis, may be reasons for the infrequent use of the dietary survey methodology in such investigations. Therefore, more reliable information on iodine content of foods, incorporating the variation within foods, is needed.

Summary Points

- The assessment of iodine intake in vegans consuming their habitual diet may be difficult.
- The use of current food tables to estimate iodine intake in vegans is limited.
- The use of dietary records and food tables may be considered inappropriate to reasonably estimate iodine intake in groups of individuals consuming unconventional foods not listed, or inconsistently listed, in food tables.
- Although the duplicate portion technique may be considered the best method for the assessment of trace element intake, it is not always practical to determine iodine intake using this method of dietary assessment.

- The techniques used in the assessment of iodine intake are indicative of the reliability of the results, although dietary analysis used in conjunction with biochemical measurements may reduce the margin of error.
- The measurement of urinary iodine excretion may be used as an indicator of iodine intake and deficiency.
- More reliable information on iodine content of foods, incorporating the variation within foods, is needed.

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Isotopes of Iodine in Thyroid and Urine: Source, Application, Level and Determination

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Abstract

Thirty four isotopes of iodine have been found and produced, but only one, ^{127}I , is stable. ^{129}I is a long-lived radioisotope of iodine occurring in the nature, but is mainly produced and released by human nuclear activity. The most frequently used radioiodines in biomedicine are ^{125}I and ^{131}I , the application of ^{123}I and ^{124}I in biomedicine has increased in recent years. The sources of ^{127}I and ^{129}I , the production methods for radioisotopes of iodine, and biomedical application of radioiodine are presented. Concentrations of iodine isotopes, especially ^{129}I in the thyroid and urine are discussed. Analytical methods for the determination of iodine isotope, especially ^{129}I and ^{127}I , are reviewed. Finally, the radiation risk of radioiodine to the thyroid, especially the radioisotopes released from nuclear accidents, ^{131}I , ^{132}I , ^{133}I and ^{135}I , are discussed.

Abbreviations

AMS	Accelerator mass spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
NAA	Neutron activation analysis
PET	Positron emission tomography
SPECT	Single photon emission computed tomography

Introduction

Iodine is an essential element to humans and presents in the human body in minute amounts (15–20 mg in adults), of which more than 80% exists in the thyroid gland. Iodine in the human body mainly comes from food intake and inhalation of atmospheric iodine. In the thyroid, iodine is added to the essential amino acid thyroxine residue on thyroglobulin,

which ultimately forms thyroid hormones. The synthesized T_4 is released into the blood and transported to organs and cells. The biological actions of thyroid hormones occur in non-thyroid tissues (Walter, 1986). In the target cells and liver, the hormones are degraded and iodide ion is released after fulfilling of the biological function. The released iodide may be reused in the thyroid or excreted to the urine via the kidneys. Since almost all the iodine in the body is eventually excreted in the urine, urinary iodine excretion provides a good index of iodine availability in a stated population, and is universally employed in epidemiological studies on goiter and iodine deficiency disorders.

The chart of nuclides summarizes 34 isotopes of iodine from ^{108}I to ^{142}I , but only one isotope, ^{127}I , is stable. Most of these isotopes are short-lived (five isotopes with a half life longer than one day). Table 46.1 lists the isotopes of iodine with a half life of more than 10 min. Of all radioactive isotopes, only ^{129}I is a naturally occurring (15.7×10^7 years) radioisotope of iodine with a long half life.

Sources and Production of Iodine Isotopes

There are only two isotopes of iodine present in nature, ^{127}I and ^{129}I . ^{127}I , as the only stable isotope of iodine, is widely distributed in the environment. The reported iodine concentration on the earth is normally quite low, 0.25 ppm in the earth's crust (Muramatsu and Wedepohl, 1998), 10 ng/ m^3 in the atmosphere and 0.05 ppm in the biosphere on the basis of fresh mass (Shinonaga *et al.*, 2001). The main reservoir of iodine in the earth's crust is the ocean and sea, approximately 70% of the iodine in the earth's crust is bound to ocean sediments (Muramatsu and Wedepohl, 1998). The iodine concentration in seawater (40–60 ng/ml) is much higher than in freshwater (<1–10 ng/ml). It is well-known that iodine is highly enriched (13–6000 ppm

Table 46.1 Nuclear properties and production model of iodine isotopes with a half life more than 10 min

Isotope	Half life	Decay	E_{max} (keV)	Main γ -ray energy, keV (abundance)	Production
^{118}I	13.7 m	EC + β^+	5402.3 (32.8%, β^+)	605.7 (78%)	Cyclotron
^{119}I	19.1 m	EC + β^+	2231 (46%, β^+), 3253 (38%, EC)	257.5 (87%)	Cyclotron
^{120}I	81.0 m	EC + β^+		506.4 (73%)	Cyclotron
^{120m}I	53 m	EC + β^+		601.1 (87%), 614.6 (67%)	Cyclotron
^{121}I	2.12 h	EC + β^+	2057.8 (76%, β^+), 1034 (10%, EC)	212.2 (84%)	Cyclotron
^{123}I	13.27 h	EC + β^+	1074.9 (97%, EC)	159 (83%)	Cyclotron
^{124}I	4.18 d	EC + β^+	2557 (25%, EC), 3160 (24%, EC), 1535 (12%, β^+), 2138 (11%, β^+)	602.7 (63%), 723 (10%), 1691 (11%)	Cyclotron
^{125}I	59.41 d	EC	150.6 (100%)	35.5 (6.68%), 27.2 (40%), 27.5 (76%)	Cyclotron/reactor
^{126}I	13.11 d	EC + β^+ , β^-	869.4 (32%, β^-), 1489 (29%, EC), 2155 (23%, EC)	338.6 (34%), 666.3 (33%)	Reactor
^{127}I	Stable				
^{128}I	24.99 m	β^- , EC + β^+	2119 (80%, β^-)	442.9 (17%)	Reactor
^{129}I	1.57×10^7 yr	β^-	154.4 (100%)	39.6 (7.5%), 29.5 (20%), 29.8 (38%)	Reactor
^{130}I	12.36 h	β^-	587 (47%), 1005 (48%)	536 (99%), 668.5 (96%), 739.5 (82%)	Reactor
^{131}I	8.02 d	β^-	606 (90%)	364.5 (82%)	Reactor
^{132}I	2.30 h	β^-	738 (13%), 1182 (19%), 2136 (19%)	667.7 (99%), 772.6 (76%)	Reactor
^{132m}I	1.39 h	IT, β^-	1483 (8.6%, β^-)	600 (14%), 173.7 (8.8%)	Reactor
^{133}I	20.8 h	β^-	1240 (83%)	529.9 (87%)	Reactor
^{134}I	52.5 m	β^-	1307 (30%)	847 (95%), 884 (65%)	Reactor
^{135}I	6.57 h	β^-	970 (22%), 1388 (24%)	1260 (29%)	Reactor

Notes: Half life of the isotopes are given as m: minutes; h: hours; d: days; and yr: years. The decay model: EC for electron capture; β^+ for positron emission; β^- for beta emission; IT for internal transfer. An isotope may decay by more than one model. The 4th column lists the maximum energy of particles emitted (E_{max}), the branching ratio is given in brackets. The main γ -ray energy and intensity are listed in the 5th column. The production method (irradiation in reactor or cyclotron) is given in the 6th column.

dry wet) in seaweed (Hou and Yan, 1998). The iodine concentration in marine fish (0.2–3 ppm wet mass) is also higher than freshwater fish (0.02–0.04 ppm wet mass). The iodine in food is normally low, and varies with the type of food and place; Table 46.2 lists the concentration of iodine in 11 types of food from China (Hou et al., 1997). Most iodine in the thyroid comes from the diet, and concentration of iodine in the diet is directly related to the status and function of the thyroid.

^{129}I is the only naturally-occurring radioisotope of iodine. All of the ^{129}I formed during primordial nucleosynthesis has decayed to ^{129}Xe (stable). Natural processes, such as spontaneous fission of ^{238}U , thermal neutron-induced fission of ^{235}U , and spallation reaction of Xe in the upper atmosphere contribute to a steady-state concentration of ^{129}I . The estimated atom ratios of $^{129}\text{I}/^{127}\text{I}$ in the marine environment is 3×10^{-13} to 3×10^{-12} , and 10^{-15} to 10^{-14} in the lithosphere (NCRP, 1983), respectively. Since 1945, the atom ratio of $^{129}\text{I}/^{127}\text{I}$ in the environment has been significantly

Table 46.2 Concentration of iodine in 11 types of food in China ($\mu\text{g/g}$, wet mass)

Type of food	Range	Average
Grain	0.060–0.165	0.143
Beans	0.070–0.193	0.104
Potatoes	0.090–0.292	0.139
Meat	0.207–0.262	0.255
Eggs	0.195–0.543	0.366
Aquatic products	0.061–0.45	0.366
Milk	0.011–0.061	0.046
Vegetables	0.102–0.161	0.123
Fruit	0.005–0.009	0.007
Sugar	0.01–0.023	0.016
Water	0.010–0.014	0.012

Notes: China is divided into four areas, namely, North1, North 2, South1, and South 2 which represent 12 of the total 23 provinces in China. 11 types of diet were collected from different places in a province. The same types of diet from the same area were mixed and analyzed for iodine. The range of iodine concentrations and the average values in the four areas are provided.

increasing, because of ^{129}I added by nuclear weapons testing, as well as the peaceful use of nuclear energy. The global release of ^{129}I due to nuclear weapons testing has increased the ratio of $^{129}\text{I}/^{127}\text{I}$ in the marine environment to $10^{-11}\sim 2\times 10^{-10}$ and $10^{-9}\sim 10^{-7}$ in the terrestrial environment (Hou *et al.*, 2000; Chao and Tseng, 1996; Handl *et al.*, 1993; Seki and Hatano, 1994). However, the largest source of anthropogenic ^{129}I to the environment by far is the reprocessing of spent nuclear fuel. Nuclear reprocessing plants at La Hague and Marcoule (France) and Sellafield (UK) had discharged 4750 kg ^{129}I to the marine system and 450 kg ^{129}I to the atmosphere by 2006 (Reithmeier *et al.*, 2006; Hou *et al.*, 2007). Besides the reprocessing plants in Europe, it was also reported that reprocessing plants in the USA and Japan have also released ^{129}I to the environment (VanMiddlesworth and Handl, 1997; VanMiddlesworth *et al.*, 2000; Muramatsu *et al.*, 1984). In addition, some nuclear accidents also released some ^{129}I to the environment. Most of this ^{129}I was however deposited in the local area, some of it was also dispersed to a large area in Europe (Raisbeck and Yiou, 1999). A higher ^{129}I level ($^{129}\text{I}/^{127}\text{I}$ ratio of 10^{-6}) from the heavily contaminated area near Chernobyl has been reported (Hou *et al.*, 2003b; Straume *et al.*, 1996, 2006; Mironov *et al.*, 2002; Michel *et al.*, 2005). Table 46.3 lists the sources and environmental levels of ^{129}I .

Except for ^{127}I and ^{129}I , all other isotopes of iodine are man-made radioisotopes. Of these, ^{123}I , ^{125}I , and ^{131}I are produced on an industrial scale and ^{124}I on a small-scale for biomedical utilization. ^{123}I is a short-lived radioisotope of iodine, with a half life of 13.27 h. It is produced by cyclotron, and 25 nuclear reactions have been investigated to produce ^{123}I . Among them, Table 46.4 lists the most significant reactions. $^{123}\text{Te}(\text{p}, \text{n})$ ^{123}I and $^{124}\text{Te}(\text{p}, 2\text{n})$ ^{123}I reactions are normally used for production of ^{123}I in the low- and medium-energy cyclotron (Qaim, 2003). The separation of ^{123}I from TeO_2 target is normally carried out by dry distillation, the separated product is either directly led into a 0.01 mol/l NaOH solution or is first adsorbed on a column and subsequently eluted with dilute NaOH (Qaim, 2003).

Table 46.3 Source and environmental level of ^{129}I

Source	Inventory/released (kg)	$^{129}\text{I}/^{127}\text{I}$ ratio in the environment
Nature	210	$\sim 1 \times 10^{-12}$
Nuclear weapons testing	50–70	$\sim 1 \times 10^{-10}$
Chernobyl accident	1.3	
Marine discharge from reprocessing plants by 2006	4750	$\sim 10^{-6}$ (North Sea water)
Air emission from reprocessing plants by 2006	450	$10^{-7}\sim 10^{-6}$ (rainwater in Europe)

Notes: The inventory of ^{129}I in nature, and the amount released from different activities are listed. The average level of ^{129}I presented as the ratio of $^{129}\text{I}/^{127}\text{I}$ in the environment is given.

^{123}I decays mainly by electron capture (>99.5%) emitting γ -rays with a main energy of 159 keV (Qaim, 2003).

^{124}I is also a short-lived radioisotope of iodine, with a relatively long half life of 4.18 days. It is produced by cyclotron via the reactions shown in Table 46.4. The best route is the $^{124}\text{Te}(\text{p}, \text{n})$ ^{124}I process, since it can be carried out in a low energy cyclotron, and the level of long-lived ^{125}I impurity is very low. A similar method as for ^{123}I is used for recovery of ^{124}I . ^{124}I decays by electron capture and emission of positrons (EC, 77% and β^+ , 23%), followed by emission of a number of high-energy gamma photons, i.e., 603 (61%), 723 (10%) and 1691 keV (11%), in addition to 511 keV annihilation photons.

^{125}I is a relative long-lived radioisotope with a half life of 59.4 days. ^{125}I can be produced by cyclotron via the reactions listed in Table 46.4. The commercial production of ^{125}I is normally carried out by irradiation of Xe with thermal neutrons via reaction $^{124}\text{Xe}(\text{n}, \gamma)$ $^{125}\text{Xe}(\text{EC})$ ^{125}I . Elemental Xe and solid XeF_2 can be used as targets. After irradiation, the XeF_2 target is dissolved in water and the evolved gaseous Xe is collected in a cold trap. After a few days decay, ^{125}Xe is cryogenically transferred to another cold trap, and ^{125}I in the first cold trap is dissolved in dilute NaOH. ^{125}I decays by 100% electron capture with emission of low energy γ -rays (35.5 keV) in only 6.7% of total disintegration and X-rays (27.2 keV, 27.5 keV).

Table 46.4 Nuclear reactions and production model of most often used radioactive isotopes of iodine

Isotope	Production	Reaction
^{123}I	Cyclotron	$^{123}\text{Te}(\text{p}, \text{n})$ ^{123}I ,
		$^{124}\text{Te}(\text{p}, 2\text{n})$ ^{123}I ,
		$^{127}\text{I}(\text{p}, 5\text{n})$ $^{123}\text{Xe}(\text{EC})$
		^{123}I , $^{124}\text{Xe}(\text{p}, \text{x})$
		$^{123}\text{Xe}(\text{EC})$ ^{123}I ,
		$^{122}\text{Te}(\text{d}, \text{n})$ ^{123}I ,
^{124}I	Cyclotron	$^{125}\text{Te}(\text{d}, 2\text{n})$
		and $^{121}\text{Sb}(\alpha, 2\text{n})$ ^{123}I
		$^{124}\text{Te}(\text{d}, 2\text{n})$ ^{124}I ,
		$^{124}\text{Te}(\text{p}, \text{n})$ ^{124}I ,
^{125}I	Cyclotron	$^{125}\text{Te}(\text{p}, 2\text{n})$ ^{124}I , and
		$^{126}\text{Te}(\text{p}, 3\text{n})$ ^{124}I
		$^{125}\text{Te}(\text{p}, \text{n})$ ^{125}I ,
		$^{124}\text{Te}(\text{d}, \text{n})$ ^{125}I ,
		$^{125}\text{Te}(\text{d}, 2\text{n})$, and
^{125}I	Reactor	$^{126}\text{Te}(\text{p}, 3\text{n})$ ^{125}I
		$^{124}\text{Xe}(\text{n}, \gamma)$
^{131}I	Reactor	$^{125}\text{Xe}(\text{EC})$ ^{125}I
		$^{130}\text{Te}(\text{n}, \gamma)$ $^{131}\text{Te}(\beta^-)$
		^{131}I , $^{235}\text{U}(\text{n}, \text{f})$ ^{131}I

Notes: The nuclear reactions for the production of iodine isotopes are listed. For $^{123}\text{Te}(\text{p}, \text{n})$ ^{123}I , ^{123}Te is the target nuclide, (p, n) is nuclear reaction which indicates bombarding with proton, with a emitting of neutron, and ^{123}I is produced. In the nuclear reactions, p is proton; n is neutron; 2n means two neutrons, d is deuterium, α is alpha particle, γ is gamma rays, f is fission products. EC means decay by electron capture, and (β^-) means decay by beta emission.

^{131}I is the main radioisotope of iodine used in medical applications with a half life of 8.02 days. The production of ^{131}I can be carried out by thermal neutron fission of ^{235}U or neutron activation reaction of $^{130}\text{Te}(n, \gamma) \text{}^{131}\text{Te}(\beta^-) \text{}^{131}\text{I}$, while commercial production is mainly carried out by thermal neutron fission reaction of ^{235}U (fission yield of 2.89%). The ^{131}I produced is easily separated from uranium and other fission products by the dry distillation method. The wet method based on the CCl_4 extraction is also used for the separation of iodine (Mirzadeh *et al.*, 2003). However, fission-produced ^{131}I is contaminated with stable ^{127}I and ^{129}I as the fission yields leading to masses 127 and 129 are 0.157 and 0.54%, respectively. The actual ratio of ^{131}I to ^{127}I and ^{129}I , however, will be a function of neutron flux, irradiation time and the post-irradiation decay period. In addition, fission yields for short-lived iodine isotopes (^{132}I , ^{133}I , ^{134}I , and ^{135}I) are substantial (most importantly ^{133}I with a yield of 6.7%), requiring post-irradiation decay to increase the radionuclide purity of ^{131}I (the life lives of these isotopes are less than 21 h). ^{131}I decays by 100% beta emission, with an average beta particle energy of 182 keV and maximum energy of 606.3 keV. The decay of ^{131}I is followed by emission of intensive γ -rays at 364.5 (81%), 637 (7.3%), 284.3 (6%) and 80.2 keV (2.65%).

^{126}I , ^{128}I and ^{130}I are neutron activation products of ^{127}I and ^{129}I by reactions $^{127}\text{I}(n, \gamma) \text{}^{128}\text{I}$, $^{127}\text{I}(n, 2n) \text{}^{126}\text{I}$, $^{127}\text{I}(3n, \gamma) \text{}^{130}\text{I}$, and $^{129}\text{I}(n, \gamma) \text{}^{130}\text{I}$, and ^{126}I can be also produced from $^{125}\text{I}(n, \gamma) \text{}^{126}\text{I}$ reaction. Due to limited applications, no commercial production of these radioisotopes is carried out. In addition, the short half lives of these radioisotopes (Table 46.1) makes them occur very seldom in the environment or human body.

^{132}I , ^{133}I , ^{134}I , and ^{135}I are fission products of uranium with fission yields of uranium, 4.31, 6.70, 7.87, and 6.54%, respectively. All of these radioisotopes of iodine decay by emitting beta particles with high energies (Table 46.1). These radioisotopes are not produced industrially or at laboratory scale because there are no real applications. However, they have been released to the environment, with ^{131}I and other fission products in some accidents, such as Chernobyl. Due to the short half life of these radioisotopes (0.9–20.8 h), they decayed very quickly and no remaining radioactivity can be measured after a few weeks of the release.

Utilization of Iodine Isotopes

Radioisotopes of iodine have been widely used in clinical diagnosis and therapy for various diseases, and in biomedical and environmental studies as a tracer of iodine or labeled compounds. The most frequently used radioisotopes of iodine are ^{131}I and ^{125}I . Due to special nuclear properties of ^{123}I and ^{124}I , the application of these two radioisotopes of iodine is increasing.

^{131}I has been used for the diagnosis and therapy of thyroid diseases for more than 60 years (Sawin and Becker,

1997), and it is still a main method used for the diagnosis of thyroid cancer, hypothyroidism, hyperthyroidism and for therapy in Graves' disease, papillary and follicular thyroid carcinoma; it has become a standard method for the treatment of persistent hyperthyroidism (Moka *et al.*, 2002). Higher energy γ -rays (364.5 and 637 keV) of ^{131}I are used for imaging of the thyroid, to show thyroid activity and, thereby show the location, size, and disease. ^{131}I therapy is based on the emission of suitable energy beta particles, and more than 1000 times higher concentration of thyroid to iodine compared to other tissues. In the thyroid cell, 90% of radiation dose of ^{131}I delivered by beta particles is absorbed within 0.8 mm from the source (mean energy of 190 keV, maximal energy of 606 keV). Virtually no beta particles escape from thyroid tissue where ^{131}I is concentrated, so large doses of radiation may be delivered by ^{131}I without damaging surrounding tissues (Klain *et al.*, 2002). At the same time, most of the central thyroid tissue is damaged when a suitable ^{131}I dose is used. In the case of thyroid cancer, the cancer cells are killed, and excessive thyroid activity is reduced in the case of hyperthyroidism.

^{125}I is another widely-used radioisotope of iodine in clinical medicine and biological studies. ^{125}I is also used for imaging of the thyroid; due to its decay by electron capture, the radiation dose to the thyroid required is much lower than ^{131}I . However, its low γ -ray energy (35.5 keV) makes its measurement and the diagnosis of some thyroid diseases more difficult. In addition, it is also used in bio distribution studies of ^{125}I -labeled drugs, peptides and antibodies, cell-targeted therapy of ^{125}I -labeled nucleic acid precursors permanent implants into the prostate with ^{125}I seeds. In the case of ^{125}I -labeled nucleic acid therapy, the radiotoxicity is due to the fact that the decay energy of ^{125}I is followed by a shower of short-range secondary electrons of only a few hundred electron volts and about 1 μm path-length in tissue. Consequently, a significant fraction of the decay energy is deposited in microscopic volumes in the vicinity of DNA, leading to multiple strand breaks (Mirzadeh *et al.*, 2003).

^{124}I decays by emitting positrons (22%), it is therefore used for positron emission tomography (PET) of thyroid (Freudenberg *et al.*, 2004). ^{124}I can be also used for imaging of the thyroid by using its γ -rays (623 (63%), 723 (10%), and 1691 keV (11%)). Due to γ -rays emission of ^{123}I , it can be also used for imaging of the thyroid. The short half life (13.27 h), electron capture decay, and low γ -ray energy (159 keV) of ^{123}I significantly reduce the radiation dose required during thyroid imaging to about 1/100 of ^{131}I when using the same amount of radioactivity. In addition, ^{123}I is a very useful analog label for producing radiotracers. Its nuclear properties are also almost ideal for single photon emission computed tomography (SPECT) studies. Compared to ^{131}I and ^{125}I , the application of ^{123}I and ^{124}I in clinical medicine and biomedical studies is very limited, not only because results from their nuclear properties, but also because of lower availability.

^{129}I , due to its very long half life, has been used as a tracer to investigate the distribution and metabolism of iodine in the body in the long-term (Hindie *et al.*, 2001). However, the difficulty of measurement due to its very low specific radioactivity ($6.5 \times 10^6 \text{ Bq/g}$) compared to ^{131}I ($4.7 \times 10^{15} \text{ Bq/g}$), the low energy of beta particles (156 keV) and γ -rays (39.6 keV) limits its application.

Level of Iodine Isotopes in Thyroid and Urine

^{127}I level in the thyroid depends on the status of iodine intake and function of the thyroid. The concentration of ^{127}I in normal thyroid is about 1 mg/g wet mass. Hou *et al.* (2000, 2003a) have reported a concentration of ^{127}I of 0.2–7.2 mg/g dry mass (or 0.04–1.4 mg/g in wet mass) in human thyroid tissue from China and Belarus, the very low ^{127}I concentration in some thyroid tissue may contribute to insufficient intake of iodine by the subject in Belarus, where iodine deficiency has been observed. A relatively higher concentration ^{127}I (0.5–3.0 mg/g) was reported in the thyroid of sheep from the coastal area of Denmark (Hou *et al.*, 2003a), which may result from the higher iodine in feed (2 ppm dry mass).

^{127}I level in the urine directly reflects iodine intake from food, because most of iodine absorbed from food is finally excreted to urine. The recommended dietary intake of iodine for adults is 120–150 mg/day by the WHO and 130 mg/day by the EU (Sumar and Ismail, 1997) and the corresponding urinary iodine level is 100–200 $\mu\text{g/l}$ (Hampel *et al.*, 2001; Delange, 2002).

^{129}I level in the thyroid is mainly related to the ^{129}I level in the environment, especially in food. Due to the same chemical and biological properties of different isotopes of iodine, ^{129}I level in the thyroid and the environment is normally expressed as an atomic ratio of $^{129}\text{I}/^{127}\text{I}$. Extensive investigation of ^{129}I levels in thyroid have been undertaken in North America (Ballad *et al.*, 1978; Oliver *et al.*, 1982), Europe (Gros *et al.*, 1975; Handl *et al.*, 1990, 1993; Aumann *et al.*, 1981, 1985; Hou *et al.*, 2003a) and Asia (Seki and Hatano, 1994; Chao and Tseng, 1996; Hou *et al.*, 2000). $^{129}\text{I}/^{127}\text{I}$ ratios in the human thyroid collected before human nuclear activity were reported to be less than 4×10^{-11} (Schmidt *et al.*, 1998; Brauer *et al.*, 1974), which reflects the natural level of ^{129}I in the environment. The ratios of $^{129}\text{I}/^{127}\text{I}$ in the thyroid increased to about 10^{-6} in the 1950s in the USA because of nuclear weapons testing in the 1950s and 1960s, and then declined to about 10^{-8} after the 1970s, due to a considerable reduction in these tests (Keisch *et al.*, 1965; Ballad *et al.*, 1978). Low $^{129}\text{I}/^{127}\text{I}$ ratios in the thyroid ($<10^{-9}$) were reported in South America, Australia and Asia (Seki and Hatano, 1994; Hou *et al.*, 2000; Handl *et al.*, 1993; Chao and Tseng, 1996). This level reflects the ^{129}I fallout from

the nuclear weapons testing in the 1950s and 1960s, and its distribution throughout the world. A higher ^{129}I level ($^{129}\text{I}/^{127}\text{I} > 10^{-8}$) has been reported in the mammalian and human thyroids in western Europe (Germany, France, Italy, the Netherlands, Austria and Norway) compared with those from Asia and South America (10^{-10} – 10^{-9}) (VanMiddlesworth and Handl, 1997; Handl *et al.*, 1993; Gros *et al.*, 1975; Seki and Hatano, 1994; Chao and Tseng, 1996; Hou *et al.*, 2000). This is attributed to very significant atmospheric release of ^{129}I from reprocessing plants in Europe. Figures 46.1 and 46.2 show the variation of $^{129}\text{I}/^{127}\text{I}$ ratio in human and animal thyroids in Europe with time. An increased $^{129}\text{I}/^{127}\text{I}$ ratio is observed in the animal thyroid since the 1990s, this trend agrees with the level of ^{129}I in precipitation (Figure 46.1), which reflects increased marine discharge of ^{129}I from reprocessing plants in La Hague and Sellafield. However, the $^{129}\text{I}/^{127}\text{I}$ ratio in human thyroid decreased since the 1990s (Figure 46.2), this may be attributed to the increased use of iodized table salt in Europe since 1990 (Delange, 2002; Hou *et al.*, 2003a, b). Table 46.5 shows the reported ^{129}I level in human and animal thyroids in different places around the world.

High $^{129}\text{I}/^{127}\text{I}$ levels in animal thyroid has been observed in the vicinity of nuclear facilities, especially reprocessing plants. $^{129}\text{I}/^{127}\text{I}$ ratios of $(1\text{--}250) \times 10^{-6}$ in bovine thyroid from North Cotentin in France, where the La Hague reprocessing plant is located (Frechou, 2000), ratios of 10^{-7} to 10^{-2} in deer thyroid from the Savannah River site in the USA, where a reprocessing plant is located (VanMiddlesworth *et al.*, 2000), and ratios of 10^{-5} in deer thyroid from the vicinity of the Karlsruhe reprocessing plant in Germany (Robens *et al.*, 1988) have been reported. Hou *et al.* (2003a) reported $^{129}\text{I}/^{127}\text{I}$ ratios of 2.65×10^{-9} to 1.10×10^{-8} with an average of 7.21×10^{-9} in human thyroid from Gomel in Belarus, where high ^{131}I and ^{137}Cs deposition have been reported. This value is almost one order of magnitude higher than those from Asia (Japan and China) and South America, which are remote from nuclear facilities and are only impacted by global fallout.

Urine has been widely used for investigation of the human nutrition status of iodine (Brussaard *et al.*, 1997). It can therefore be used as an indicator of the ^{129}I level and exposure status of humans. Hou *et al.* (2003a) determined ^{129}I concentration in human urine samples collected from Denmark. The $^{129}\text{I}/^{127}\text{I}$ measured in human urine ($(0.9\text{--}2.9) \times 10^{-8}$) is around 10 times lower than the $^{129}\text{I}/^{127}\text{I}$ ratio in sheep thyroid ($(5\text{--}40) \times 10^{-8}$), grass ($(19\text{--}26) \times 10^{-8}$) and rainwater ($(14\text{--}107) \times 10^{-8}$) from Denmark (Hou *et al.*, 2003a; Hou, 2004).

Except for ^{127}I and ^{129}I , other isotopes of iodine occur in the thyroid and urine only for a short time, due to their short half life (<60 days). ^{126}I , ^{128}I and ^{130}I are very seldom used for medical purposes except for the use of ^{130}I in the very early period of medical applications of radioisotopes (Becker and Sawin, 1996). ^{123}I , ^{124}I , ^{125}I and ^{131}I

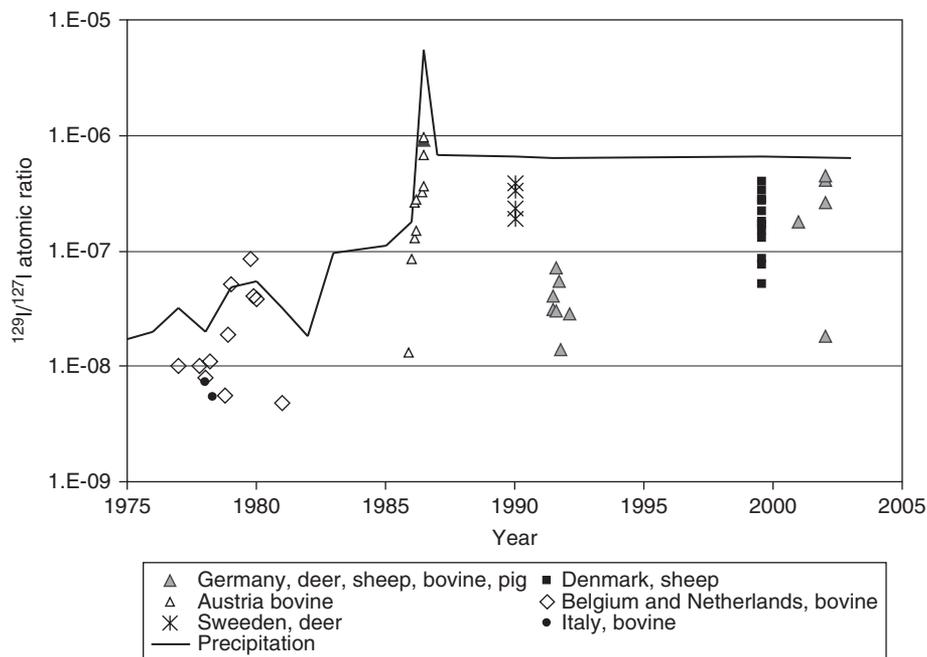


Figure 46.1 Variation of $^{129}\text{I}/^{127}\text{I}$ ratios in animal thyroid from Europe (1978–2002). The reported $^{129}\text{I}/^{127}\text{I}$ ratios in animal thyroid from different countries were plotted vs. time. The variation in individual location is shown as the measured values. The variation in $^{129}\text{I}/^{127}\text{I}$ ratio in precipitation is also shown as a line.

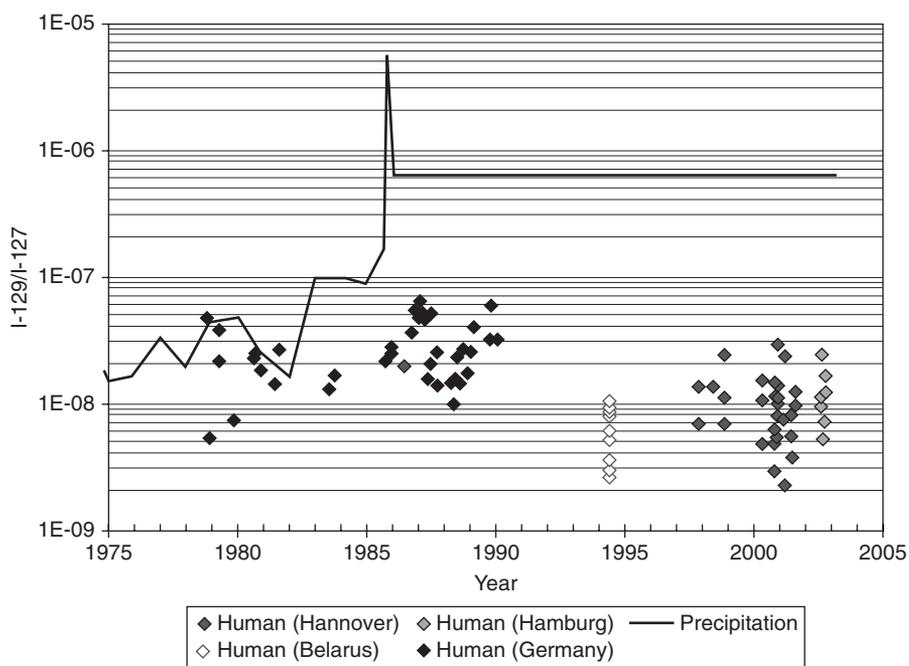


Figure 46.2 Variation of $^{129}\text{I}/^{127}\text{I}$ ratios in human thyroid from Europe (1978–2003). The reported $^{129}\text{I}/^{127}\text{I}$ ratios in human thyroid from different countries were plotted vs. time. The variation in $^{129}\text{I}/^{127}\text{I}$ ratio in precipitation is also shown as a line.

Table 46.5 Comparison of ^{129}I level in human and animal thyroids from different places

Sample	Date	Location	$^{129}\text{I}/^{127}\text{I}$ ratio		References
			Range	Mean	
Human thyroid					
Human thyroid	<1936	USA	$<4 \times 10^{-11}$		Brauer <i>et al.</i> , (1974)
Human thyroid	1936	USA	4×10^{-11}		Edwards, (1962)
Thyroid powder	1943	USA	7.0×10^{-12}		Schmidt <i>et al.</i> , (1998)
Horse thyroid	1947	USA	7.6×10^{-10}		Oliver <i>et al.</i> , (1982)
Human thyroid	1974–1975	Missouri, USA	2.4×10^{-10} – 1.33×10^{-8}	2.8×10^{-9}	Oliver <i>et al.</i> , (1982), Ballad <i>et al.</i> , (1978)
Human thyroid	1983	Tokyo, Japan	4.1×10^{-10} – 1.3×10^{-9}	7.9×10^{-10}	Seki and Hatano, (1994)
Human thyroid	1979–1984	Lower Saxony, Germany	2.1×10^{-9} – 4.7×10^{-8}	2.0×10^{-8}	Handl <i>et al.</i> , (1990)
Human thyroid	1985–1986	Chile	1.1 – 2.0×10^{-9}		Handl <i>et al.</i> , (1993)
Human thyroid	1986–1990	Lower Saxony, Germany	3.6×10^{-9} – 6.0×10^{-8}	2.9×10^{-8}	Handl <i>et al.</i> , (1990, 1993)
Human thyroid	1994–1995	Tianjin, China	4.1×10^{-10} – 2.0×10^{-9}	1.1×10^{-9}	Hou <i>et al.</i> , (2000a)
Human thyroid	1995	Gomel, Belarus	0.265 – 1.10×10^{-8}	7.21×10^{-9}	Hou <i>et al.</i> , (2003b)
Animal thyroid					
Animal thyroid	1974–1977	Missouri, USA	4.0×10^{-10} – 1.4×10^{-8}	3.6×10^{-9}	Oliver <i>et al.</i> , (1982), Ballad <i>et al.</i> , (1978)
Cow thyroid	1973–1981	France, Italy, Belgium and the Netherlands	7.6×10^{-9} – 1.0×10^{-7}		Handl <i>et al.</i> , (1990), Aumann <i>et al.</i> , (1985), Gros <i>et al.</i> , (1975) Aumann <i>et al.</i> , (1985)
Bovine and Hog thyroid	1979–1981	Germany	4 – 900×10^{-10}		Aumann <i>et al.</i> , (1985)
Bovine thyroid	1983	Japan	3.5×10^{-10} – 1.38 $\times 10^{-9}$	7.9×10^{-10}	Seki and Hatano, (1994)
Deer	1983	WAK, Karlsruhe, Germany	9 – 15×10^{-6}		Robens <i>et al.</i> , (1988)
Bovine thyroid	1985–1986	Chile	1.2×10^{-10} – 6.0×10^{-9}		Handl <i>et al.</i> , (1993)
Bovine thyroid	1986	Ulm, Germany	3.3 – 9.0×10^{-7}	6.15×10^{-7}	VanMiddlesworth and Handl (1997)
Bovine thyroid	1986	Bad Hall, Austria	0.85 – 9.0×10^{-7}	2.54×10^{-7}	VanMiddlesworth and Handl (1997)
Bovine thyroid	1986	Norway	1.0 – 2.1×10^{-8}	1.55×10^{-8}	VanMiddlesworth and Handl (1997)
Sheep thyroid	1957 and 1989	Australia	5 – 6×10^{-10}		Handl (1996)
Reindeer	1989–1990	Gävle, Sweden	1.9 – 4.0×10^{-7}		Handl (1996)
Sheep, hog and bovine thyroid	1995–1996	Taiwan	2.3×10^{-11} – 8.2×10^{-9}	1.46×10^{-9}	Chao and Tseng (1996)
Bovine thyroid	1999	North Contentin, France	0.9 – 250.7×10^{-6}		Frechou (2000)
Sheep thyroid	2000	Ribe, Denmark	5.28 – 41.1×10^{-8}	1.81×10^{-7}	Hou <i>et al.</i> , (2003b)

Notes: The range of $^{129}\text{I}/^{127}\text{I}$ ratio reported and the average is listed.

in the thyroid and urine mainly result from medical applications for radioiodine therapy and diagnosis. The concentration of these radioisotopes of iodine in the thyroid and urine therefore very much depends on the amount and chemical form of these isotopes, the status of iodine nutrition and the thyroid status. Iodine administered as iodide is quickly absorbed and stored in the thyroid, and then gradually excreted to urine. For a normal thyroid status, 30–40% of iodide administered is absorbed in the thyroid 24 h after the administration. A high uptake of iodide in the thyroid occurs with hyperthyroidism or

deficiency of iodine intake from diet (40–80% for 24 h thyroidal uptake), while a low uptake (<20% for 24 h thyroid uptake) occurs with hypothyroidism. If radioisotopes of iodine are administered as labeled compounds, it may not be concentrated in the thyroid, instead of other target tissues. Radioiodine used for medical treatment should only occur in the patient's body. However, a large amount of ^{131}I (more than 1.5×10^9 Bq per person for the therapy) is used in the hospital, and some of this ^{131}I may enter into the waste system by urinary excretion of ^{131}I from patients, although most urine from the

patients may be collected in the first few days after administration. The author has measured a significant amount of ^{131}I in slug from a waste treatment station. The people working in the waste treatment station and public nearby the station may be exposed to ^{131}I because of the release of ^{131}I into the air during treatment (dry and incineration).

As an impurity in the production of ^{131}I by neutron fission of ^{235}U , ^{129}I presents in the thyroid and urine of patients who were administered ^{131}I . The ^{129}I concentration in ^{131}I products depends on the irradiation time and neutron flux. The reported isotopic ratio of $^{129}\text{I}/^{131}\text{I}$ in the medical ^{131}I solution ranges from 2.6×10^{-13} to $1.8 \times 10^{-12} \text{ g } ^{129}\text{I}/\text{kBq } ^{131}\text{I}$ (Aumann, 1981; Muramatsu *et al.*, 1984; Chao *et al.*, 1998; Hou *et al.*, 1999). An administration of $3.7 \times 10^9 \text{ Bq}$ of ^{131}I in therapy will inject $(9.6\text{--}66.6) \times 10^{-7} \text{ g } ^{129}\text{I}$ to the patient, and will increase $^{129}\text{I}/^{127}\text{I}$ in the thyroid and urine of the patient to $(0.6\text{--}4) \times 10^{-4}$ (total 15 mg iodine in the body). This value is comparable to the highest value found in the vicinity of reprocessing plants (Frechou, 2000).

^{131}I , ^{132}I , ^{133}I , ^{134}I and ^{135}I are fission products; they may be released from nuclear facilities. During the Chernobyl accident, a large amount of ^{131}I ($1.76 \times 10^{18} \text{ Bq}$) was released to the atmosphere (UNSCEAR, 2000) and transferred to human thyroids. A 4.3 Gy radiation dose to the thyroid from ^{131}I has been reported in the Gomel region, Belarus (Gayrilin *et al.*, 2004). At the same time ^{132}I , ^{133}I , ^{134}I and ^{135}I are also released. The estimated activity ratios of ^{132}I , ^{133}I and ^{135}I to ^{131}I in the core of the Chernobyl accident reactor are 1.33, 1.48, and 0.91 (Gayrilin *et al.*, 2004), which implies there is also a large amount of ^{132}I , ^{133}I , ^{134}I and ^{135}I released from the Chernobyl accident and transferred to the thyroid. Due to the short half lives ($<21 \text{ h}$), ^{132}I , ^{133}I , ^{134}I and ^{135}I released from the Chernobyl accident may only be deposited in the local area, and very small amounts could be transferred a long distance after a few days.

Determination of Iodine Isotopes in the Thyroid and Urine

Radioisotopes of iodine, except ^{129}I , in the thyroid and urine are easily determined by measurement of their γ -rays, which can be carried out by a γ -detector, such as NaI and high purity germanium (HpGe) detector. In thyroid imaging using ^{131}I , ^{125}I , or ^{123}I , a gamma camera is normally used. A good picture of ^{123}I can be obtained using a SPECT system. A better space resolution of ^{124}I imaging can be obtained using PET by measuring 511 keV γ -rays emitted from the annihilation of positrons.

^{129}I is a β -emitter with emission of 39.6 keV γ -rays and X-rays. It can therefore also be measured by γ -detector. ^{129}I in thyroids collected from a heavily contaminated area at La Hague has been successfully measured using a high efficiency

HpGe detector (Frechou *et al.*, 2002). However, in the normal environment, ^{129}I concentration in the thyroid and urine are normally very low ($<10 \text{ mBq}$ in the whole thyroid for a $^{129}\text{I}/^{127}\text{I}$ ratio of 10^{-7}), and it is difficult to be measured by γ -detector. The determination of ^{129}I by γ -detector in the urine of workers in the La Hague reprocessing plant was a failure (Le Guen *et al.*, 2002). Due to β^- -emission of ^{129}I , it can also be detected by liquid scintillation counter; however, the detection limit of LSC (10 mBq) for ^{129}I is similar to a γ -detector. Inductively coupled plasma mass spectrometry (ICP-MS) has been investigated for the determination of ^{129}I . However, the very serious interference of ^{129}Xe existing in Ar_2 gas, $^{127}\text{I} + 2\text{H}^+$ and tailing of ^{127}I make the detection limit of ^{129}I no better than 10^{-6} for $^{129}\text{I}/^{127}\text{I}$ (Izmer *et al.*, 2004).

For ^{129}I in thyroid and urine at environmental levels, only neutron activation analysis (NAA) and accelerator mass spectrometry (AMS) are sensitive enough; both of these methods require the separation of iodine from the sample. The separation of iodine from thyroid is normally carried out by combustion or alkaline fusion, followed by water leaching. Iodine is released from the sample during combustion at high temperatures (800–900°C) in a tube oven, and trapped in an alkaline solution. In the fusion method, the sample is mixed with alkaline solution, such as KOH, and then fused at 500–600°C. The fused sample is then leached with water. The iodine in the trap solution or leachate is then separated by CCl_4 extraction. For NAA, the separated iodide is irradiated in a nuclear reactor to convert ^{129}I to ^{130}I for γ -counting using an HpGe γ -detector. Additional ^{129}I , ^{127}I in the sample is also activated by fast neutrons in the reactor to ^{126}I via reaction $^{127}\text{I}(n, 2n) ^{126}\text{I}$. Therefore, both ^{129}I and ^{127}I can be determined by this method. For the AMS, iodine in the back-extracted aqueous phase is precipitated as AgI. The dried AgI precipitate is mixed with niobium or Ag powder and pressed into a holder. ^{129}I is then measured in an AMS system. A ratio of $^{129}\text{I}/^{127}\text{I}$ as low as 10^{-10} has been successfully measured by the radiochemical NAA method. While the detection limit of AMS is 3–4 orders of magnitude lower than NAA, a ratio of $^{129}\text{I}/^{127}\text{I}$ less than 10^{-13} can be measured by AMS.

The author has developed an analytical procedure for the determination of ^{129}I in urine, which is based on the fact that iodine in urine mainly exist as inorganic iodine. The urine sample is acidified with HNO_3 , and Na_2SO_3 is added to convert all iodine to iodide, the sample is then loaded to an anion exchange column, and the column is washed with water and 0.2 mol/l NaNO_3 , the iodide absorbed on the column is eluted by 2 mol/l KNO_3 . The iodine in the eluate is then concentrated by CCl_4 extraction. The separated iodine is finally determined using NAA or AMS (Hou *et al.*, 2003b).

^{127}I , as the only stable iodine, is often determined in urine to investigate iodine nutritional status. The available methods

for urine iodine determination have been reviewed by Dunn *et al.* (1998). The most practical and simple method involves mild acid digestion and colorimetry of the Sandell–Koltoff reaction. Urinary iodine concentration determined by ICP-MS is normally more accurate and sensitive (with a lower limit of detection of 1 µg/l). NAA is also a sensitive method for the determination of urinary iodine, a detection limit of 1 µg/l has been reported (Hou *et al.*, 2000).

Determination of ^{127}I in thyroid *in vitro* can easily be carried out by the methods used for urine (Hou *et al.*, 2000, 2003a; Imanishi *et al.*, 1991). However, only a few methods can be used for the determination of ^{127}I *in vivo*. An NAA method has been reported for the *in vivo* determination of ^{127}I in 1960s by Comar and his coworkers (Comar *et al.*, 1967). This method is based on the irradiation of thyroid directly by neutrons from a nuclear reactor or neutron generator, and the γ -counting of ^{128}I produced from the reaction $^{127}\text{I}(n, \gamma) ^{128}\text{I}$. The estimated radiation dose from neutrons was about 0.45 Gy at the thyroid and 1.2 Gy at the thyroid cartilage. Although this method is introduced 40 years ago, the clinic application is still very seldom. This may result from inconvenient access to a neutron irradiation facility and insufficient investigation of the irradiation damage of thyroid by neutrons.

An X-ray fluorescence method has been developed for *in vivo* determination of iodine in thyroid (Aubert *et al.*, 1981; Jonckheer and Deconinck, 1982). This method is based on the irradiation of iodine in the thyroid by γ -rays provided by a γ -source, such as ^{241}Am . The excited iodine atoms emit a characteristic X-ray fluorescence radiation, which is proportional to the amount of iodine present in the gland. The reported detection limit reaches 0.01 mg/ml thyroid, this value is much lower than the iodine concentration in thyroid. The reported radiation dose equivalent is only 60 mSv per measurement. This method has been successfully used for the clinical determination of ^{127}I in thyroid (Milakovic *et al.*, 2006; Reiners *et al.*, 1996, 2006; Briancon *et al.*, 1992). An indirect method was also reported to determine of thyroid iodine *in vivo* (Imanishi *et al.*, 1991), which is based on the relationship of CT attenuation values with iodine concentration in the thyroid. It was reported that the CT value correlated linearly with iodine concentration in thyroid nodules when iodine concentration was higher than 0.02 mg/g.

Radiation Risk of Radioisotopes of Iodine in Thyroid

Radioisotopes of iodine have been used in clinical medicine for the diagnosis and therapy of thyroid diseases for 60 years; it was considered that the risk linked to radioisotopes of iodine, especially ^{131}I , radiation was much lower. There has been no firm evidence of an excess radiation risk reported using radioiodine for imaging, in which a lower radioiodine

dose is normally delivered to the thyroid (~ 1 Gy). Radioiodine therapy for the treatment of hyperthyroidism, Graves' disease, thyroid autonomy, euthyroid multinodular goiters and thyroid carcinoma requires a much higher ^{131}I dose (> 50 Gy) (Lind, 2003; Klain *et al.*, 2002; Reiners and Schneider, 2003; Manders and Corstens, 2003). The risk of thyroid cancer from radioiodine therapy is still unclear and highly debatable. Some studies show no significant increase of thyroid cancer in patients treated with ^{131}I (Tan, 2003). A study of large cohorts of thyroid cancer patients treated with ^{131}I has demonstrated a significant increase in the risk of solid tumors and leukemias with high cumulative activity of radioiodine (De Vathaire *et al.*, 1997). It has been stated that no therapeutic activity of ^{131}I below which an increase in the risk of tumor and leukemia can be excluded, and the risk of leukemia increases with higher cumulative activities, especially when associated with external radiation therapy (Klain *et al.*, 2002).

The Chernobyl accident released a large amount of radioiodine, including ^{131}I , ^{132}I , ^{133}I , ^{134}I and ^{135}I . The ^{131}I activity in some people in the contaminated area were measured after the accident, and the absorption dose in the thyroid was estimated. The reported average thyroid dose per individual was 0.01–0.06 Gy in the heavily contaminated area. In the Gomel region in Belarus, 30% of children less than 4 years' old had received a thyroid dose higher than 2 Gy, but less than 5 Gy (Gavrilin *et al.*, 2004). Based on the experience of the radiation risk in medical use of ^{131}I , it was considered that the risk linked to ^{131}I is not a problem. However, an increased incidence of childhood thyroid cancer was reported in the contaminated territories, starting as early as 4 years after the accident (Hindie *et al.*, 2003).

The reason for the higher incidence of childhood thyroid cancer in the Chernobyl area is still not very clear. The following factors were considered to have enhanced the risk of thyroid cancer: (1) age at exposure, it was shown that thyroid irradiation increased with decreasing age, the number of thyroid cancer cases recorded in Belarus was three times higher in infants aged less than 5 years at exposure than in children aged 10–15 years (Heidenreich *et al.*, 1999). (2) It is well known that a high uptake of radioiodine occurs among people who have a deficient intake of dietary iodine. It has been reported that in some contaminated areas, such as Gomel in Belarus, there was a low dietary intake of iodine (Mityukova *et al.*, 1995). In addition, the microscopic distribution of radioiodine in cases of iodine deficiency showed a highly heterogeneous distribution of iodine from one follicle to another, this suggests that in cases of iodine deficiency, thyroid cells lining follicles with the highest radioactive iodine content would receive a dose substantially higher than the average cell dose value. (3) Short-lived radioisotopes of iodine, from ^{132}I to ^{135}I , were released in comparable amounts as ^{131}I in the Chernobyl accident. Due to a short half life, the accumulated radiation dose of these radioisotopes is only about

10% of ^{131}I . However, the dose rate delivered by these radioisotopes is much higher than ^{131}I . For example, the dose resulting from ^{133}I and ^{132}I in a given dose to the thyroid is delivered about 10 and 275 times faster than that resulting from ^{131}I . This means the initial dose rate from these short-lived radioisotopes would be much higher than in the case of pure ^{131}I . In addition, the heterogeneous distribution of radioiodine in the first period (hours to a day) after uptake also significantly increases the radiation dose from these short-lived radioisotopes in some cells (Hindie *et al.*, 2001).

Due to long half life and low concentrations of ^{129}I in the environment and thyroid (lower than 10^{-7} for $^{129}\text{I}/^{127}\text{I}$ ratio in normal environment, Table 46.5), the radiation dose to the thyroid from ^{129}I is very small. For the reported highest ^{129}I level (10^{-2} for $^{129}\text{I}/^{127}\text{I}$ ratio) in environmental samples and thyroid from the vicinity of reprocessing plants, the absorption dose of thyroid is estimated to be only 1.4, 1.2 and 0.7 mSv/yr for a 1-year-old, 10-year-old child, and adult, respectively (Le Guen *et al.*, 1998; Robkin and Shieien, 1995). It is therefore foreseen that there is no significant radiation risk of thyroid from ^{129}I .

Frechou C.(2000). Optimisation of the measurement protocols of ^{129}I and $^{129}\text{I}/^{127}\text{I}$. Methodology establishment for the measurement in environmental matrices, CEA-R-5947 pp.139.

Summary Points

- 34 isotopes of iodine have been found and produced, of which ^{127}I and ^{129}I occur in nature, and only ^{127}I is stable, all others are radioactive.
- The most frequently used radioisotopes of iodine are ^{131}I and ^{125}I . ^{131}I is normally produced by neutron irradiation of uranium, while ^{125}I is produced by neutron irradiation of ^{124}Xe .
- ^{131}I is widely used for radiotherapy and imaging, while ^{125}I , ^{124}I and ^{123}I are mainly used for imaging. ^{125}I and ^{131}I were also applied for radiolabeling and tracer studies.
- ^{127}I level in the thyroid depends on the status of iodine intake and function of the thyroid. The concentration of ^{127}I in normal thyroid is about 1 mg/g wet mass.
- ^{127}I level in the urine directly reflects iodine intake from food, the recommended dietary intake of iodine for adults is 120–150 mg/day, the corresponding urinary iodine level is 100–200 $\mu\text{g}/\text{l}$.
- $^{129}\text{I}/^{127}\text{I}$ ratios in the human thyroid have increased to 10^{-10} – 10^{-8} from $\sim 10^{-12}$ in the pre-nuclear era. The highest $^{129}\text{I}/^{127}\text{I}$ ratio of 10^{-2} was observed in animal thyroid collected from the vicinity of nuclear reprocessing plants.
- The reported $^{129}\text{I}/^{127}\text{I}$ in human urine from non-contaminated areas is $\sim 10^{-8}$.
- Radiometric methods are, mainly γ -detector, used for the determination of radioisotopes of iodine. For environmental levels of ^{129}I , only NAA and AMS are sensitive enough.
- NAA and X-ray fluorescence are used for *in vivo* determination of stable iodine in the thyroid; the most frequently used method for iodine in urine is colorimetry of the Sandell–Koltoff reaction, while ICP-MS is a more accurate and simple method compared to others.
- No significant radiation risk was reported for diagnosis using radioiodine, while high incidence of thyroid cancer was reported in heavily contaminated areas of the Chernobyl accident. The estimated radiation dose of thyroid is only <1.4 mSv/yr for the highest ^{129}I reported (10^{-2} for $^{129}\text{I}/^{127}\text{I}$ ratio).

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The U-Shaped Curve of Iodine Intake and Thyroid Disorders

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Abstract

Thyroid disorders are common in all populations, but the occurrence and the pattern of disease depend on the iodine intake of the population. The association between iodine intake level and the risk of disease is U-shaped, as both low and high iodine intakes are associated with an increase in the risk of thyroid problems. The curve is nonsymmetrical with the most serious problems associated with iodine deficiency, which should be corrected. However, the iodine intake should only be brought to the level where iodine deficiency disorders are avoided. Optimally, the iodization program should be planned to keep population iodine intake within a relatively narrow range around the recommended level.

Abbreviations

IQ	Intelligence quotient
NIS	Sodium iodide symporter
TSH	Thyroid stimulating hormone
UIE	Urinary iodine excretion

Introduction

Prevention of disease by modification of risk factors is important in many areas of medicine. For optimal planning and execution of prevention programs it is important to know the dose–response relationship between the risk factor that is modified and the disease that is to be prevented. Such a relationship may take several forms (Rose, 1992; Laurberg *et al.*, 2001). For example, there seems to be a more or less linear association between irradiation of the thyroid in childhood and later development of thyroid cancer. Irradiation of the thyroid in childhood should be as low as possible. The relationship between iodine intake and the risk of disease is more complicated. Even if the most severe problems are seen when iodine intake is insufficient, a high iodine intake may also lead to disease. The relationship is U-formed (Laurberg *et al.*, 2001) (Figure 47.1).

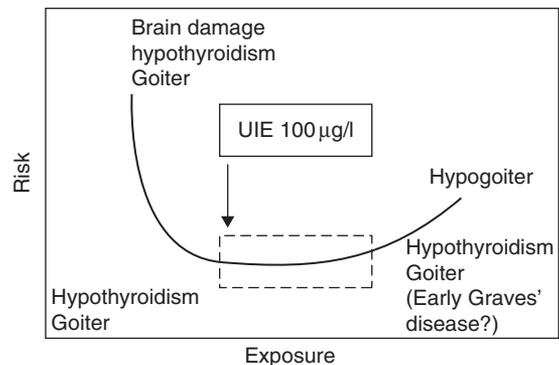


Figure 47.1 Theoretical curve showing the U-shaped relationship between exposure to a certain level of iodine intake over a long period and the risk of developing a thyroid disease. The stippled box illustrates the optimal level of iodine intake, with the lower level in homogenous population groups being a median urinary iodine concentration of 100 µg/l (World Health Organization, 2001). Reproduced from Laurberg *et al.* (2007) with permission.

Iodine Deficiency and Disease

In severe iodine deficiency there is insufficient substrate to meet the increase in need for thyroid hormone production in pregnant women and the relatively high iodine demands of young children. This is associated with a risk of developmental brain damage in the child, as well as a series of pregnancy complications. In severely iodine-deficient populations a proportion of the population may be affected by the combination of mental and physical disabilities of cretinism, and in addition, the distribution of IQ in the entire population may be shifted to the left (Boyages *et al.*, 1989). Moreover, goiter will be highly prevalent and thyroid function abnormalities common.

In mild and moderate iodine deficiency, endemic cretinism is not observed. However, in a population there is a certain spread of iodine intake, and women at the lower end of the range of iodine intake may still be at risk of insufficient iodine intake during pregnancy. Deficiency of other nutrients and intake of goitrogens from food or from tobacco smoking may increase the risk.

The obvious consequence of mild-to-moderate iodine deficiency in a population is a high rate of goiter and thyroid multinodularity that will increase with age (Knudsen *et al.*, 2000a). The most important factor behind this development is probably not insufficient thyroid hormone production caused by iodine deficiency, but autoregulation of thyroid iodide utilization (Laurberg, 2000).

It has often been hypothesized that the factor involved in goitrogenesis, when iodine intake is below the recommended level, is an increase in serum TSH caused by insufficient thyroid hormone production. However, at the level of iodine deficiency observed in Denmark before the Danish iodization program we found no evidence of a high TSH in the population. On the contrary, serum TSH was on average lower in people with moderate than in people with only mild iodine deficiency (Knudsen *et al.*, 2000b). The low TSH pattern developed gradually over the years, as illustrated in Figure 47.2.

The findings that have been reproduced in North Germany (Völzke *et al.*, 2005) suggest that the cause of the high frequency of goiter in the population is not an increase in serum TSH, but rather that goiter is a secondary phenomenon to iodide autoregulation. Low iodine intake leads to upregulation of many thyroidal processes, and hence thyroid growth. Another important factor may be the upregulation of the thyroidal production of H_2O_2 to facilitate thyroid hormone production (Song *et al.*, 2007). H_2O_2 excess may be involved in the development of multinodular goiter by promoting mutations and necrosis of thyroid cells.

Over the years, people living with iodine deficiency tend to develop multifocal thyroid autonomy, and multinodular toxic goiter is a common cause of hyperthyroidism. The difference in relative distribution of the four most common causes of hyperthyroidism in Iceland, with high iodine intake, and Jutland, Denmark, with mild-to-moderate iodine deficiency, is shown in Figure 47.3. In Iceland, Graves' disease was the dominating cause of hyperthyroidism, with few cases of solitary toxic adenoma, multinodular toxic goiter and subacute thyroiditis. In Jutland, Graves' disease was also a common cause of hyperthyroidism, but less common than multinodular toxic goiter. Because of the high incidence of multinodular toxic goiter in elderly people, the life-time risk of developing hyperthyroidism was two to three times higher in Jutland than in Iceland (Laurberg *et al.*, 1991).

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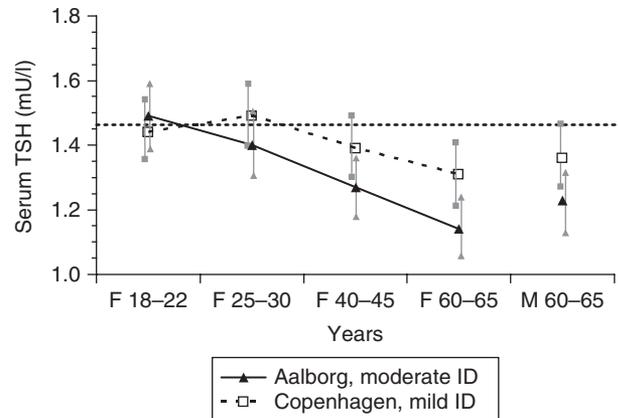


Figure 47.2 Age and region-dependent differences in s-TSH in the Dan Thyroid cohort study before the increase in iodine intake. Mean serum thyrotropin (TSH) after logarithmic transformation and 95% confidence intervals for the mean in 4356 participants of the Dan Thyroid C1 population study (Laurberg *et al.*, 2006). The calculation was based on participants who had not been treated for thyroid disorders. The Copenhagen area had mild iodine deficiency with median urinary iodine excretion in subjects not taking iodine supplements of $61 \mu\text{g}/\text{l}$. The Aalborg area was moderately iodine-deficient (urinary iodine $45 \mu\text{g}/\text{l}$). In each area the study included four groups of women (F) and one group of men (M) within the age intervals indicated. Data from Knudsen *et al.*, (2000b).

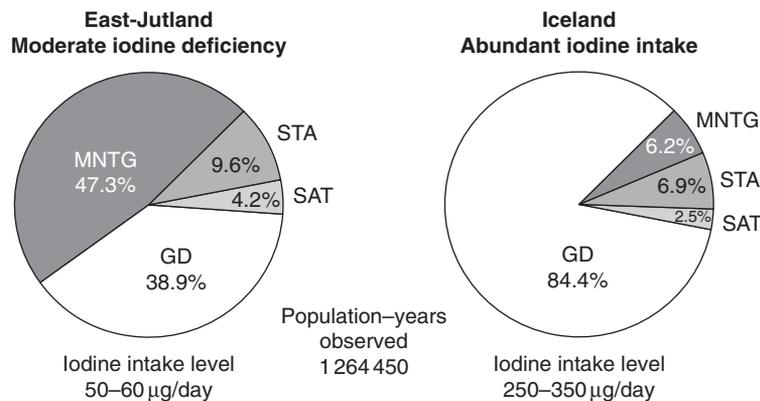


Figure 47.3 Nosological types of hyperthyroidism with different iodine intake levels. Relative frequency of the four most common nosological types of hyperthyroidism in Iceland, with relatively high iodine intake from consumption of fish and high iodine content of dairy products, and from East Jutland, Denmark, with mild-to-moderate iodine deficiency. MNTG, multinodular toxic goiter; GD, Graves' disease; STA, solitary toxic thyroid adenoma; SAT, subacute thyroiditis. Data from Laurberg *et al.*, (1991).

Table 47.1 (left column) lists some health problems that may become less common with eradication of iodine deficiency. We have indicated our evaluation of the validity of the documentation for the associations from possibly true to evident. As indicated, we consider the evidence to be good.

Diseases that may be More Common with a Higher Iodine Intake

It is well-documented that high iodine intake of an individual may lead to hyperthyroidism (e.g., in a previously euthyroid patient with autonomous thyroid nodules, presumably caused by an increase in iodide substrate for thyroid hormone production) (Laurberg, 2000), to hypothyroidism (e.g., in a euthyroid patient with Hashimoto's thyroiditis, presumably caused by defective escape from iodide inhibition of thyroid hormone synthesis and secretion) (Markou *et al.*, 2001), or to goiter (e.g., in the fetus after excessive maternal iodine intake, presumably caused by insufficient escape from iodide inhibition of fetal thyroid hormone secretion with a secondary increase in fetal serum TSH) (Markou *et al.*, 2001). Excessive iodine intake may also be associated with relapse of hyperthyroidism caused by Graves' disease after previous remission during antithyroid drug therapy (Alexander *et al.*, 1965; Roti *et al.*, 1993), and hypothyroidism after excessive iodine intake is common in patients who have previously had various thyroid diseases (Roti *et al.*, 1990). Moreover, in countries with a high iodine intake from the diet (seaweed), such as Japan

and Korea, a substantial proportion of patients with autoimmune hypothyroidism will normalize the thyroid function after a reduction in dietary iodine intake (Kasagi *et al.*, 2003; Yoon *et al.*, 2003).

Based on such evidence on iodine-induced disease in individual patients, it would be expected that an increase in population iodine intake would lead to an increase in the incidence and prevalence of certain thyroid disorders. To evaluate this in more detail, and to obtain information on the level of iodine intake where such an increase will take place, epidemiological studies are necessary. As shown in **Table 47.1** there is evidence to suggest that a number of abnormalities may be more common when iodine intake becomes high. However, in general the evidence is less strong, compared with the evidence for less disease with eradication of iodine deficiency (**Table 47.1**), and the sum of burdens is lower (**Figure 47.1**).

In a population-based study of 68-year-old people living in Iceland and in Jutland, subclinical hypothyroidism was much more prevalent in Iceland, with sufficient to excessive iodine intake, than in an area of Jutland, with moderate iodine deficiency (**Figure 47.4**) (Laurberg *et al.*, 1998). Subclinical hyperthyroidism was much more common in Jutland, as discussed above (**Figure 47.4**).

A similar finding of more subclinical hypothyroidism with abundant iodine intake compared with mild iodine deficiency was reported in nursing home residents living in Hungary (Szabolcs *et al.*, 1997). In China, people having excessive iodine intake from a combination of high groundwater

Table 47.1 Abnormalities that theoretically may be less or more common after an increase in population iodine intake

<i>Less common</i>		<i>More common</i>	
A.	Brain damage caused by maternal/fetal/neonatal iodine deficiency ^c	A.	Brain damage caused by maternal hypothyroidism ^a
B.	Goiter caused by iodine deficiency ^c	B.	Goiter caused by autoimmunity ^b
C.	Hyperthyroidism caused by thyroid autonomy ^c	C.	Hyperthyroidism caused by Graves' disease ^a
D.	Hypothyroidism caused by iodine deficiency ^c	D.	Hypothyroidism caused by autoimmune thyroiditis ^b
E.	Follicular and anaplastic thyroid cancer ^b	E.	Papillary thyroid cancer ^b
F.	Thyroid cancer after radioactive iodine fall-out ^b		

Notes: The actual change in risk would depend on the level at which the change in iodine intake occurs. A rough estimate of confidence is given. Reproduced from Laurberg *et al.*, (2007) with permission.

^aPossibly true.

^bProbably true.

^cEstablished.

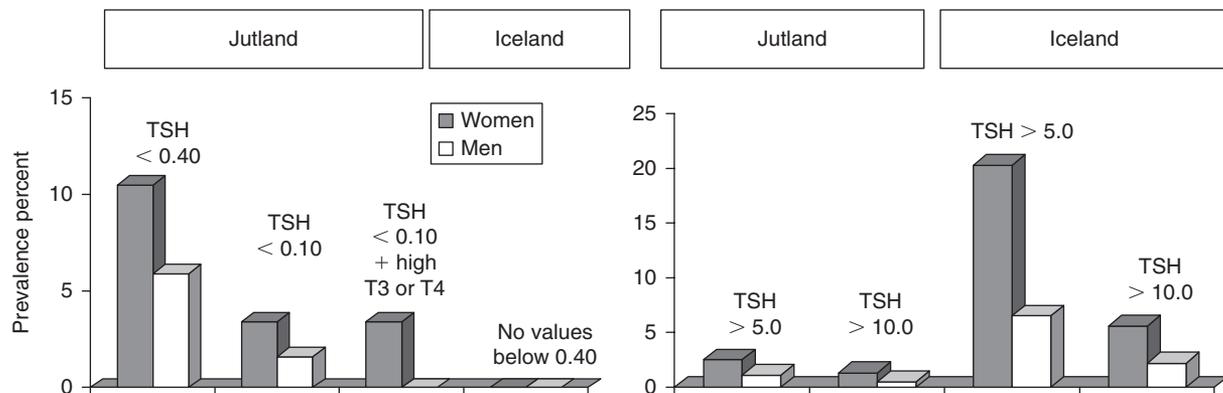


Figure 47.4 Serum TSH (mU/l) below and above reference in 68-year-old people from the population in Iceland and in East Jutland. Prevalence rates of thyroid hyperfunction with serum TSH below the reference range and thyroid hypofunction with TSH above the TSH reference range (0.4–4.0 mU/l) in 68-year-old people living in Iceland with relatively high iodine intake and in Jutland with moderate iodine deficiency. Data from Laurberg *et al.*, (1998).

iodine content and iodization of salt had a higher rate of subclinical hypothyroidism than people with iodine intake within the recommended level (Teng *et al.*, 2006). Subclinical hypothyroidism during high iodine intake developed especially in people with circulating thyroid antibodies.

In Denmark, we studied the incidence of overt hypothyroidism before the Danish iodine program in two areas with a small difference in iodine intake caused by different iodine contents of groundwater. The population living in the area with only mild iodine deficiency had a considerably higher incidence of overt hypothyroidism than the population with moderate iodine deficiency, whereas the lower iodine intake was associated with more hyperthyroidism (Figure 47.5) (Bülow Pedersen *et al.*, 2002). Subtyping of disease revealed that the difference in hypothyroidism was caused by 50% more cases of spontaneous autoimmune hypothyroidism in the area with the highest iodine intake (Carlé *et al.*, 2006).

Taken together, these studies may suggest that any increase in iodine intake of a population living with mild-to-moderate iodine deficiency may be associated with an increase in the occurrence of hypothyroidism in the population. The higher the iodine intake, the more cases of hypothyroidism will develop.

The mechanism leading to more hypothyroidism with higher iodine intake has not been fully elucidated. A high iodine load to the thyroid leads to inhibition of many thyroidal processes involved in hormone synthesis and secretion. Normally the thyroid will escape from this inhibition via downregulation of NIS and a subsequent fall in thyroidal iodide uptake (Eng *et al.*, 1999). Apparently, this escape process is not fully effective in many people, especially people with a partly defective function of the thyroid because of thyroid autoimmunity or previous thyroid disease.

From an evolutionary point of view, such defective escape with a tendency to the development of hypothyroidism has probably had little impact, as the major part of the earth's

population previously lived with relatively low iodine intake (Kelly and Snedden, 1960).

A higher iodine intake may also possibly lead to more thyroid autoimmunity. Such a mechanism has been experimentally documented in animals genetically prone to develop autoimmunity, but convincing epidemiological evidence for this mechanism being operative in human populations is lacking (Pedersen and Laurberg, 2007).

In our comparative study of the incidence of overt hyperthyroidism in Iceland and Jutland, many more young people developed Graves' disease in Iceland than in Jutland (Laurberg *et al.*, 1991). People in Jutland on average developed Graves' disease 15–20 years later than in Iceland, but the life-time risk of Graves' disease was no different between the areas. This pattern may suggest that Graves' disease develops on a background of a rather strong genetic predisposition, as also suggested by studies of twins (Brix *et al.*, 2001), but that a high iodine intake may lead to earlier development of the disease.

Finally, it should be mentioned that excessive iodine intake was found to be associated with a high prevalence of goiter in school children, both in the US Ten-State Nutritional Survey (Trowbridge *et al.*, 1975) and in studies performed in China (Li *et al.*, 1987). In the US study, the high iodine intake mostly came from the use of iodine-containing chemicals in bread by bakeries, whereas the high iodine intake in China was caused by a high iodine content in drinking water. Apparently the thyroid growth in Chinese children had been caused by a high serum TSH (Li *et al.*, 1987).

Practical Consequences for Iodization Programs

The consequence of the U-shaped association between iodine intake level and the risk of disease is that iodine intake should be brought to the level where iodine deficiency disorders are avoided, but not much higher (Laurberg, 1994).

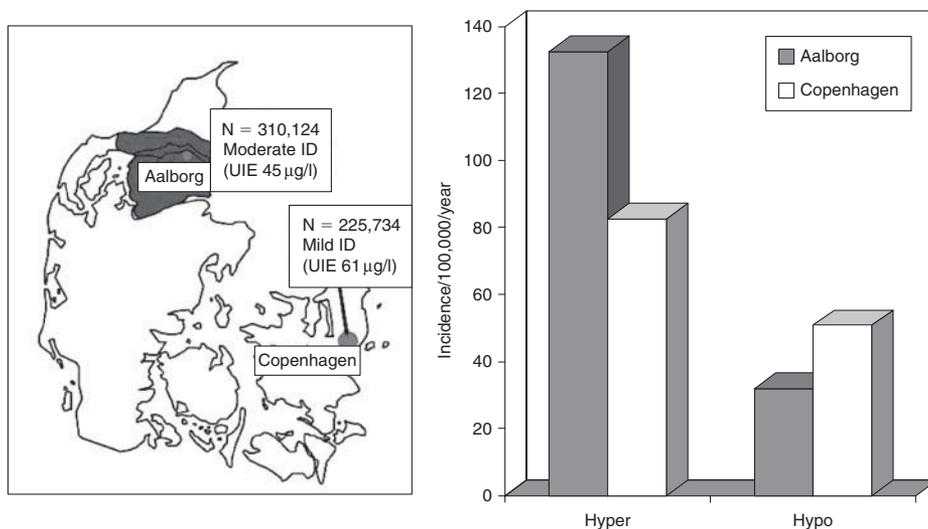


Figure 47.5 Incidence rates of hyper- and hypothyroidism in the Dan Thy Register study. Three years time period, 1997–2000. Incidence rates of overt hyper- and hypothyroidism in two open Danish population cohorts with mild (Copenhagen, median urinary iodine concentration (UIE) 61 µg/l) and moderate (Aalborg, UIE 45 µg/l) iodine deficiency. All new cases of overt hyper- and hypothyroidism were identified during a 3-year period and incidence rates calculated. Hyperthyroidism was statistically significantly more common than hypothyroidism in both areas. Hyperthyroidism was significantly more common in Aalborg than in Copenhagen and hypothyroidism significantly more common in Copenhagen than in Aalborg. Data from Bülow Pedersen *et al.*, (2002).

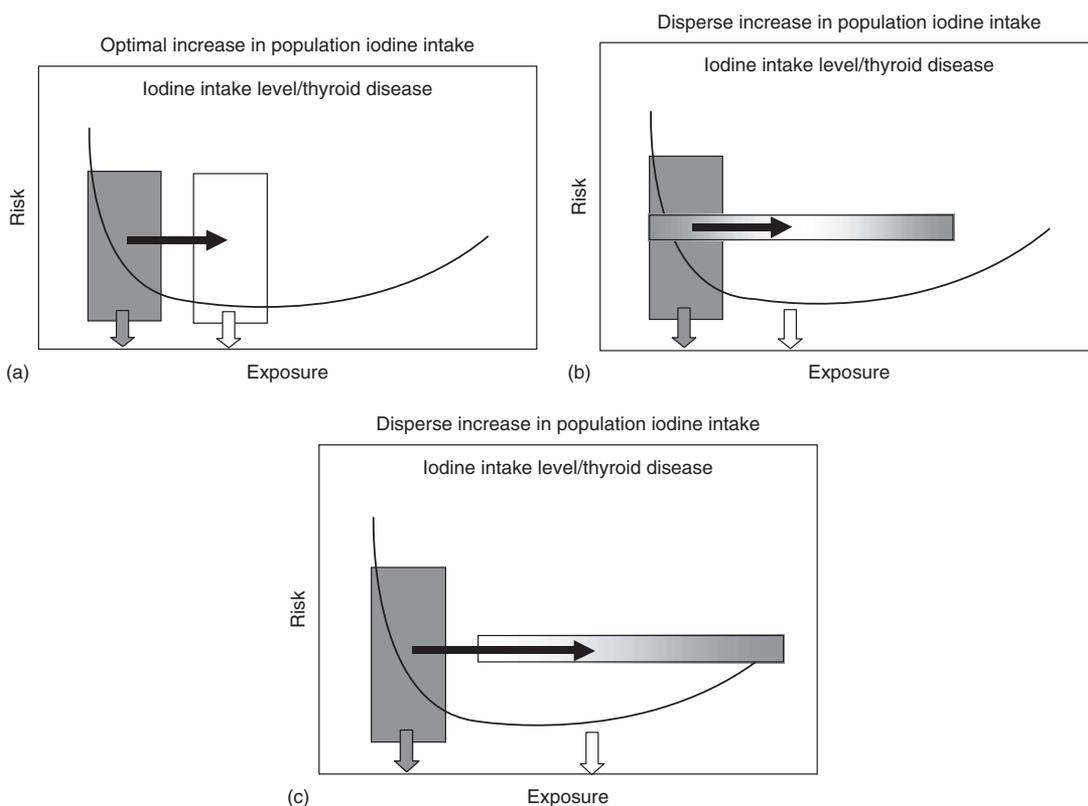


Figure 47.6 Illustration of the principle in an optimal increase in iodine intake of an iodine-deficient population. (a) The boxes represent the distribution of iodine intake in the population before and after a good iodization program, with nearly unaltered distribution. (b) Illustrates a situation with the same average increase in iodine intake, but a much larger dispersion in individual iodine intake, because iodine has been added in larger amounts to a narrower set of foods. (c) The same dispersion if iodine deficiency should be entirely avoided in the population; the average increase in iodine intake has to be considerably higher than in (a) and (b), and many more people will be exposed to excessive iodine. Reproduced from Laurberg *et al.*, (2007) with permission.

If iodine intake in the population is around the lower limit of the recommended range (median urinary iodine concentration of 100 µg/l), it may be appropriate to supplement pregnant and lactating women with iodine as part of the vitamin/mineral supplements taken by the majority of pregnant women in many countries. Such individual iodine supplements may compensate for the increased need for iodine during pregnancy and lactation (World Health Organization, 2007) and it seems not to increase the risk of postpartum thyroiditis (Nohr *et al.*, 2000).

Another point of importance is careful planning of the program introduced to increase the iodine intake of iodine-deficient populations. Optimally, such a program should distribute iodine evenly, which is probably best achieved by a more or less general low-level iodization of salt for households and for production by food industries. Figure 47.6a illustrates how the dispersion in population iodine intake will be nearly unchanged after such a type of program, with few people having insufficient or excessive iodine intake. If, on the other hand, average iodine intake is brought to the same level by adding iodine to a much smaller proportion of salt which is only taken by some people, then the amount of iodine added per gram salt will have to be high, and the dispersion in iodine intake of the population much larger, as illustrated in Figure 47.6b. In such a population, people who do not use iodized salt may still be iodine-deficient. In order to cover sufficiently people who only sporadically use iodized salt, the iodine content of salt and consequently the median iodine intake of the population will have to be even higher, as shown in Figure 47.6c. In such a population few will suffer from iodine deficiency, but excessive iodine intake will be common.

Conclusion

Iodine deficiency with impairment of thyroid hormone production may have severe consequences. To compensate for the low iodine supply that was previously highly prevalent in the world, complex mechanisms have been developed in the thyroid gland. On the one hand, mechanisms are able to accumulate and utilize even very small supplies of iodine. On the other hand, the thyroid immediately reacts to a sudden load of iodine to avoid overproduction of thyroid hormone. As usual when complex mechanisms are involved, this leads to a risk of malfunction – and disease. To minimize such a risk at the level of the population, iodine intake is best kept within a relatively narrow range around the recommended level.

Summary Points

- Thyroid disorders are common in all populations, but the occurrence and the pattern of disease depend on the iodine intake of the population.

- Both low and high iodine intakes are associated with an increase in the risk of thyroid problems.
- The most serious problems are associated with iodine deficiency, which should be corrected.
- The iodine intake should only be brought to the level where iodine deficiency disorders are avoided.
- Optimally, the population iodine intake should be kept within a relatively narrow range around the recommended level.

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Section 3

Pathological Aspects of Iodine Deficiency

Section 3.1

General Aspects of Pathology

Iodine Deficiency: The Extent of the Problem

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Abstract

Iodine deficiency remains a major threat to the health and development of populations worldwide, particularly among pre-school children and pregnant women in low-income countries. Iodine deficiency is the world's greatest single cause of preventable brain damage, and this is the primary motivation behind the current worldwide drive to eliminate iodine deficiency through universal salt iodization (USI). Until the 1990s, total goiter prevalence (TGP) in school-age children was the primary indicator for the assessment of functional consequences of iodine deficiency in the population; however, urinary iodine (UI) is now recommended by the World Health Organization (WHO) as the main indicator to assess iodine status in a population and to track progress toward the elimination of iodine deficiency. Estimates of the current worldwide iodine deficiency situation were made using both indicators (UI and TGP) in the time frame from 1993–2003. These estimates suggest that 36.5% of the school-age population and 35.3% of the general population have insufficient iodine intake, resulting in almost 2 billion individuals worldwide at risk of iodine deficiency. The current TGP estimate suggests that 15.8% of the global population is affected by goiter. Most countries with history of iodine deficiency have implemented USI programs. Over the last decade, the number of countries where iodine deficiency is considered a public health problem has decreased by more than half, from 110 in 1993 to 54 in 2003. Although progress has been made in the global fight against iodine deficiency, there is still much room for improvement in iodized salt coverage; especially in the 54 countries where iodine deficiency remains a public health problem. Periodic monitoring of population iodine status is important to ensure that salt iodization is effective in the control of iodine deficiency, and does not expose susceptible groups to levels of iodine intakes that are too high.

Abbreviations

IQ Intelligence quotient
Tg Thyroglobulin

TGP Total goiter prevalence
TSH Thyroid-stimulating hormone
UI Urinary iodine
USI Universal salt iodization
WHO World Health Organization

Introduction

Most countries in the world are affected by iodine deficiency to some extent, and therefore it remains a major threat to the health and development of populations worldwide; particularly among pre-school children and pregnant women in low-income countries. Iodine deficiency results in hypothyroidism that can occur at any stage of life, but its most devastating consequences take place during fetal development and childhood, with stillbirth, miscarriages, poor growth and cognitive impairment. While cretinism is the most extreme manifestation, of considerably greater significance are the more subtle degrees of mental impairment that lead to poor school performance, reduced intellectual ability and impaired work capacity. For iodine-deficient communities, between 10 and 15 intelligence quotient (IQ) points may be lost when compared to similar, but noniodine-deficient populations (Bleichrodt and Born, 1994). The adverse effects of iodine deficiency on cognitive performance and motor function may be partly reversible, as shown in moderately iodine-deficient school-age children following iodine supplementation (Zimmermann *et al.*, 2006a).

Iodine deficiency is the world's greatest single cause of preventable brain damage, and also the primary motivation behind the current worldwide drive to eliminate iodine deficiency.

Universal salt iodization (USI) is the main intervention strategy for iodine deficiency control, and was adopted by the International Conference on Nutrition in 1992, reaffirmed by the World Health Assembly in 1993, and established as a World Summit for Children mid-decade goal in 1995. Salt has been chosen as a vehicle, because of its widespread consumption and the extremely low cost of iodization. In high-risk areas, where populations cannot

easily be reached by iodized salt, the alternative is to administer iodine supplements – either as iodide or as iodized oil – focusing on women and children.

Until the early 1980s, only a few countries were known to be affected by iodine deficiency, but over the last two decades, knowledge of the global magnitude of iodine deficiency has improved considerably. The surveillance of iodine deficiency worldwide and the current extent of the problem is the focus of this chapter.

Assessment Techniques: Measuring the Extent of the Problem

Urinary iodine (UI) concentration is currently the most widely used biochemical marker of iodine nutrition (WHO *et al.*, 2001). As most iodine ingested by the body is excreted in the urine (>90%), UI reflects iodine intake at the time of measurement. By measuring UI in the population, it can be determined if iodine requirements are being met, and thus, whether a population is at risk of iodine deficiency. Within individuals, iodine excretion may vary on a day-to-day basis, but these variations even out across populations. A single specimen of urine is sufficient to measure iodine output when using it to assess the iodine status of populations. Only a small urine volume is required for UI measurement, which makes it easy to collect under field conditions. Because this indicator is sensitive to recent intake and is relatively easy to measure, UI is now recommended by the WHO as the main indicator to assess iodine status in a population, and to track progress toward the elimination of iodine deficiency. School-age children are the recommended population group for iodine intake surveillance, as school-based surveys are practical to perform, and the iodine status in school-age children is usually assumed to reflect the status of the general population (WHO *et al.*, 2001). The most recent estimates of the national, regional and global prevalence of iodine deficiency are consequently based on UI in school-age children. **Table 48.1** provides the criteria to define the public health significance of iodine deficiency based on median UI (WHO *et al.*, 2001).

Until the 1990s, total goiter prevalence (TGP) in school-age children was the primary indicator for the assessment of functional consequences of iodine deficiency in the population (WHO *et al.*, 2001). Thyroid size was traditionally determined by palpation, but the reliability of this method is limited by high inter-observer and intra-observer variations. The measurement of thyroid size by ultrasound has therefore been an important step in the detection of mild-to-moderate iodine deficiency. International reference values for normal thyroid size are now available from iodine-sufficient children (Zimmermann *et al.*, 2004). Because TGP is not a sensitive indicator of recent changes in iodine status in the

Table 48.1 Classification of iodine deficiency as a public health problem in a population

Median urinary iodine ($\mu\text{g/l}$)	Iodine intake	Iodine status
<20	Insufficient	Severe iodine deficiency
20–49	Insufficient	Moderate iodine deficiency
50–99	Insufficient	Mild iodine deficiency
100–199	Adequate	Adequate iodine nutrition
200–299	Above requirements	Risk of iodine-induced hyperthyroidism in susceptible groups
≥ 300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)

population, it is currently recommended for the baseline assessment of iodine status, but not as the main indicator for regular monitoring of the response to an intervention. A TGP of $\geq 5\%$ in school-age children indicates a public health problem (WHO *et al.*, 2001).

Serum thyroid-stimulating hormone (TSH) and thyroglobulin (Tg) are other promising biochemical markers of thyroid function. Serum TSH is elevated in iodine-deficient populations, but in school-age children and adults changes in concentration are small. Conversely, in neonates, as a result of low thyroid iodine and high iodine turnover, elevated blood TSH is a good indicator of the severity of population iodine deficiency (WHO *et al.*, 2001). The increase in the number of neonates with moderately elevated TSH concentrations (above 5 mIU/l whole blood) is proportional to the degree of iodine deficiency. Permanent sporadic congenital hypothyroidism, with extremely elevated neonatal TSH, occurs in approximately 1 of 4000 births in iodine-sufficient countries. Neonatal screening should preferably include all neonates to reflect population iodine status.

Tg is a protein produced by the thyroid that is involved in thyroid hormone synthesis. An increased serum Tg in thyroid hyperplasia occurs when insufficient amounts of iodine are available to the gland. It is more sensitive to changes in iodine status than goiter, but not as sensitive to recent changes in iodine intake as UI. A dried blood spot assay that makes sampling more practical in remote areas has recently been developed and validated (Zimmermann *et al.*, 2006b). As the general use of TSH and Tg, and their use in assessing and monitoring iodine deficiency at the population level are still limited, data are not yet included in the WHO databank on iodine deficiency.

Historical Perspective on the Magnitude of Iodine Deficiency Throughout the World

Attempts to track progress in the fight against iodine deficiency have been made for at least 50 years. The first iodine deficiency estimates were published by the WHO in 1960 (Clements *et al.*, 1960). They were based on goiter prevalence, and covered a limited number of countries. It was estimated that the total number of cases of goiter was 200 million (Clements *et al.*, 1960). Several reviews of surveys within countries, and descriptions of probable progress toward the elimination of goiter, followed (Stanbury, 1969; Stanbury and Hetzel, 1985; Dunn *et al.*, 1986). In the early 1990s, following a resolution of the World Health Assembly (WHO, 1990), the WHO established a global databank on micronutrient status and published another set of estimates quantifying the prevalence of goiter (WHO *et al.*, 1993). Data came from 121 countries, and neighboring country information was used to make estimates for the remaining countries. It was the first time that iodine deficiency was estimated at global and regional levels.

In 1993, an attempt was made to quantify the number of individuals afflicted with iodine deficiency and estimate the prevalence in various regions of the world. These estimates were based on TGP, and utilized national data in school-age children and the general population, and neighboring country information for countries with no data (WHO *et al.*, 1993). An estimate was generated for all WHO member states. In countries without data, one-third of the estimates were based on a combination of subnational data and neighboring country information. The population at risk of iodine deficiency was determined by using the number of individuals affected by goiter within a country and a multiplication factor based on the geographical distribution of iodine deficiency within the country. It was estimated that 12.0% of the world's population (655 million individuals) suffered from goiter, and that 28.9% (1.57 billion) were at risk of iodine deficiency. Based on a TGP above 5%, it was considered that iodine deficiency was a public health problem in 110 countries.

The WHO Global Database on Iodine Deficiency was refined and updated in the early 2000s. The database is now being continuously updated, and compiles information on iodine nutritional status – specifically UI and TGP – from all countries in the world. Survey data are collected from literature searches, contacts with other United Nations (UN) organizations, ministries of health, and other national agencies, nongovernmental organizations, and research and academic institutions. Papers and reports published in any language are entered into the database. In some cases, authors must be contacted for clarification or for additional information; an archive of this correspondence is available from the WHO. This database is a valuable resource, since it can be used to track progress

at global, regional and country levels. The 1993 estimates were updated in 2004, and the WHO published a second set of global iodine deficiency estimates described in the following section (Andersson *et al.*, 2005).

Current Extent of Iodine Deficiency

The estimate of the current worldwide iodine deficiency situation is based primarily on UI data. However, TGP is also included in order to compare prevalence figures to previous estimates. The most recent estimates for both indicators utilized several types of data in the time frame of 1993–2003, but prioritized recent data (1998–2003). Thus, data were selected as follows: recent national surveys (1998–2003); recent subnational data (1998–2003); older national surveys (1993–1997); and older subnational data (1993–1997). When subnational data were utilized, surveys within the time frame were combined and weighted by the sample size of each survey. Data for school-age children were prioritized. In the absence of data for this group, data from the next closest population groups were utilized as follows: children closest to school-age; adults; the general population; preschool-age children; and other population groups. The estimates utilized 2002 UN population estimates to determine the number of individuals affected (United Nations Population Division, 2003).

The UI data covered 781 million school children (92.1% of this population group), and were extrapolated to the general population. The coverage was highest in the WHO region of Southeast Asia (98.8%), and lowest in the Eastern Mediterranean (83.4%) (Table 48.2). Nationally representative data were used for 75 countries, while subnational data were used for 51 countries; no data were available for 66 countries. Although the number of countries without data seems high, it only represents 7.9% of the world's school-age population.

It is estimated that 36.5% of the school-age population and 35.3% of the general population have insufficient iodine intake, resulting in almost 2 billion individuals worldwide at risk of iodine deficiency (Table 48.3).

The prevalence varied by region, and was highest in the WHO regions of Europe (59.9%) and the Eastern Mediterranean (55.4%), followed by Africa (42.3%) and Southeast Asia (39.9%), with the lowest prevalence in the Western Pacific (26.2%) and the Americas (10.1%). Within WHO regions, there are important disparities across countries – especially in the Americas where the prevalence is much higher in Caribbean countries than in central, northern, or southern countries of that region. The current TGP estimate suggests that 15.8% of the global population is affected by goiter. The prevalence is highest in the Eastern Mediterranean (37.3%), and lowest in the Americas (4.7%) (Figure 48.1).

There are 54 countries where iodine deficiency is considered to be a problem of public health significance (median

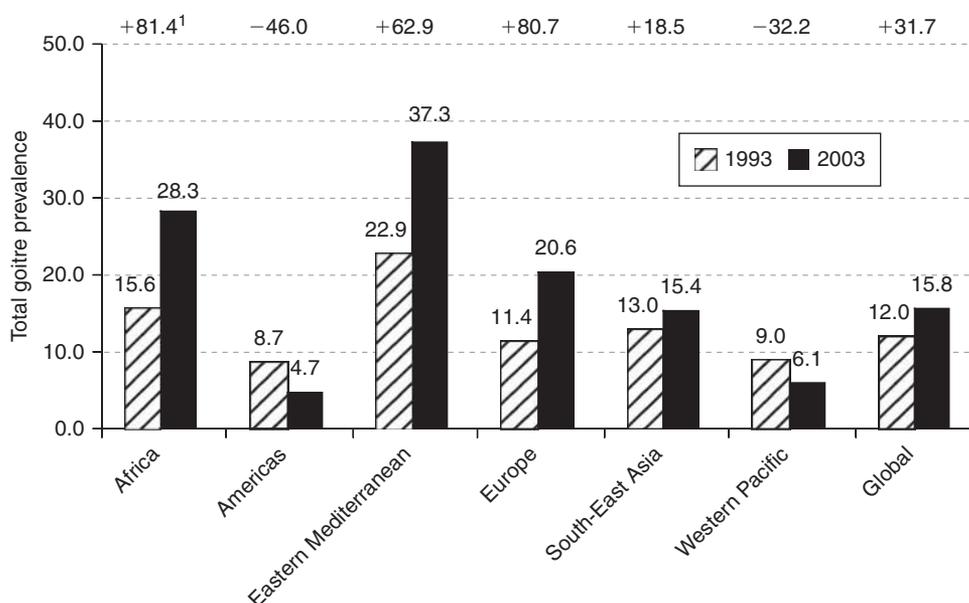
Table 48.2 Population covered by urinary iodine surveys in 2003 (de Benoist *et al.*, 2004)

WHO region	Number of school-age children	School-age children covered by data	Coverage (%)
Africa	128.9	116.9	90.7
Americas	109.0	98.8	90.6
Southeast Asia	242.4	239.4	98.8
Europe	81.2	70.5	86.8
Eastern Mediterranean	87.1	72.6	83.4
Western Pacific	199.4	183.0	91.8
Global	848.0	781.2	92.1

Abbreviations: UI, urinary iodine; WHO, World Health Organization.

Table 48.3 Proportion of population and number of individuals with insufficient iodine intake in school-age children (6–12 years) and in the general population (all age groups), by WHO region, 2003

WHO region	Insufficient iodine intake (UI < 100 µg/l)			
	School-age children		General population	
	Proportion (%)	Total number (millions)	Proportion (%)	Total number (millions)
Africa	42.3	49.5	42.6	260.3
Americas	10.1	10.0	9.8	75.1
Southeast Asia	39.9	95.6	39.8	624.0
Europe	59.9	42.2	56.9	435.5
Eastern Mediterranean	55.4	40.2	54.1	228.5
Western Pacific	26.2	48.0	24.0	365.3
Total	36.5	285.4	35.2	1988.7

**Figure 48.1** Total goitre prevalence (TGP) in 1993 and 2003 and percent change in 2003 by WHO region. ¹Percent change in TGP from 1993 to 2003.

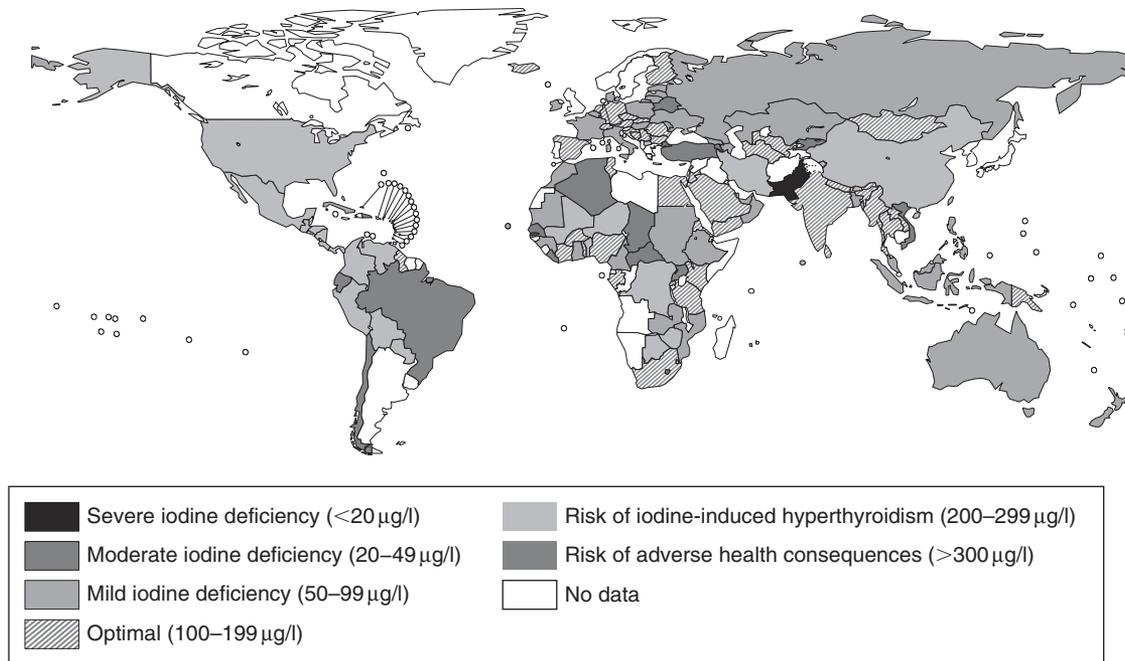


Figure 48.2 Degree of public health significance of iodine nutrition based on median UI by country. The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city, or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate borderlines for which there may not yet be full agreement. © WHO 2007. All rights reserved.

UI <100 µg/l) (Figure 48.2). Furthermore, there are a considerable number of countries, 24, in which the population may be at risk of increased susceptibility to iodine-induced hyperthyroidism in susceptible groups (median UI of 200–299 µg/l), or at risk of adverse health consequences (median UI \geq 300 µg/l) (Andersson *et al.*, 2005).

If compared to 1993 estimates, the current TGP data suggest that there is an overall increase of 31.7% in goiter prevalence throughout the world. The prevalence increased in the WHO regions of Africa, the Eastern Mediterranean, Europe and Southeast Asia, but decreased in the Americas and the Western Pacific (Figure 48.1). However, these data must be interpreted with much caution, as TGP does not reflect recent changes in iodine intake. Furthermore, the majority of the available TGP data in the time span of 1993–2003 were collected before 1998 – a time when many programs of iodine deficiency control had not yet been implemented (WHO *et al.*, 1993).

The data show an increase in the number of individuals at risk of iodine deficiency from 1.57 billion in 1993 to 2 billion in 2003. However, this increase is apparently because during this time, the global population increased by about 16%. In addition, the methods used to calculate the number of individuals at risk were different, with the 1993 estimate basing it on an extrapolation of goiter data and the 2003 estimate basing it on UI data. However, when comparing the number of countries where iodine deficiency is no longer considered a public health problem,

it appears that the number of countries in which iodine deficiency is a public health problem dropped considerably between 1993 and 2003. In 1993, the countries were classified based on TGP rather than on UI, and 110 countries were considered to have a public health problem with respect to iodine deficiency. Compared to the current figure of 54, and even while considering the change in methodology, this represents remarkable progress in the fight against iodine deficiency.

The estimates presented are based primarily on data from school-age children, who generally reflect the status of the whole population. However, because the effects of iodine deficiency are most critical during brain development, iodine sufficiency is critical in the infant and young child, especially in the fetus. Sufficient iodine intake during pregnancy is essential, and surveillance of pregnant women is now recommended by WHO, alongside surveillance of school-age children (Table 48.4) (WHO, 2007a). Pregnant women have higher iodine requirements, since they must ingest enough iodine for themselves and for the developing fetus. The normal range for the median UI is therefore higher for pregnant women than for the general population (WHO, 2007a). Reference ranges for UI concentration during pregnancy are listed in Table 48.5. At present, there are few nationally representative surveys conducted in pregnant women, making global evaluation of the extent of iodine deficiency in pregnant women worldwide difficult to assess at this time.

Table 48.4 Recommended iodine intakes for individuals. WHO, (2007a).

Population group	Age	Recommended iodine intake ($\mu\text{g}/\text{day}$)
Infants	0–59 months	90
School-age children	6–12 years	120
Adolescents and adults	>12 years	150
Pregnant and lactating women	14–50 years	250

Table 48.5 Classification of iodine deficiency as public health problem in pregnant women. WHO, (2007a).

Median urinary iodine ($\mu\text{g}/\text{l}$)	Iodine intake	Iodine status
<150	Insufficient	Iodine deficiency
150–249	Adequate	Adequate iodine nutrition
250–499	Above requirements	No added health benefits
≥ 500	Excessive	No added health benefits

Strategies for the Control of Iodine Deficiency

The recommended strategy for the control of iodine deficiency is universal salt iodization (USI), which requires the iodization of all salt for both human and animal consumption. The success of USI lies in the fact that it is a cost-effective and sustainable method for delivering iodine to the population. In countries where there are many small local salt producers, implementation of USI is more challenging, but still achievable. Distribution of iodized salt to impoverished and geographically remote areas may require extra efforts, but it is vital for the goal of covering more than 90% of households. Nationally, representative monitoring is important to identify pocket areas within countries not reached by USI. Global efforts to strengthen USI were initiated in 1998. The remarkable impact on iodine deficiency between 1993 and 2003 must be attributed to these efforts.

More recently, concerns have been raised about the potential for mixed messages created by campaigns to reduce salt intake and those to consume iodized salt (WHO, 2006). It has been recommended that the amount of iodine added to salt may need to be adjusted depending on the recommended level of salt intake within a country, and that these two public health messages need to be coordinated (WHO, 2007b). This highlights the importance of assessing a population's salt consumption, since too little or too much iodine ingested may have health

consequences in the population. This also makes regulation an important component of any USI program.

An alternative to USI is iodine supplementation. This strategy is more costly, but is the only option in areas of severe endemicity or where USI cannot be implemented. A recent WHO report recommends that where USI is not fully implemented, the most vulnerable population groups – pregnant women and young infants – be given additional iodine supplied through targeted supplementation (WHO, 2007a). Options for supplementation include iodized oil, which is given yearly or as daily iodine supplements.

Conclusions

Significant progress has been made in the global fight against iodine deficiency. The number of countries where iodine deficiency is a public health problem has decreased by more than half over the last decade, and most countries have implemented USI programs and are monitoring the iodine status of their populations. However, there is still much room for improvement. Efforts need to be intensified to reach the populations still at risk, especially in the 54 countries where iodine deficiency remains a public health problem. The presence of an effective, consistent monitoring system of population iodine status, in combination with monitoring of iodized salt quality, is important to ensure that salt iodization is effective in the prevention and control of iodine deficiency, and does not expose susceptible groups to levels of iodine intakes that are too high. Continued focus on these points should yield measurable improvements at the next assessment.

Summary Points

- Most countries in the world are affected by iodine deficiency to some extent, and it remains a major threat to the health and development of populations worldwide; particularly among preschool children and pregnant women. Iodine deficiency is the world's greatest single cause of preventable brain damage, and the primary motivation behind the current worldwide drive to eliminate iodine deficiency.
- USI is the main intervention strategy for iodine deficiency control.
- Until the 1990s, TGP in school-age children was the primary indicator for the assessment of functional consequences of iodine deficiency in the population.
- UI is now recommended by the WHO as the main indicator to assess iodine status in a population, and to track progress toward the elimination of iodine deficiency. School-age children are the recommended population group for iodine intake surveillance.
- The WHO Global Database on Iodine Deficiency compiles information on UI and TGP from all countries in the world.

- The current worldwide estimates of iodine deficiency, based on UI concentrations from 1993 to 2003, suggest that almost 2 billion individuals worldwide are at risk of iodine deficiency.
- The number of countries where iodine deficiency is considered a public health problem has decreased by more than half over the last decade. Iodine deficiency, however, still remains a problem of public health significance in 54 countries worldwide.
- The iodine intake is too high in 24 countries, and the population may be at risk of increased susceptibility to iodine-induced hyperthyroidism in susceptible groups or those at risk of adverse health consequences.
- Regular monitoring of iodized salt quality and population iodine status is important to ensure that salt iodization is effective in the prevention and control of iodine deficiency, and that it does not expose susceptible groups to levels of iodine intake that are too high.

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Iodine Deficiency in Pregnancy: Iodine Deficiency and Supplementation in Pregnancy

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Abstract

Iodine deficiency is still a major public health concern in the world, although substantial progress in the household use of iodized salt has been achieved in the past decade. Iodine deficiency is of critical importance in the pregnant woman, because it results in lowering of maternal thyroxine levels leading to less thyroxine availability for the developing fetus. As thyroxine is vital for nervous system maturation, its deficiency results in cretinism (in severe cases) to mild psychomotor impairment in cases of mild iodine deficiency. Assessment of iodine deficiency in pregnancy is obtained by the evaluation of urinary iodine excretion, although there is a debate about iodine loss in early pregnancy. An excretion of at least 185 mcg/l should occur, which would correspond to the recently recommended iodine intake of 250 µg/day. There is continuing debate as to whether women are in negative iodine balance during gestation. Iodide supplementation during pregnancy is recommended in cases of iodine deficiency, and can be given as oral potassium iodide or iodized oil. Routine iodine supplementation is by iodized salt.

Abbreviations

CNS	Central nervous system
	International Council for Control of Iodine Deficiency Disorders
TSH	Thyrotropin-stimulating hormone
WHO	World Health Organization

Introduction

Iodine deficiency is still a major public health problem worldwide, although substantial progress has been made towards its eradication in many countries. It is of particular importance in the pregnant woman, because of the sequelae that may be observed in the fetus and the neonate ([Table 49.1](#)). Epidemiological demonstration of the association

of cretinism with goiter has been known for around 150 years, and goiter was also found in about 30% of cases. In a seminal study in New Guinea ([Pharoah and Connolly, 1994](#)), it was discovered that cretinism could be prevented by iodine administration, but only if it was given (as iodized oil in this case) before the onset of pregnancy. The reduction in cretinism in Europe in the early twentieth century was due to the increase in iodine intake from a variety of foodstuffs, as well as specific supplementation programs.

In another critical study, it was observed that thyroid hormone does indeed cross the placenta. Therefore, if insufficient iodine is available to the mother it results in reduced maternal synthesis of thyroid hormone with insufficient placental thyroxine transport for fetal requirements.

This chapter will discuss the physiology of iodine deficiency in pregnancy, in addition to outlining the magnitude of the problem and its assessment in clinical practice.

Pathophysiology of Iodine Deficiency in Pregnancy

The response of the thyroid gland to iodine deficiency is characterized by an increase in the trapping mechanism of iodine uptake into the follicular cell, as well as an increase

Table 49.1 The spectrum of IDD

Fetus	Abortions Stillbirths Congenital anomalies Increased perinatal mortality Endemic cretinism Deaf mutism
Neonate	Neonatal goiter Neonatal hypothyroidism Endemic mental retardation Increased susceptibility of the thyroid gland to nuclear radiation

Note: Adapted from [Hetzel \(1994\)](#).

in the subsequent steps in the intrathyroidal metabolism of iodine, leading to a preferential secretion of T3. These responses are initiated and maintained by an increase in thyrotropin-stimulating hormone (TSH), although autoregulatory thyroid effects are also seen. The net result of this stimulation is the development of goiter.

The iodine requirement during pregnancy is increased to provide for the needs of the fetus, and to compensate for the increased loss of iodine in the urine, due to increased renal clearance of iodide during pregnancy as a consequence of an increased glomerular filtration rate. This leads to increased activity of the thyroid gland, which is evidenced by an elevation in thyroid iodide clearance and thyroid enlargement. In iodine-deficient areas, this process is more marked and when fetal thyroid function commences (around mid-gestation) maternal iodine deficiency becomes more severe as fetal requirements are met.

Iodine deficiency results in lowered maternal circulating thyroid hormone concentrations, leading to reduction in placental transfer of thyroxine. The possibility of iodine storage by the placenta compensating for diminished dietary iodine intake has been suggested by [Delange \(2004\)](#), and placental iodine storage has been demonstrated ([Smyth et al., 2006](#)).

During the early twentieth century, it was noted that studies of endemic cretinism had suggested that the fetal developing thyroid was dependent on factors that may impair the maternal thyroid reserve ([Hetzel, 1993](#)). Subsequently, a cause–effect relationship was shown between maternal iodine deficiency and the birth of neurological cretins. Furthermore, there was evidence that the degree of maternal hypothyroxinemia correlated with the central nervous system (CNS) damage of the progeny. These data were difficult to explain at that time, due to the fact that it was not thought that transplacental thyroid hormone transport took place. The view was that the placenta was impermeable to iodothyronines, and that the small amounts possibly transferred would be of no physiological importance. It has now been shown convincingly that this does indeed occur, not only before the fetal thyroid starts to synthesize thyroid hormones (i.e. up to 12 weeks gestation), but right through pregnancy ([Vulsma et al., 1989](#)). Aspects of the thyroid hormone delivery to the fetus are summarized in [Table 49.2](#).

The realization that iodine deficiency in pregnancy has a pronounced effect on fetal, neonatal and childhood brain function has resulted in a large body of knowledge on the effects of thyroid hormone on brain and nervous-system development (see [Grave, 1977](#); [DeLong et al., 1989](#); [Stanbury, 1994](#); [Bernal, 2002](#)).

Although fetal thyroid function does not commence until the equivalent of 12 weeks gestation in man, the presence of functional fetal nuclear receptors for T3 is noted in early pregnancy, indicating that triiodothyronine is exerting an action at this time ([De Nayer and Dozin, 1989](#)).

Maternal thyroid hormone is necessary before onset of fetal thyroid function, as shown by interference of cortical cell migration ([Table 49.3](#)) and the cortical expression of several genes in the fetuses of mothers rendered hypothyroid by goitrogens. Maternal thyroxine still contributes to the thyroid hormone available to the fetal tissues at term, even after fetal thyroid function is present. Indeed the maternal T4 is sufficient to prevent fetal cerebral T3 deficiency until birth in a hypothyroid fetus. The T3 at this stage is locally produced in cerebral structures by deiodination of T4 by type 2 iodothyronine deiodinase, hence the requirement for T4. Both type 2(D2) and type 3(D3) deiodinases are critical in producing and modulating the supply of T4 to the fetus, as well as producing locally derived T3. The type 3 enzyme is mainly placentally located and inactivates T4 and T3, thereby regulating the influx of T4 to the fetus ([Bianco and Larsen, 2005](#)).

Corroborative data have been obtained by extensive studies of iodine deficiency in sheep ([Hetzel and Mano, 1994](#)). In these animals, induced iodine deficiency results in reduced brain weight, a reduction in brain DNA and retarded myelination. In keeping with earlier observations, morphological changes in the cerebellum accompanied by delayed maturation were noted ([Hetzel et al., 1989](#)).

Table 49.2 Physiology of thyroid hormone availability to the fetal brain

(a) Before onset of fetal thyroid function
T4 and T3 present in embryonic and fetal fluids and tissues
T4 and T3 are of maternal origin
Nuclear receptors present and occupied by T3
D2 and D3 expressed in brain
(b) Between onset fetal thyroid function and birth
Maternal transfer continues
Brain T3 dependent on T4 and D2 and 3, NOT on systemic T3
Normal maternal T4 protects fetal brain from T3 deficiency
Normal T3 in low T4 mother does not prevent cerebral T3 deficiency

Note: From [Lazarus \(2005\)](#).

Table 49.3 Effect of thyroid hormone on neural development

Altered migration and differentiation of neurons
Cell migration – cerebellum
Cerebral cortex and hippocampus
Stunted dendritic and axonal growth and maturation
Purkinje cells
Dendritic spine number
Delayed and poor deposition of myelin
Reduced axonal number
Thyroid hormone receptors
Presence of receptors in neural tissue at early developmental stage suggests fetal brain is a target for TH even before onset of fetal gland function

Note: From [Bernal \(2002\)](#).

The effect of the thyroid hormone on brain development has been reviewed by Bernal (2002), who has indicated the many neurobiological actions of the hormone on developing neural tissue, in addition to documenting some of the genes affected by T3 (Table 49.4).

The situation in humans is similar to that observed in animals, although placentation is different in some anatomical respects. Nuclear receptors have been demonstrated in brains of 10-week-old fetuses; low amounts of T4 have been found in coelomic fluid, which provides enough for transport into the fetal nervous system. The molecular control of thyroid hormones by deiodinases for the activation of thyroid hormones is critical to ensure that the correct amounts of these hormones are supplied to the fetus (Pemberton *et al.*, 2005). The first trimester surge of maternal T4 has been proposed as a biologically relevant event controlled by the conceptus to ensure its developing cerebral cortex is provided with the necessary amounts of substrate for the local generation of adequate amounts of T3 for binding to its nuclear receptor. Therefore, women unable to increase their production of T4 in early pregnancy would constitute a population at risk for neurological disabilities in their children (Morreale de Escobar *et al.*, 2004). Iodine deficiency, the commonest cause of maternal hypothyroxinemia, should be corrected with iodine supplements. The most damaging disorders induced by iodine deficiency are irreversible mental retardation and cretinism. A meta-analysis conducted in severely iodine-deficient areas showed that iodine deficiency is responsible for a mean reduction in Intelligence Quotient (IQ) of 13.5 points in the population (Bleichrodt and Born, 1994). A more recent meta-analysis of 37 studies comparing IQ in children from iodine-sufficient areas to those in iodine-deficient areas or children in deficient areas born before or after iodine supplementation has been conducted by Chinese workers (Qian *et al.*, 2005). The studies comprised 12291 children, and showed that there was a loss of 12.45 IQ points in children exposed to severe iodine deficiency, but there was a recovery of 8.7 IQ points when iodine supplementation was introduced before or during pregnancy.

Table 49.4 Genes affected by thyroid hormone

Myelin genes
Mitochondrial genes
Neurotrophins and their receptors, e.g. NGF receptor, BDNF in Purkinje cells
Cytoskeletal components, e.g. tubulin isotypes, mitochondrial associated proteins
Transcription factors
Splicing regulators
Extracellular matrix proteins and adhesion molecules
Intracellular cell-signaling genes
Cerebellar genes, e.g. pcp-2

Note: From Bernal (2002).

This study has added evidence to the view that the level of iodine nutrition plays a crucial role in the intellectual development of children.

Clinical Studies of Iodine Deficiency in Pregnancy

The observations relating to iodine deficiency in pregnancy are firstly those concerned with maternal thyroid function and maternal goiter. Maternal urinary iodine (UI) excretion is the usual method of assessing iodine status in the population at risk or the individual, and is discussed below. Neonatal indicators of maternal iodine deficiency are goiter and neurointellectual impairment.

Urinary iodine excretion in pregnancy

Early work on UI excretion in pregnancy (Aboul Khair *et al.*, 1964) reported increased renal iodine clearance as a result of an increase in glomerular filtration rate, which was thought to lead to a decline in plasma inorganic iodine (PII) concentration. The latter finding has been disputed by Liberman *et al.* (1998), at least in areas of diminished dietary iodine intake. Earlier studies reported both diminished (Pedersen *et al.*, 1993) or unchanged UI excretion in pregnancy (Glinoe *et al.*, 1990; Liesenkotter *et al.*, 1996). In contrast, a report from one of the authors (Smyth *et al.*, 1997) demonstrated an increase in UI excretion in pregnancy as early as the first trimester, which remained elevated relative to nonpregnant controls in the second and third trimesters, but fell precipitously at delivery and was indistinguishable from nonpregnant controls at 40 days postpartum (see Figure 49.1).

A more recent report (Smyth *et al.*, 2007) on acute changes in UI at delivery confirmed the previously reported fall in UI at delivery, and showed that median

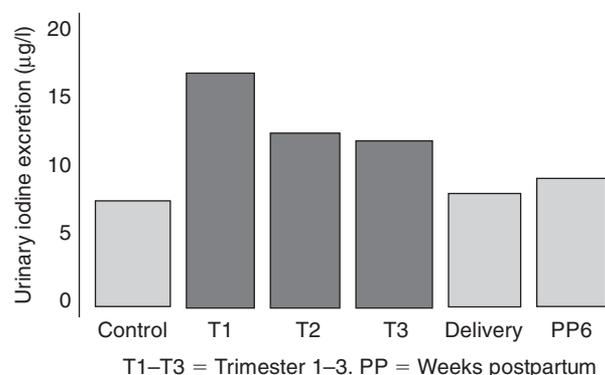


Figure 49.1 Changes in UI during pregnancy in 38 women sampled sequentially during each trimester (T1–T3) and at delivery. PP = Postpartum. Thirty-eight women were sampled sequentially during each trimester (T1–T3), and at delivery and postpartum. Note: From Smyth *et al.*, (1997).

UI returned to nonpregnant levels at 10 days following delivery.

The demonstration of increased UI during pregnancy was supported by more recent reports (Kung *et al.*, 2000; Brander *et al.*, 2003). Despite these studies, the late Francois Delange, in 2004, disputed the concept of “systematically increased urinary loss of iodine during pregnancy.” The divergence between the various reports may reflect different levels of dietary iodine intake during pregnancy (Smyth, 1999). In this review, it was postulated that differences in the pattern of UI excretion during pregnancy could be explained by the existence of a urinary iodide threshold. In iodine deficiency this threshold may not be reached, while in areas replete in iodine the threshold may be obscured. Indeed such a threshold (termed an iodostat) was proposed (Dworkin *et al.*, 1966), and it was suggested that the level at which the iodostat is set depends on customary dietary iodine. In pregnancy the iodostat or threshold may not alter to conserve iodine, despite increased urinary loss. This may result in depletion of thyroidal iodine stores. This depletion does not appear to have deleterious consequences for the mother or neonate, as euthyroidism was maintained throughout gestation. However, if the dietary iodine intake during gestation was restricted or the pregestational thyroidal iodine stores were inadequate, the repercussions could be more serious. All of these findings raise the question as to the significance of maternal or neonatal thyroid function of iodine loss in pregnancy. The World Health Organization (WHO) has recommended a daily intake of 250 µg iodine for pregnant and lactating women (Benoist and Delange, 2007). For population estimates, daily iodine intake can be extrapolated from UI by assuming that 90% of ingested iodine

appears in the urine, and that 24h urinary volume is arbitrarily set at 1.5l (IOM, 2001; Zimmerman and Delange, 2004). Using this estimate, a UI concentration of 185 µg/l would approximate to a daily iodine intake of 250 µg. Applying these criteria would indicate that the great majority of pregnant women, at least in Europe, were ingesting suboptimal amounts of iodine (Zimmerman and Delange, 2004). Recent data from the UK have shown low UI in first trimester women, although other evidence of iodine deficiency has not been noted (e.g. goiter). Kibirige *et al.* (2004) found low iodine excretion in a study in north east England. In Wales, UK, up to 50% of woman recruited for a controlled antenatal thyroid screening study (Lazarus and Premawardhana 2005) had UI < 100 µg/l (Figure 49.2).

Iodine balance

The possible consequences in terms of iodine loss from the increased UI excretion in different study populations have been investigated in Ireland and the UK, areas of borderline dietary iodine intake. Data have been compared to the values obtained from Sri Lanka, in which a program of iodine prophylaxis via iodized salt has been in operation for some years, and are shown in Table 49.5 (Smyth, 1999).

In this table, admittedly crude calculations based on median UI excretion in the three study populations revealed a negative iodine balance of -30% and -28.5% in the Irish and UK study populations, respectively, with a positive balance of +8% in the iodine-replete Sri Lankan population. However, it must be emphasized that despite providing useful comparisons between iodine-replete regions and those

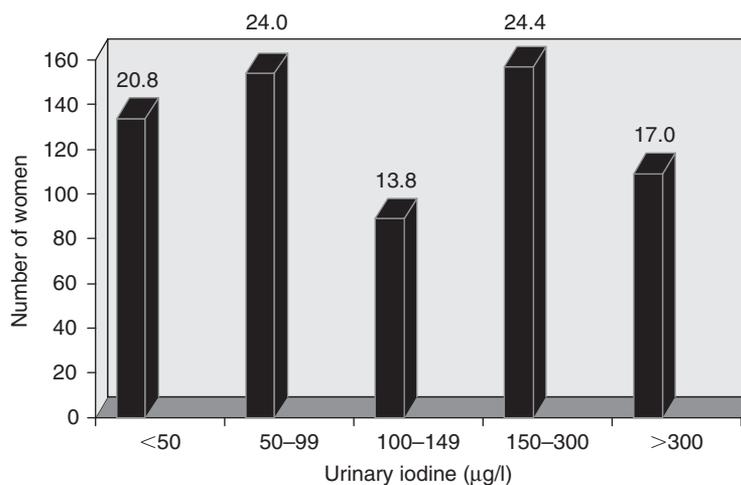


Figure 49.2 Urinary iodine (UI) excretion in first-trimester women recruited for a controlled antenatal thyroid screening study. The figure shows random “spot” UI measurements in 643 women from Wales, UK, studied at 11–15 weeks gestation. Numbers on top of columns indicate % of total. Note that only about 41% of women appear to excrete an amount of iodine consistent with the recommended daily intake in pregnancy.

Table 49.5 Urinary iodine excretion in pregnancy

	IRL		UK		SL	
UI excretion (280 days × daily UI)	T1	{ 42 × 80 56 × 163	T1	{ 42 × 82 56 × 126	T1	{ 42 × 146 56 × 196
	T2	98 × 141	T2	98 × 170	T2	98 × 122
	T3	84 × 135	T3	84 × 147	T3	84 × 102
Total nonpregnant (μg)	22,316		28,000		40,880	
Iodine balance	~ -30%		~ -28.5%		~ +8%	

Note: UI excretion during pregnancy measured in groups of pregnant women from Ireland (IRL), the United Kingdom (UK) and Sri Lanka (SL) during three trimesters (T1–T3). Values for T1 assume nonpregnant UI levels for the first 42 days and measured levels for 56 days.

with borderline iodine intake, these are crude estimates that may not accurately reflect iodine balance (Dworkin *et al.*, 1966).

Iodine Requirements During Pregnancy

The iodine requirement during pregnancy is increased, in order to provide for the needs of the fetus and to compensate for the increased loss of iodine in the urine due to increased renal clearance. From early gestation, maternally-produced thyroid hormones pass across the placenta to stimulate cell division and differentiation in the fetal brain (Morreale de Escobar *et al.*, 2004). As pregnancy is associated with a rapid increase in thyroid hormone secretion, the maternal need for iodine increases, because the mother is making additional thyroid hormones for herself and the fetus. The mother also loses iodine in the urine while trying to provide the fetus with an iodine store. The figures have been derived from studies of thyroid function during pregnancy and in the neonate under conditions of moderate iodine deficiency. In such an environment, thyroid function during pregnancy is characterized by a progressive decrease in the serum concentration of thyroid hormones and an increase in serum TSH and thyroglobulin. Thyroid volume increases steadily and is above the upper limit of normal in 10% of these women at the end of pregnancy. These abnormalities can only be prevented when the mother receives a daily iodine supplement of 165 μg. This would correspond to a UI concentration of about 250 μg/l.

Recently, a WHO technical consultation group met to review the maternal iodine requirements during gestation (Benoist *et al.*, 2007). Consideration was given to the fact that the amount recommended should be sufficient to prevent brain damage or thyroid function disorders due to iodine deficiency during pregnancy. There is a lack of data to indicate an optimal intake. Therefore, conclusions were reached after evaluation of the efficiency of gut absorption of iodine, the estimated metabolic needs, and the typical daily losses in the feces and urine.

Current Studies of Iodine Status

There are many studies documenting iodine deficiency in different areas of the world with consequent effects on maternal and fetal thyroid status. In southwest France, low iodine intake, as shown by low UI excretion, was associated with reduced thyroid hormone levels and thyroid gland growth during pregnancy (Caron *et al.*, 1997). In southern Italy, measurement of the impact of moderate iodine deficiency on maternal thyroid function during pregnancy showed that the incidence of isolated hypothyroxinemia increased significantly (from 30 to 70%) between mid-gestation and term, suggesting that moderate iodine deficiency may cause maternal thyroid failure during the later stages of gestation (Vermiglio *et al.*, 1999). In West Africa, reproductive failure (repeated miscarriage and stillbirth) was associated with low iodine status, thus emphasizing the need for iodine supplementation (Dillon and Milliez, 2000). In Saudi Arabia, 28.8% of pregnant women at term were iodine deficient and maternal UI excretion correlated with neonatal total thyroxine and birth weight. The results underpinned the need for an increased iodine supply in Saudi women (Ardawi *et al.*, 2002). In a study of UI excretion in 403 pregnant women, Azizi *et al.* (2003) concluded that recommended values for dietary iodine through universal salt iodization may not be adequate for pregnant women, and revision was indicated. In the Silesian region of central Europe, gestational iodine deficiency (urinary I < 100 μg/l) was found in 29% and was seen more often in women with imminent premature delivery (Bodzek *et al.*, 2006). Despite previous iodization programs a re-emergence of iodine deficiency in Australia has been documented by Li *et al.* (2006), who noted a reduced iodine content in milk which would impact negatively on iodine intake in the pregnant woman.

A new recommended daily iodine intake of 250 μg/day was arrived at that represents an increase from the previous figure of 200 μg/day. In the pregnant woman, a median UI of <150 μg/l is regarded as insufficient, an excretion of 150–249 as adequate, that of 250–499 as more than adequate and >500 as excessive.

Iodine Supplementation in Pregnancy

Iodine may be administered as potassium iodide or as an iodized oil preparation. Women from countries whose iodine status is satisfactory, and where salt is normally iodized, do not usually require supplementation. In countries with borderline iodine sufficiency where the distribution of iodized salt is uneven or has lapsed, there is a strong case for giving a daily dose of 200–250 µg potassium iodide during pregnancy. In countries where iodine deficiency is severe because iodized salt is not available, oral iodide may be given. Alternatively, if there are logistic difficulties in administration, a single injection of iodized oil (200–400 mg) will ensure adequate iodization through gestation.

The above methods are emergency expedients, and should only be used when the normal processes of distribution and salt consumption are deficient or absent. The WHO database on iodine deficiency is a comprehensive survey that allowed the criteria for iodine nutrition to be established. Thus, a median UI of <20 µg/l is classed as severe I deficiency, a UI of 20–40 as moderate, of 50–99 as mild, and 100–199 as optimal in the nonpregnant population.

The effects of iodine supplementation on pregnant women and neonates have been studied in many areas of the world. In Denmark, a randomized prospective trial showed that maternal thyroid hormone concentrations did not alter, but the cord thyroglobulin concentration was much lower in mothers who had received iodine (Pedersen *et al.*, 1993). Clearly, the thyroid has good adaptation powers in pregnancy. A similar study in Belgium showed lower thyroid volume and lower serum Tg in newborns whose mothers had received iodine supplements in a randomized protocol (Glinoe *et al.*, 1995). This study emphasized the potential risk of goitrogenic stimulation in both the mother and the newborn in the presence of mild iodine deficiency, and the amelioration of these features by iodine supplementation. Diminished thyroid stimulation in pregnant woman following iodization was also noted in Poland (Kaminski *et al.*, 2003). Maberly *et al.* (2003) noted that although elimination of iodine deficiency by production, marketing and universal consumption of iodized salt was a significant public health achievement, 20–30% of pregnancies and newborns still did not have the benefit of this strategy. It is clear that iodization in a community produces significant benefit in relation to maternal thyroid function during gestation and fetal and neonatal outcomes. A problem exists, however, in that it is easy to be complacent after iodization has occurred in the expectation that iodine sufficiency will continue. Several studies have shown that this is not the case, and regular monitoring of iodine status is essential to maintain optimal iodine nutrition. The monitoring depends mainly on the UI concentration in representative population samples, usually from schoolchildren.

In a recent large Chinese study in 11 provinces covering a wide geographic and socioeconomic range, it was noted that iodine excretion was significantly less in pregnant and lactating women compared to schoolchildren from the same community (Yan *et al.*, 2005). This means that, although there may be effective iodization programs in different areas of the world, pregnant women may still risk deficiency in these areas and therefore require further supplementation at this time.

Conclusions

The main impact of iodine deficiency is on pregnant and lactating women and young infants, due to the role of maternal, fetal and neonatal hypothyroxinemia in the development of brain damage resulting in irreversible mental retardation (Glinoe and Delange, 2000). While substantial progress has been made to eradicate iodine deficiency worldwide, there is still a lot to be achieved. The WHO estimates that 740 million people are at present affected by goiter. However, it is estimated that 68% of the populations of affected countries currently have access to iodized salt, compared to 10% a decade ago (Delange *et al.*, 2001). A more recent survey indicated that iodine deficiency is still a public health problem in 54 countries (Andersson *et al.*, 2005). In addition to iodization programs, iodine supplementation to appropriate groups of pregnant women should be considered in iodine-deficient areas until the iodization programs have ensured adequate ambient iodine concentrations.

Summary Points

- Iodine deficiency is still common in many areas of the world.
- Even mild iodine deficiency results in suboptimal psychomotor performance in childhood.
- Adequate maternal thyroxine concentration is essential for fetal nervous system maturation.
- The recommended daily intake of iodine in pregnancy is 250 µg.
- Acute iodine supplementation is by oral potassium iodide or iodized oil. Long-term iodization is optimally achieved by iodized salt.

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Iodine Nutrition and Iodine Deficiency in Term and Preterm Newborns: Iodine Nutrition in Newborns

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Abstract

Iodine is required for the synthesis of thyroid hormones, thyroxine (T4) and 3,5,3'-triiodothyronine (T3), which are necessary for adequate growth and development throughout fetal and extrauterine life. The iodine intake of newborns is entirely dependent on the iodine content of breast milk and the formula preparations used to feed them. An inadequate iodine supply (deficiency or excess) might be especially dangerous in the case of premature babies. The minimum recommended dietary allowance (RDA) is different depending on age group. The iodine intake required is at least 15 µg/kg/day in full-term infants and 30 µg/kg/day in preterms. Premature infants are in a situation of iodine deficiency, precisely at a stage of psychomotor and neural development which is extremely sensitive to changes in of thyroid function.

Abbreviations

BW	Body weight
ELBW	Extremely low birth weight
FT4	Free T4
GA	Gestational age (in weeks)
ICCIDD	International Council for the Control of Iodine Deficiency Disorders
PMA	Postmenstrual age
T3	3,5,3'-Triiodothyronine
T4	Thyroxine
TBG	Thyroid-binding globulin
Tg	Thyroglobulin
TSH	Thyroid-stimulating hormone

Introduction

Iodine is a trace element which is essential for the synthesis of thyroid hormones. The thyroid hormones, thyroxine

(T4) and 3,5,3'-triiodothyronine (T3), are necessary for adequate growth and development throughout fetal and extrauterine life. Their effects on the central nervous system are mediated by regulation of the expression of genes that synthesize proteins implicated in cerebral neurogenesis, neuronal migration and differentiation, axonal outgrowth, dendritic ontogeny and synaptogenesis, cerebellar neurogenesis, gliogenesis, and myelinogenesis. If maternal iodine deficiency during pregnancy is severe, fetal brain damage will occur. This damage is irreversible after birth. Mild/moderate iodine deficiency during early postnatal life is associated with neuro/psycho-intellectual deficits in infants and children. The severity is related not only to the degree of iodine deficiency, but also to the developmental phase during which it occurs, the most severe being the consequence of iodine deficiency during neonatal life. An inadequate iodine supply might be especially dangerous in the case of premature infants, who are prematurely deprived of the maternal supply of hormones and iodine before their own thyroid is able to accumulate as much iodine as in term newborns. The incidence of permanent congenital hypothyroidism in preterm newborns is not higher than in children at term, but the frequency of hypothyroxinemia and transitory hypothyroidism is high during the neonatal period. There are several causes of changes in thyroid function: incomplete maturation of the hypothalamic-pituitary-thyroid axis, interruption of maternal transfer of thyroid hormones to the fetus across the placenta, changes in thyroid function that accompany severe illness, and the effect of neonatal medication (dopamine, heparine, corticoids, etc.).

Iodine deficiency during the perinatal period and exposure to an iodine excess is quite conspicuous. The iodine intake of newborns is entirely dependent on the iodine content of breast milk and formula preparations used to feed them. The minimum recommended dietary allowance (RDA) for different age groups is summarized in [Table 50.1](#). To meet such requirements, the iodine content of formulas for premature newborns should contain 20 µg/dl, and that of first and follow-up preparations 10 µg/dl. We refer here to these new

recommendations as those of the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) (Delange *et al.*, 1985; Delange, 1985, 1993, 2001, 2004; Morreale de Escobar *et al.*, 2004; WHO, UNICEF, ICCIDD, 2001).

Iodine Requirement in Neonates

The recommended intake of iodine in neonates reflects the observed mean iodine intake of young infants exclusively fed human milk in iodine-replete areas. However, it is well-established that the iodine content of breast milk is critically influenced by the dietary intake of the pregnant and lactating mother (Delange, 1985, 1993; Semba and Delange, 2001; Dorea, 2002). The iodine requirement in neonates was evaluated from metabolic studies, by determining the values that resulted in a situation of positive iodine balance which is required in order to ensure a progressively increasing intrathyroidal iodine pool in the growing infant (Delange *et al.*, 1993). In our unit, we studied thyroid gland volume by ultrasound, and found that the volume varied from 0.3 to 1.3 ml in preterm infants during the first month of life and 0.9 ml in term infants at birth (Ares *et al.*, 1995) (Table 50.2). These studies indicate that the iodine intake required in order to achieve a positive iodine balance is at least 15 µg/kg/day in full-term infants and 30 µg/kg/day in preterms. This corresponds to approximately 90 µg/day and is consequently twice as high as the 1989 recommendations of 40–50 µg/day (National Research Council, 1989; Delange, 2004).

Table 50.1 Daily iodine requirements in infants and children (RDA)

Group	Age	Iodine RDA
Premature infants	0–5 months	>30 µg/kg/day
Term infants		15 µg/kg/day
Children	6–12 months	90 µg/day
	1–3 years	90 µg/day
	4–6 years	90 µg/day
	7–10 years	120 µg/day

Role of Thyroid Hormones during Neonatal Life

The thyroid hormones, T4 and T3, are necessary for adequate growth and development (Greenberg *et al.*, 1974; Legrand, 1986) throughout fetal and extrauterine life. These hormones regulate many metabolic processes: somatic growth; cardiac, pulmonary, and bone maturation; central nervous system maturation; and neuronal differentiation. They also regulate oxygen consumption and protein, lipid, and carbohydrate metabolism. There is evidence that thyroid hormones are necessary for surfactant synthesis and lung maturation (Biswas *et al.*, 2002). Brain and lung maturation have received special attention because of the potentially irreversible or life-threatening consequences associated with early thyroid hormone deficiency (Kester *et al.*, 2004; De Vries *et al.*, 1986).

Effects of Thyroid Hormones on the Human Central Nervous System during Fetal and Postnatal Life

The close involvement between human brain development and thyroid hormones is widely accepted (Morreale de Escobar *et al.*, 2004). The effects of T3 on the central nervous system are mediated by regulation of the expression of genes that synthesize proteins implicated in cerebral neurogenesis, neuronal migration and differentiation, axonal outgrowth, dendritic ontogeny and synaptogenesis. They are also necessary for cerebellar neurogenesis (predominantly during early postnatal life), gliogenesis (predominantly during late fetal life to 6 months postnatally), and myelogenesis (during the second trimester of gestation to 2 years of postnatal life). Low T4 levels during neonatal life, especially if persistent, could be a negative factor contributing to neurodevelopmental problems of extremely preterm infants. Indeed, retrospective studies have shown a relationship between hypothyroxinemia and developmental delay and an increased risk of disabling cerebral palsy (De Vries *et al.*, 1986; den Ouden *et al.*, 1996; Lucas *et al.*, 1996, 1988; Meijer *et al.*, 1992; Reuss *et al.*, 1996).

Table 50.2 Distribution of weight, height, surface area and thyroid volume (mean ± SEM) in the preterm infants studied

Range of weight (kg)	n	Postmenstrual age (weeks)	Body weight (kg)	Body height (cm)	Body surface area (m ²)	Thyroid volume (ml)
1.0–1.5	5	33.6 ± 0.5	1.30 ± 0.05	38.50 ± 1.00	0.123 ± 0.007	0.32 ± 0.04
1.6–2.0	4	35.0 ± 0.8	1.85 ± 0.08	42.68 ± 1.45	0.150 ± 0.008	0.48 ± 0.10
2.1–2.5	6	35.8 ± 0.7	2.30 ± 0.06	46.15 ± 0.60	0.187 ± 0.006	0.51 ± 0.11
2.6–3.0	5	38.5 ± 1.1	2.85 ± 0.05	45.20 ± 1.90	0.191 ± 0.009	0.68 ± 0.13
3.1–3.5	6	39.0 ± 0.5	3.35 ± 0.07	50.00 ± 0.10	0.240 ± 0.020	0.77 ± 0.11
3.6–4.0	12	40.8 ± 0.5	3.75 ± 0.30	51.00 ± 0.20	0.250 ± 0.100	0.92 ± 0.18

Note: n = number of observations.

Iodine Deficiency and Thyroid Function in Newborns

We carried out a cohort study on thyroid function and iodine intake in 115 premature infants and 28 term infants born in the Neonatology Unit of Hospital La Paz in Madrid (Spain) during 1991–1995. The premature infants, born between 27 and 36 weeks of gestation, were studied during the first week of life and every 15 days for up to 2–4 months after birth. On each occasion samples of the different formulas or maternal milk given to each infant were kept for direct determination of iodine content, and the ingested volume over a 24-h period was recorded in each case, as described previously in more detail (Ares *et al.*, 1994, 1995, 1997). We then studied 67 infants born before 30 weeks of gestation during 1999–2001; the study was repeated in 2006.

Iodine Content in Human Milk

Iodine concentration was determined using a modification of the chloric acid digestion method described by Benotti and Benotti for serum. The iodine content of mother's milk is shown in Table 50.3. Seventy-six percent of the samples had an iodine concentration between 10 and 30 µg/100 ml. No statistically significant differences were found between samples of women with premature and term delivery, or between different times during the lactation period. Mean values for the iodine content of breast milk are quite variable. Breast milk is the best source of iodine for the newborn baby.

Iodine Content in Premature and Infant Formulas: Iodine Intake in Newborns

Data were obtained from samples of formulas for premature infants, starting formulas and special (medical) formulas.

Table 50.3 Mean iodine content in mother's milk (\pm SEM)

Study year 1992 (<i>n</i> = 84)	Study years 1998–2001 (<i>n</i> = 54)	Study year 2006 (<i>n</i> = 32)
10.0 \pm 1.0 µg I/dl (range 3–35)	25.8 \pm 1.4 µg/dl (range 7.4–60)	13.7 \pm 1.9 µg/dl (range 4.5–48)

In the majority of preparations, iodine content was significantly lower than that of human milk. The iodine content of 68 formulas from 13 different brands used in Spain was determined (mean iodine content = 10 \pm 1.3 µg/dl). In most of the preparations, the average iodine content was 10 µg/dl, significantly lower than that of human milk. However, different types of formulas may have different iodine content. Although the iodine content of most starting, follow-up, and special formulas was similar to the ESPGAN recommendation (5 µg I/dl), it was usually lower than the recommendation for premature infants (7 µg I/dl). Iodine intake is lower than the recommendations in most groups of premature infants on formula alone for at least 1 month after birth, especially in the case of 27–33 weeks gestational age (GA) group (Tables 50.4 and 50.5). In contrast, the recommended iodine intake was reached sooner by premature babies on 50% breast milk or more. The iodine intake of sick premature infants was even lower than that of weight-matched healthy infants. None of the premature infants had an iodine intake near the ICCIDD recommendation of 30 µg/kg/day. Eighty percent of the babies had an iodine intake <30 µg I/kg, and only 20% of the babies had an iodine intake >30 µg I/kg (Table 50.6). Premature babies do not ingest the 150–200 ml/kg/day recommended for term babies until they are approximately 1 month old and weigh 2 kg. Because of the small volume of milk ingested, the iodine intake of the newborns was less than the recommended dietary allowance. Infants on total parenteral nutrition have a mean iodine intake of only 3 µg/kg/day. In the 1992 study, the iodine intake of the infants born at 27–30 weeks of gestation and that of children with serious neonatal pathology was less than the iodine intake of healthy children (Table 50.4). In the 1999–2001 study, analyzing the data of iodine intake by kilograms of weight, it was observed that the average ingestion of premature infants was below the new recommendations of >30 µg I/kg until 2 months of life. Infants on parenteral nutrition have an iodine intake of <3 µg/day.

“Peditrace” parenteral solution contains 1.3 µg/ml potassium iodide, equivalent to 1 µg iodide/kg/day. The iodine intake of newborn babies is entirely dependent on the iodine content of breast milk or formula preparations. Breast milk contained more iodine than most of the formulas, especially those for premature babies. It is

Table 50.4 Gestational age, weight, height, volume of milk and iodine intake of very low birth weight preterm infants (27–30 weeks of gestation) at different postnatal ages

Parameters	1st sample	2nd sample	3rd sample	4th sample	5th sample
Number of babies	54	55	52	49	6
Age in days	0.3 \pm 0.0	9.1 \pm 0.5	18.5 \pm 0.7	28.4 \pm 1.0	28.7 \pm 0.7
Gestational age (weeks)	27.0 \pm 0.25	28.9 \pm 0.2	30.3 \pm 0.2	31.8 \pm 0.27	31.6 \pm 0.6
Weight (g)	987.0 \pm 30.9	938.7 \pm 30.0	1035.9 \pm 34.4	1221.5 \pm 50.3	1196.6 \pm 34.7
Height (cm)	35.3 \pm 0.4	36.1 \pm 0.1	36.6 \pm 0.3	38.6 \pm 0.42	40.5 \pm 2.5
Volume of formula (ml)	0	61.8 \pm 8.4	113.1 \pm 11.0	161.4 \pm 12.5	
Volume of breast milk (ml)	0	73.0 \pm 11.5	115.7 \pm 17.3	122.8 \pm 18.4	198.0 \pm 42.0
Iodine intake (µg/day)	0.2 \pm 0.1	13.8 \pm 3.0	23.5 \pm 3.1	23.6 \pm 2.0	45.7 \pm 12.7

Table 50.5 Iodine intake of preterm (31–36 weeks of gestation) and term newborns ($\mu\text{g}/\text{day}$) at different post-natal ages

Group	<7 days	30 days
Sick preterm infants	7.5 ± 1.8	13.1 ± 2.2
Healthy preterms	16.5 ± 1.6	19.3 ± 2.5
Term infants	31.3 ± 4.3	25.1 ± 2.8

Table 50.6 Iodine intake ($\mu\text{g}/\text{kg}$) of newborns according to body weight

Iodine intake	Mean \pm SE	Infants with iodine intake <30 $\mu\text{g}/\text{kg}/\text{day}$ (%)	Infants with iodine intake >30 $\mu\text{g}/\text{kg}/\text{day}$ (%)
Total	15.7 ± 1.06	80	20
From breast milk	20.1 ± 2.1	74	26
From formula preparations	12.9 ± 1.0	87	13

concluded that newborn and premature babies who are formula-fed are at risk of being iodine deficient. Preterm babies on formula preparations and with parenteral nutrition are at high risk of iodine deficiency.

The volume of food ingested by the infant is small, iodine content in formula preparations is insufficient, and parenteral nutrition does not supply enough iodine. This problem is not exclusive to Spanish premature babies, as the iodine content of many formulas in other countries is also inadequate. Therefore, supplements should be added if iodine intake is found to be inadequate. Breast milk appears to be the best source of iodine for the premature infant.

Iodine Excretion into the Urine

Urinary iodine excretion was calculated from the volume and iodine concentration of a 24 h urine sample. During the first weeks of life, iodine excretion was higher in the majority of 27–30-week-old GA infants than for the same infants at later postnatal ages. It was observed that the 27–33-week-old GA sick infants excreted approximately 1.5 times the amount of iodine in urine than healthy term infants, despite their lower iodine intake. The daily iodine balance was calculated as the difference between iodine intake and iodine excretion. The iodine balance of very premature infants appears to be negative during the first week of life, whereas at about 31–32 weeks GA and later the iodine balance is clearly positive. This negative balance increases with the maturity of the preterm infant. This condition is transient, with the balance changing from negative to positive with increasing GA. Many of the 31–36 weeks GA preterms who

Table 50.7 Mean (\pm SEM) iodine intake and iodine excretion into the urine, at different postnatal ages, of preterm babies

Age in days	Iodine intake ($\mu\text{g}/\text{day}$)	Iodine ($\mu\text{g}/\text{dl}$)	Excretion ($\mu\text{g}/\text{day}$)
4.5 ± 0.4	5.9 ± 1.7	2.7 ± 0.5	3.9 ± 0.6
17.6 ± 1.3	19.5 ± 4.3	9.9 ± 3.2	4.3 ± 2.1
30.5 ± 5.0	20.9 ± 6.1	12.8 ± 4.0	8.8 ± 3.4
44.2 ± 1.6	38.9 ± 1.1	12.1 ± 4.0	9.8 ± 3.4
60.0 ± 1.0	66.4 ± 1.1	13.0 ± 3.0	11.5 ± 2.8

Note: The daily iodine excretion of the babies at 44.2 and 60.0 days of postnatal age was calculated from iodine concentration in casual urine samples, as most were no longer in the neonatology unit.

were sick, and many of the small-for-gestational-age (SGA) neonates also excreted more iodine into the urine for the first weeks of life than healthy infants of the same GA. This finding suggests that the premature infants in negative balance are unable to “retain” all the iodine they are ingesting, and that an increased intake would not correct their negative iodine balance. Most of the 31–36 weeks GA healthy preterms and term infants were in positive iodine balance, which increased with the iodine intake and, to a lesser degree, with postmenstrual age (PMA), retaining 80–90% of the iodine intake; iodine excretion in urine was lower than intake (Table 50.7). The premature and term babies were in positive iodine balance (Ares *et al.*, 1994, 1997) (Table 50.8).

Iodine Intake and Thyroid Function

As prematurity is not a normal physiological situation, circulating levels of T₄, FT₄, T₃, and thyroid-stimulating hormone (TSH) are usually compared to those of healthy term newborns. T₄, FT₄ and T₃ levels are lower in preterm neonates as compared to term newborns, increasing progressively with maturation, whereas TSH levels are the same. Iodine intake does affect the circulating levels of FT₄, T₃, Tg and TSH in preterm infants, independent of their age. Circulating levels are lower in preterm than in term neonates of comparable age and iodine intake (or balance), at least up to 44 weeks PMA and an intake of 80 $\mu\text{g}/\text{day}$. T₄, free T₄ (FT₄) and T₃ of preterm and term neonates increased with age, whereas thyroglobulin (Tg) decreased and TSH did not change. The percentage contribution of iodine deficiency to hypothyroxinemia may be greater in the more immature infants who have a very low iodine supply: serum FT₄, T₃, Tg and TSH of preterm neonates were affected negatively, independent of age, by a low iodine intake. Iodine deficiency contributes to about 30% of the hypothyroxinemia in enterally and parenterally fed preterm infants of 27–30 weeks gestation (Figures 50.1 and 50.2) (Ares *et al.*, 1994, 1995, 1997, 2004; Morreale de Escobar and Ares, 1998).

Table 50.8 Percentage of preterm and term neonates in negative iodine balance^a

Group by GA (weeks)	27–30 (%)	31–33 (%)	31–33 (%)	34–36 (%)	34–36 (%)	Term (%)
Postnatal age		Sick	Healthy	Sick ^b	Healthy	
5 days	75	28	11	21	11	7
15 days	33	14	0	0	0	0
30 days	0	0	0	0	0	0

^aCalculated using the number of infants in each group with complete 24 h urine excretion data as 100%.

^bThe SGA infants are included in this subgroup.

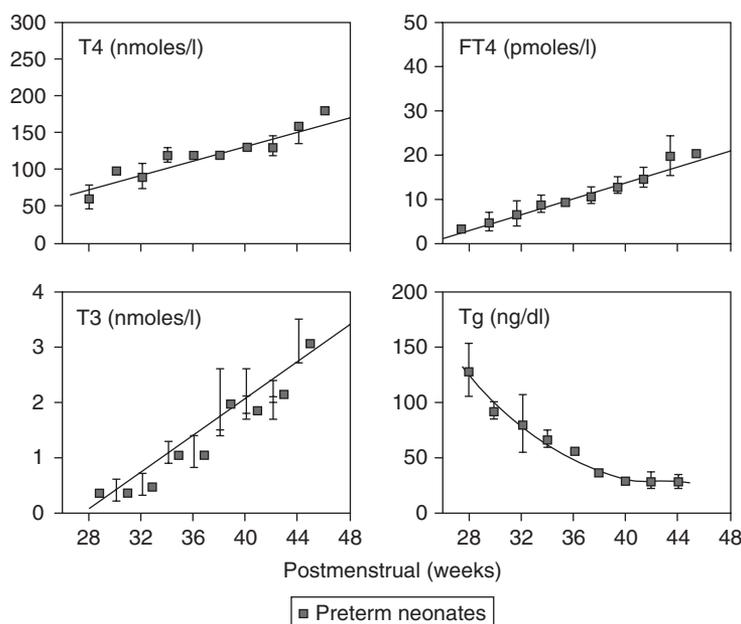


Figure 50.1 Mean (\pm SEM) circulating concentrations of T4, FT4, T3 and Tg are plotted against postmenstrual age, both for preterm and term neonates.

Iodine Deficiency in Preterm Babies Born at less than 30 Weeks of Gestation and Alterations in their Neurodevelopment

Neonates, and especially preterm infants, are a population at risk of suffering the consequences of iodine deficiency because of the impact of neonatal hypothyroxinemia on brain development. We evaluate the possible association between mental development scores at different ages and iodine intake during the neonatal period. Sixty-seven preterm infants were subdivided into GA groups for data analysis. The mental development scores reported here are those of the Brunet–Lezine scale index for children (0–24 months of age). The children were tested at 6, 9, 12, 18 and 24 months of postnatal age, and results were corrected for their GA. The test assesses: P, motor abilities and postural

control; C, eye motricity coordination and adaptability to new objects; L, language; and S, sociability. For these tests the mean (\pm SD) value in normal children is 100 ± 16 points. Taking into account the value of the global intelligence quotient and the WHO rankings, the children were subdivided into groups according to the degree of neurological damage. Group 1, very severe (0–20); Group 2, severe (21–35); Group 3, moderate (36–50); Group 4, mild (51–68); Group 5, borderline (69–85); Group 6, normal (86–100); and Group 7, high (>100). At 6 months, 34% of the babies had an index (global intelligence quotient) <68 points; values at 9 months were 15.8%, and 6.6% at 12 and 18 months, respectively. Iodine intake was variable, but mostly less than RDA. At 6 months, none of the indices were related to iodine intake. At 9, 12, 18 and 24 months, those babies who had a lower iodine intake during the first 30 days of life had indices <68 points.

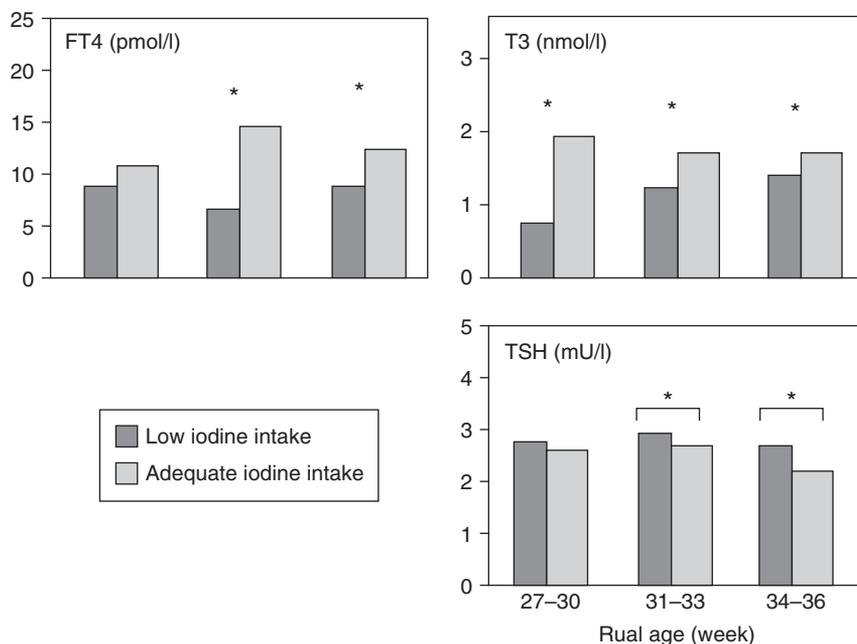


Figure 50.2 Mean (\pm SEM) circulating concentrations of FT4, T3 and TSH are plotted related to iodine intake for preterm neonates.

The most affected indices were motor abilities and postural control, language and the general cognitive index. After correction for age and degree of illness, there were statistically significant differences in psychomotor development of children that were related to their lower iodine intake: neonates with iodine deficiency during the first 60 days of life were correlated with poorer developmental indices at 24 months of age. It is important to follow the psychomotor development of all premature infants born at <30 weeks GA for at least the first 2 years of life, with special attention to those who were iodine deficient during the neonatal period, in order to determine whether early stimulation methods might improve their later development and in order to disclose the importance of avoiding iodine deficiency during the early postnatal period in preterm infants.

Excess of iodine during the Neonatal Period

There are several causes for exposure to an iodine excess, caused by iodine-containing antiseptics ($10000\mu\text{g}$ of iodine/ml) and radiologic contrast media ($250\text{--}370\text{mg}$ of iodine/ml). The aim is to bring to attention the evidence that even a minor iodine overload may impair thyroid function during a period of development when thyroid hormones are very important for the brain (Ares *et al.*, 1995, 2007). The precocious diagnosis and treatment of changes in thyroid function associated with iodine excess could have beneficial effects in the prevention of developmental abnormalities. We suggest a protocol to evaluate thyroid function when iodine

compounds are used – topically, as enema, or intravenously – because of the blocking effect of iodine excess in the thyroid gland. The risk of transient hypothyroidism may be avoided by the use of iodine-free compounds. Chlorhexidine should be used for skin disinfection and surgery. In our study population, those babies with elevated iodine urinary excretion presented with lower T4 and T3 serum concentrations (total T4 = $70.4 \pm 6.4\text{nmol/l}$ and T3 = $1.23 \pm 0.0\text{nmol/l}$), compared those babies with low iodine excretion (total T4 = $93.9 \pm 5.4\text{nmol/l}$ and T3 = $1.56 \pm 0.1\text{nmol/l}$) ($p < 0.039$; $p < 0.008$) (Figure 50.3a, b).

The purpose of the present protocol is systematically to include the determination of T4 in blood spotted on DBS paper, in order to detect hypothyroxinemia without elevation of TSH and to establish the necessity to incorporate a routine into the Neonatal Thyroid Screening Program that would obtain a special screening specimen in infants at high risk of suffering changes in their thyroid function (Figure 50.4). We follow up newborns with congenital heart disease ($n = 91$) who needed angiocardigraphy in the neonatal period and evaluated thyroid hormone levels once per week. The occurrence of hypothyroidism (diagnosed as TSH $> 10\text{mU/l}$ and FT4 $< 10\text{pmol/l}$) after angiography was high (Table 50.9).

Summary

Pregnant and lactating women and neonates are the main targets of the effects of iodine deficiency, because of the impact of maternal, fetal and neonatal hypothyroxinemia on

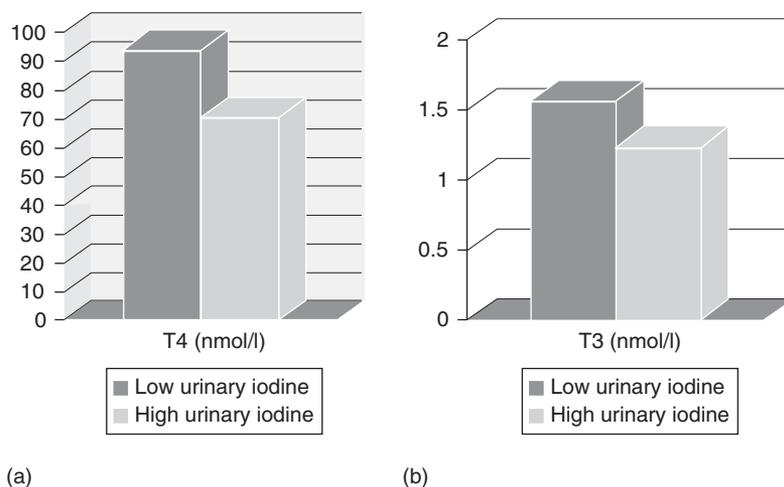


Figure 50.3 Mean total T4 (a) and T3 (b) serum concentration in preterm babies related to low or high iodine urinary excretion. Effect of iodine excess in the neonatal period.

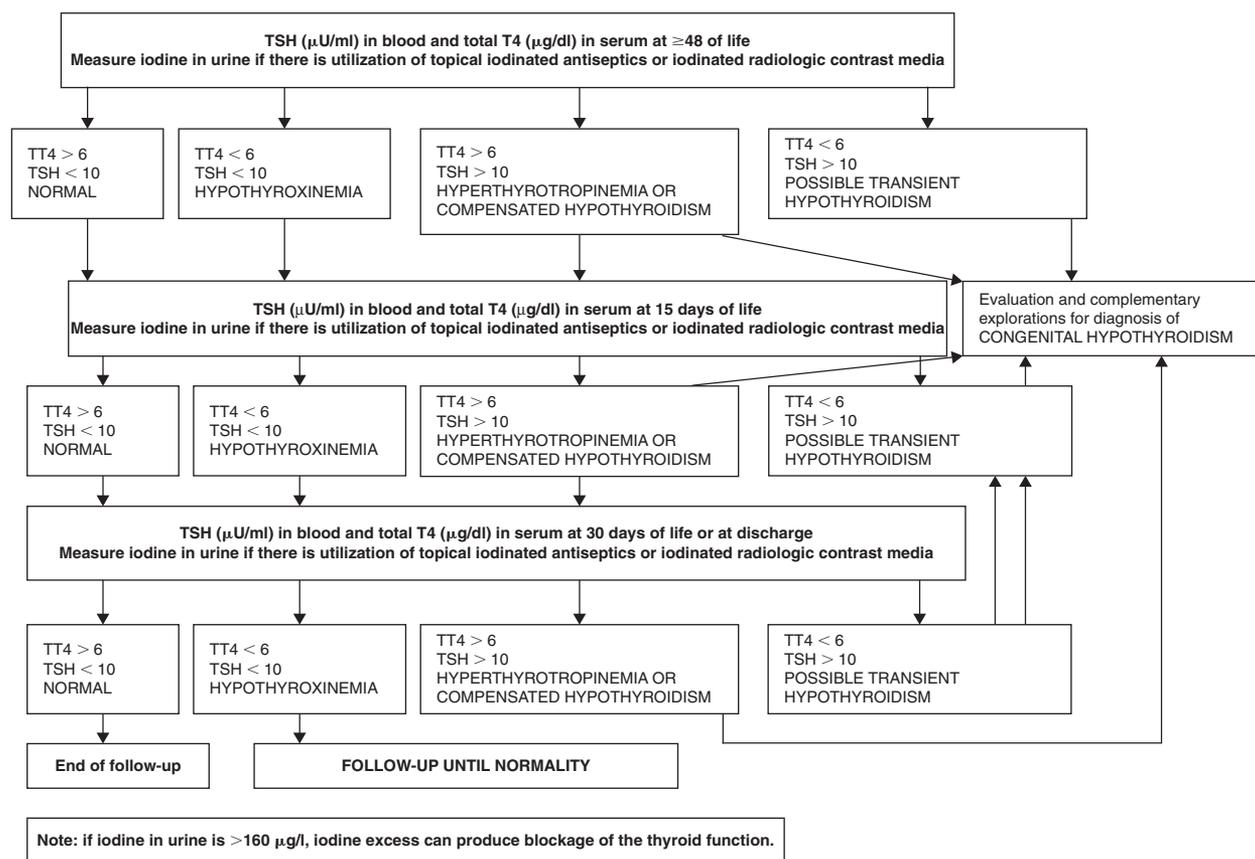


Figure 50.4 Proposed protocols for monitoring neonatal thyroid function under special circumstances.

brain development of the progeny (Morreale de Escobar and Ares, 1998; Morreale de Escobar *et al.*, 2000, 2004; Lavado-Autric *et al.*, 2003). Neurological damage is clearly preventable if pregnant mothers are tested for thyroid function during the first trimester, and by giving pregnant women, or even

before pregnancy, sufficient iodine to avoid hypothyroxinemia. If the mother has adequate iodine nutrition, breast milk is the best source of iodine for the newborn. However, based on data from the literature and on metabolic considerations, it is proposed that the recommended dietary intake of iodine

Table 50.9 Percentage of normal thyroid function and incidence of hypothyroidism in newborns with iodine excess due to angiocardigraphy in the neonatal period

Newborns	n	Hypothyroidism (%)	Normal thyroid function (%)
Term	75	11 (14.7)	64 (85.35)
Preterm	16	6 (37.5)	10 (62.5)

is 250–300 µg/day for pregnant women, 225–350 µg/day for lactating women and 90 µg/day for neonates and young infants (Zimmermann and Delange, 2004; Hollowell *et al.*, 1998).

Changes in thyroid function in premature infants, leading to low circulating levels of T₄ or T₃, have been associated with impairment of neural maturation, as measured by nerve conduction velocity and by lower scores in the Bayley mental and motor scales (De Vries *et al.*, 1986; den Ouden *et al.*, 1996; Lucas *et al.*, 1996, 1988; Meijer *et al.*, 1992; Reuss *et al.*, 1996). Iodine deficiency may well be one of the causes leading to inadequate thyroid hormone levels, and should be avoided. The recommended intake of iodine for preterm infants, based on balance studies, is 30 µg /kg/day. Enteral and parenteral nutritional fluids are the principal sources of iodine intake in these infants. The volume of food ingested by the infant is small, iodine content in formula preparations is insufficient and parenteral nutrition does not supply enough iodine. This problem is not exclusive to Spanish premature babies, as the iodine content of many formulas in other countries is also inadequate. Therefore, supplements should be added if iodine intake is found to be inadequate. Breast milk appears to be the best source of iodine for the premature infant (Ares *et al.*, 1994, 1995, 1997; Ibrahim *et al.*, 2003). Prevention of iodine deficiency and follow-up is recognized as a priority. The number of extremely low birth weight (ELBW) infants is high. Correction of their iodine deficiency and hypothyroxinemia and its consequences appears, at present, to be an intervention with promising possibilities (Ares *et al.*, 1995; Cools *et al.*, 2000; van Wassenaeer *et al.*, 1997; Vanhole *et al.*, 1997; La Gamma *et al.*, 2006). However, too little is known of the different factors involved in the metabolism of iodine and thyroid hormones during late fetal life, and their adjustment to the conditions faced by ELBW infants, to be able to standardize possible treatment protocols. Future research would be facilitated if preterm babies were followed during their stay in intensive care units with respect to their iodine nutrition and thyroid function [T₄, FT₄, T₃, TSH, thyroid-binding globulin (TBG), Tg] as carefully as they are followed for other organ functions (Morreale de Escobar and Ares, 1998; Rapaport, 2002; Rapaport *et al.*, 2001; Ares *et al.*, 2007).

In conclusion, in view of more reliable recent information on thyroid function and the physiology of preterm

infants, the iodine content of many formulas for feeding premature infants appears to be inadequate. Most premature babies do not ingest the amount of iodine recommended since 1992 by the ICCIDD, the WHO, and the European Community (Delange, 2001, 2004; WHO, UNICEF, ICCIDD, 2001). Producers of such formulas should be urged to comply with the new recommendations and ensure that their products do so irrespective of the country where they are being used. Premature infants in many countries are now in a situation of iodine deficiency, precisely at a developmental stage that is very sensitive to alterations of thyroid function.

Summary Points

- An adequate iodine intake should be ensured in preterm infants.
- Enteral and parenteral nutrition fluids are the principal sources of iodine intake in these infants. If the mother has adequate iodine nutrition, breast milk is the best source of iodine for the newborn.
- The volume of food ingested by the infant is low.
- The iodine content in formula preparations must be taken into account. Parenteral nutrition does not supply the preterm newborn with sufficient iodine to meet the recommendations. Supplements should be added if iodine intake is found to be inadequate.
- Most preterm babies are at high risk of iodine deficiency; neonates, and especially preterm infants, are a very important population at risk of suffering the consequences of both iodine deficiency and excess, because of the impact of neonatal hypothyroxinemia on brain development.
- Iodine deficiency and excess should be avoided.
- Correction of hypothyroxinemia and its consequences appears, at present, to be an intervention with promising possibilities.
- Prevention and follow-up in pediatrics is recognized as a priority.
- The number of ELBW babies is increasing.
- Future research would be facilitated if very premature infants were tested for thyroid function (T₄, FT₄, T₃, TSH, TBG, and Tg) immediately after birth and repeatedly during their stay in intensive care units, and as carefully as they are followed for other organ functions.

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Abstract

Severe iodine deficiency may impair thyroid hormone synthesis and cause a compensatory increase in H₂O₂ concentration in thyrocytes. This may result in more prolonged exposure to oxygen-free radicals, which in turn may extend reactive oxygen species (ROS)-mediated oxidative damage including DNA, induce mutations, and possibly contribute to degenerative changes in tissues. High levels of cell proliferation and higher replication rates during thyroid enlargement may also prevent mutation repair and implement mutations into the genome. A history of benign thyroid diseases, mostly goiter and nodules, and long-term residency in iodine-deficient areas have been considered as established risk factors for thyroid cancer. Our experimental data showed the availability of an effective mechanism to regulate antioxidative response in the thyroid glands of severely iodine deficient rats by adaptive increases in antioxidant enzymes (cGPx, SOD and CAT) during severe iodine deficiency. Our studies on high-school children from a severely iodine deficient area indicated the presence of oxidative activity and enhancement of free radical reactions in severely plus moderately iodine deficient goitrous children, and suggested that goiter development is more likely to occur in individuals having lower status of antioxidant enzymes and selenium.

Abbreviations

*OH	Hydroxyl radical
2-OH-Ade	2-Hydroxyadenine
5-OH-Cyt	5-Hydroxycytosine
8-OH-Ade	8-Hydroxyadenine
8-OH-Gua	8-Hydroxyguanine
AOE	Antioxidant enzymes
BPH	Benign prostatic hyperplasia
CAT	Catalase
cGPx	Cytosolic glutathion peroxidase
D1	Type I 5'-deiodinase

D2	Type II 5'-deiodinase
FapyAde	4,6-Diamino-5-formamidopyrimidine
FapyGua	2,6-Diamino-4-hydroxy-5-formamidopyrimidine
GPx	Glutathion peroxidase
IDD	Iodine-deficiency disorders
IL	Interleukin
LP	Lipid peroxidation
NMID-C	Nongoitrous children, with normal urinary iodine levels plus mild iodine deficiency
pGPx	Plasma or extracellular glutathion peroxidase
PH-GPx	Phospholipids hydroperoxide glutathion peroxidase
ROS	Reactive oxygen species
SePP	Selenoprotein P
SMOID-C	Severely iodine deficient nongoitrous children within the control group
SMOID-G	Severely plus moderately iodine deficient goitrous children
SOD	Superoxide dismutase
T ₃	3,5,5'-triiodothyronine
T ₄	Thyroxin
TBARS	Thiobarbituric acid reactive substances
TPO	Thyroid peroxidase
TrxR	Thioredoxin reductase
TSHR	TSH receptor
UI	Urinary iodine

Introduction

According to the WHO Health Report (2002), the deficiency of iodine is one of the most serious health factors in the world, along with iron, zinc and vitamin A deficiencies. While iodine is primarily required for thyroid hormone

synthesis and regulation, thyroid hormones are essential for the maintenance of normal metabolic functions and growth and development of living organisms. The physiological role of thyroid hormones is to ensure the timed coordination of different developmental events through specific effects on the rate of cell differentiation and gene expression (Delange and Hetzel, 2006). Iodine deficiency is a major public health problem, particularly in young children and women, and is a significant threat to national, social and economic development in many parts of the world. The spectrum of iodine deficiency disorders (IDD) includes abortion, stillbirths and congenital abnormalities in the fetus; increased infant mortality, endemic cretinism and mental retardation in neonates; hypothyroidism, goiter, retarded physical development and impaired mental function in children and adolescents; decreased fertility and iodine-induced hyperthyroidism in adults; and spontaneous hyperthyroidism in the elderly (Delange and Hetzel, 2006). Brain damage and irreversible mental retardation are the most severe IDDs, and iodine deficiency is considered as the leading cause of preventable mental retardation (WHO, UNICEF and ICCIDD, 1994). The main risk factor for iodine deficiency is low dietary supply of iodine, which arises from depletion of iodine from soil, mainly due to distant past glaciation and the leaching effect of snow, water, or heavy rainfall. High consumption of natural goitrogens, including thiocyanate, which inhibits iodide intake, and organification at higher doses are other factors (Delange, 1994; Köhrle *et al.*, 2005). Iodine deficiency is a worldwide problem. One-third of the world's population subsists on a diet that is deficient in iodine, which puts them at risk for IDD (WHO, UNICEF and ICCIDD, 1994). Goiter, or the enlargement of the thyroid gland, is the earliest and predominant clinical sign of iodine deficiency. Endemic goiter may be said to exist in a population when more than 5% of the preadolescent school-age (6–12 years) children have enlarged thyroid glands (WHO, UNICEF and ICCIDD, 2001). About 13% of the world's population suffers from goiter; it continues to be a major public health problem in many of the developing countries, and it also exists in localized regions in some of the economically advanced countries of the west, including central and eastern Europe (Delange *et al.*, 1993; WHO, UNICEF and ICCIDD, 1999).

Selenium-dependent glutathion peroxidase (GPx)s provide general defense against oxidative stress; however, recent discoveries of new selenoproteins have revealed further relationships among selenium, iodine and thyroid hormones. Classical GPx and several other selenoproteins participate in the protection of thyrocytes from oxidative damage by H_2O_2 produced for thyroid hormone biosynthesis. Three isozymes of iodothyronine 5'-deiodinase, the enzymes that catalyze the metabolic conversion of thyroxin (T_4) to the major biologically active hormone, 3,5,5'-triiodothyronine (T_3), are selenoenzymes, and their main function is to regulate

the levels of thyroid hormones (Arthur *et al.*, 1990; Behne *et al.*, 1990; Berry *et al.*, 1991). Therefore, besides iodine, selenium is also involved in thyroid functions, more specifically, in the regulation of the thyroid hormone metabolism and the protection of the thyroid gland from oxidative damage. Selenium deficiency certainly plays a role in the etiology of the type of myxedematous endemic cretinism seen in central Africa, but does not by itself constitute a cause for endemic goiter (Delange and Hetzel, 2006).

Oxidative Stress and the Thyroid Gland

The association between iodine deficiency and oxidative damage originates from the process of production of thyroid hormones in the thyroid gland. The biosynthesis of thyroid hormones involves several phases, including trapping and organification of serum iodide, incorporation of iodine into tyrosine, coupling of iodinated tyrosyl residues of the thyroglobulin, and proteolytic cleavage to release iodothyronine under the control of pituitary hormone, TSH (Gentile *et al.*, 1995; Taurog, 1996). Organification, or oxidation, of iodide to iodine is accomplished by H_2O_2 catalyzed by the enzyme thyroid peroxidase (TPO), and leads to the eventual formation of T_3 and T_4 . Thyroid hormone synthesis thus requires an adequate supply of iodide, as well as continuous production of H_2O_2 , the electron acceptor for thyroperoxidase reaction (Corvilain *et al.*, 1991). Provision of substrate H_2O_2 in the thyroid occurs via TSH-mediated NADPH oxidase induction, and through dismutation of superoxide radicals thus formed by the enzyme superoxide dismutase (SOD) (Dumont *et al.*, 1992). The action of TSH on the thyroid follicular cell is mediated via intracellular signaling systems, including the G-protein-linked cAMP and phosphatidylinositol- Ca^{2+} cascades (Caillou *et al.*, 2001). The system controlling H_2O_2 generation is the limiting step for thyroid hormone synthesis, when the iodine supply is sufficient. However, K_M of TPO for H_2O_2 is high, and much higher amounts of H_2O_2 are produced than are consumed by the iodination process (Corvilain *et al.*, 1991, 1994). H_2O_2 is toxic to cells, can be the precursor of highly reactive peroxides (Halliwell and Gutteridge, 1989), and if not properly reduced to H_2O by intracellular defense mechanisms, can expose the thyroid gland to free radical damage (Farber *et al.*, 1990). In order to resist to this lifelong oxidative stress, the thyroid has several lines of antioxidative defense mechanisms. The production of H_2O_2 in the extracellular space, i.e., the colloidal lumen at the surface of the apical membrane, and the orientation of the active site of the integral membrane enzyme TPO toward this compartment are the first components of this defense strategy, which avoids the exposure of intracellular compartments and membranes to H_2O_2 and other reactive oxygen species (ROS) (Köhrle *et al.*, 2005). Nevertheless, H_2O_2 can

easily diffuse through the membranes and provoke direct damage to cytoplasmic macromolecules and even to DNA. Thyrocytes are further protected by antioxidant enzymes (AOE) such as catalase (CAT), SOD and selenium-containing enzymes. Originally, GPx was the only identified selenoenzyme that is present and functions in the thyroid (Flohe *et al.*, 1973). Recent evidences suggest the existence of various selenoproteins with distinct regulation of expression and secretion or function, including various GPx enzymes (cytosolic GPx [cGPx], plasma or extracellular GPx [pGPx], phospholipids hydroperoxide GPx [PH-GPx]), thioredoxin reductase (TrxR), type I 5'-deiodinase (D1), type II 5'-deiodinase (D2), selenoprotein P (SePP) and selenoprotein 15 (Bermano *et al.*, 1996; Howie *et al.*, 1998; Köhrle, 1999; Köhrle *et al.*, 2005; Meinhold *et al.*, 1992; Mitchell *et al.*, 1996; Zagrodzki *et al.*, 1998). In fact, human thyroid contains the highest concentration of selenium in comparison with all other organs (Dickson and Tomlinson, 1967; Köhrle, 1999), even in the case of selenium-deficient nutrition (Zimmermann and Köhrle, 2002), and this implicates the important role of selenium in thyroid function. Like iodine, selenium is inadequately available for humans and livestock in many parts of the world (Chanoin *et al.*, 1993), and the relationships between selenium and thyroid function are complex and dual. On the one hand, selenium involves itself in thyroid hormone metabolism and provides sparing of iodine by decreasing the catabolism of prohormone T₄ when a shortage of iodine intake exists (Goyens *et al.*, 1987). On the other hand, the effects of selenium deficiency on the thyroid gland are also related to the peroxidative damage induced by H₂O₂, the levels of which increase in thyroid cells by lack of protection due to defective selenoenzymes, particularly GPx. Therefore, although the high concentration of intracellular H₂O₂ allows a higher efficiency of thyroid hormone synthesis, the thyroid gland is a source of oxygen radicals in iodine and selenium deficiency.

Thyroid Hormones and Oxidative Stress

It is evident that variations in thyroid hormone levels are among the main physiological modulators of *in vivo* cellular oxidative stress. The hypermetabolic state in hyperthyroidism is associated with increases in free radical production and lipid peroxidation (LP), and the hypometabolic state in hypothyroidism is generally associated with a decrease in free radical production and LP in most tissues (Fernandez *et al.*, 1985; Venditti *et al.*, 1997). The development of a hyperthyroid state in vertebrates leads to enhancement of their basal metabolic rate due to an increase in the rate of O₂ consumption in most tissues, excluding the spleen, testis and adult brain (Barker and Klitgaard, 1952). Thyroid hormones were shown

to influence several mitochondrial functions, including oxygen consumption, oxidative phosphorylation and proton leak, and to increase the metabolic activity of almost all tissues of the body (Fernandez *et al.*, 1985; Guerrero *et al.*, 1999; Nishiki *et al.*, 1978; Venditti and Di Meo, 2006). T₃ hormone exerts significant action on energy metabolism, with mitochondria being the major target for its calorogenic effects. Acceleration of O₂ consumption by T₃ leads to an enhanced generation of reactive oxygen and nitrogen species in target tissues, with a higher consumption of cellular antioxidants and inactivation of AOE, thus inducing oxidative stress. Administration of T₃ hormone to hypothyroid rats was associated with increased oxidative capacity, as shown by several studies (Das and Chainy, 2001; Venditti *et al.*, 1997). An increase in LP levels and a reduction of total antioxidant capacity were observed in hyperthyroid patients (Bednarek *et al.*, 2004; Komosinska-Vashev *et al.*, 2000). It was also reported that T₃ induces the expression of redox-sensitive genes as a nongenomic mechanism of T₃ action (Fernandez *et al.*, 2006; Tapia *et al.*, 2003, 2006). It was shown that T₃ administration to rats induces a calorogenic response and liver glutathione depletion as an indication of oxidative stress, with higher levels of interleukin (IL)-6 in serum and hepatic STAT3 DNA binding (Tapia *et al.*, 2006).

Iodine Deficiency and Oxidative Damage

Animal studies

It is known that the generation of H₂O₂ is inhibited by iodide *in vivo* and *in vitro* (Cardoso *et al.*, 2001; Morand *et al.*, 2003). This implies higher generation of H₂O₂ in the thyroid gland in iodine deficiency. We previously investigated the antioxidant and oxidant status of various organs in an iodine-deficient rat model (Giray *et al.*, 2004). We introduced iodine deficiency by supplying male Wistar rats with 1% sodium perchlorate-containing water for 5 weeks. Activities of AOE (cGPx, SOD and CAT) and LP levels (as measured thiobarbituric acid reactive substances [TBARS]) were measured in thyroid, liver, kidney, brain tissues and plasma and erythrocytes. Iodine deficiency and the resulting hypothyroidism in rats was evidenced by increased weight of the thyroid gland, higher levels of TSH, and low levels of circulating T₄ and T₃. There was no enhancement of LP in any of the tissues examined, and except for significant elevation of SOD activity in the kidneys and reduction in CAT activity in liver and kidney, iodine deficiency did not cause any changes in tissues examined, other than the thyroid gland (Giray *et al.*, 2004). Marked changes in AOE activities, including ~2.5-fold increase in cGPx, ~100% enhancement in CAT and (although nonsignificant) ~50% increase in SOD, were observed in the thyroid (Figure 51.1). These results suggested the development of

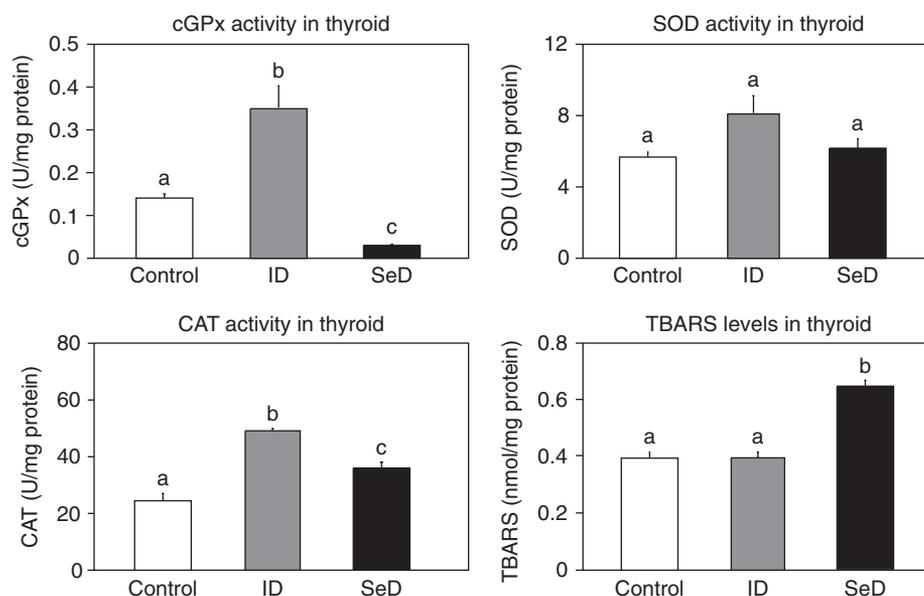


Figure 51.1 Antioxidant enzyme activities and LP levels in thyroid tissues of iodine- and selenium-deficient rats. Source: Giray *et al.*, 2004, with kind permission from Wiley-Liss. Superscripts of different letters differ significantly ($p < 0.05$) from each other. Abbreviations: C, control group; ID, iodine-deficient group; SeD, selenium-deficient group; cGPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; TBARS, thiobarbituric acid reactive substances; LP, lipid peroxidation.

an adaptive response in the AOE system of rats within a period of 5 weeks of severe iodine deficiency, to protect the thyroid against oxidative stress induced by the high levels of H_2O_2 and the ROS derived thereof. Our results were in line with an earlier report that showed marked increases in thyroidal cGPx (two- to fourfold) and D1 (10- to 12-fold) activities in iodine-deficient heifers, while no changes in liver, kidney, pituitary and brain activities (Zagrodzki *et al.*, 1998) were reported. Increases in cGPx expression (fivefold) and activity in second-generation iodine-deficient rats were also reported (Mitchell *et al.*, 1996). Investigators pointed out the occurrence of compensatory mechanisms and concluded that iodine deficiency produces oxidant stress in the thyroid gland and increases the requirement for selenium to maintain the activities of selenoenzymes. In fact, in a selenium- plus iodine-deficient rat model, we also observed increases in cGPx, SOD and CAT activities in the thyroid. But the increase in cGPx was relatively low, and indicating the importance of selenium-dependent antioxidative defense mechanisms, a high level of LP was found in the thyroid, as well as in liver and brain tissues (Giray *et al.*, 2004). Thus, the elevation of thyroidal cGPx, CAT and SOD activities in severely iodine deficient rats seems to provide effective means of elimination and control of H_2O_2 and prevent LP when selenium intake is not inadequate.

The data presented in a recent report (Maier *et al.*, 2007) were also in line with our findings, and supported the hypothesis that although thyrocytes very likely have an effective mechanism to regulate antioxidative response that counter the potential threat of oxidant stress, the

compensatory increase in H_2O_2 during severe iodine deficiency may extend ROS-mediated oxidative damage including DNA. The study was conducted on mice and rats fed an iodine-controlled diet, and the time course of adaptive changes was determined. Extracellular SOD, PH-GPx and peroxiredoxine 3 and 5 showed increased mRNA expression within 2–3 months of iodine restriction. Serum TSH levels were found to be unchanged or only little changed, but an increase in TSH receptor (TSHR) mRNA expression was observed. Additional oxidized base adducts in thyroidal genomic DNA were detected, indicating increased radical burden. The authors suggested that iodine deficiency initiates a transient oxidative attack and DNA modifications, and the thyroid response to iodine deficiency is not only restricted to enlargement, but also very likely involves cellular hyperfunction, as suggested by increased mRNA expression of the TSHR, TPO and sodium-iodine symporter.

Besides enzymatic systems, cells are protected by several intracellular vitamins. Vitamin E plays a major role in the maintenance of membrane integrity, by being both a strong free radical scavenger and a structural stabilizer (Gutteridge, 1978), and its depletion results in increased LP. Since the radical hypothesis is difficult to test *in vivo*, analyzing the effects of free radical scavengers or of antioxidants known to stop LP can be an alternative approach. In order to assess the implications of free radical generation in the induction of thyroid cell necrosis, such a study was conducted by Mutaku *et al.* (1998). They investigated the effects of vitamin E, vitamin C and β -carotene

(separately or in combination), and demonstrated that while the concentration of vitamin E in thyroid of normal rats was as high as in liver and higher than in plasma, during iodine deficiency it increased significantly (more than twofold) in the thyroid and plasma, but decreased in the liver. The hepatic effect was in line with a previous report (Warren and Reed, 1991) showing the mobilization of hepatic stores of vitamin E during response to an oxidative stress *in vivo*. In addition, the level of increase in thyroidal vitamin E concentration strongly suggested a tissue-specific effect during goitrogenesis. Treatment of iodine-deficient rats with vitamin E alone, or in association with other vitamins, led to a five-fold increase in vitamin E in the thyroid, a 24-fold increase in the liver, and a three-fold increase in the plasma, and reduced goiter development without any change in thyroid hormone metabolism. The effect was at the level of thyroid follicular cell proliferation and did not depend on TSH variations. Vitamin E thus showed an antigoitrogenic effect during iodine deficiency, but did not interfere with either endothelial cell proliferation or iodine metabolism. The other free radical scavengers, β -carotene or vitamin C, did not show a similar effect, suggesting a specific rather than an antioxidant action of vitamin E. Although further studies would be needed to confirm the exact site of vitamin E action, the authors have discussed the probability of a direct effect of vitamin E on intracellular signaling pathways.

Cell death plays a central role in regulating thyroid cell mass; coexistence of necrosis and apoptosis, and thus a balance between cell death and proliferation, have been reported to occur during goiter development (Denef *et al.*, 1981; Rognoni *et al.*, 1987; Tamura *et al.*, 1998). In a study where the extent of intrathyroidal necrosis and apoptosis was investigated in vitamin E-deficient and sufficient rats during goiter formation and involution, Mutaku *et al.* (2002) showed that necrosis predominates in the goitrous gland, while apoptosis is significantly induced in goiter involution. They found an increase in LP and a decrease in GPx in the thyroid, and a concomitant rise in LP in liver, and attributed the necrotic effect to free radical formation that was potentiated in vitamin E-deficient rats having reduced antioxidative protection.

Human studies

With the aim of investigating the possible changes in oxidant and antioxidant status in iodine-deficient goitrous children, we conducted a study in two towns of the eastern Black Sea region where the prevalence of endemic goiter is one of the highest in Turkey (Hatemi and Urgancioğlu, 1993). As described elsewhere (Giray *et al.*, 2001), the subjects were all from the urban population of the two towns (~50 km apart from each other) and socioeconomically homogenous (lower-middle class). Dietary information

relevant to selenium and antioxidant nutrients, and possible goitrogenic food intake including vegetables of the *Brassicaceae* family, was collected through a standard food-frequency questionnaire (but no significant difference was found). After screening the whole student populations of the two towns ($n = 502$) for goiter by palpation, the overall prevalence of goiter was found to be 39.6%. Groups of goitrous ($n = 48$, 15–18 years) and nongoitrous ($n = 49$) children were selected by a simple random technique. An out-of-region control group ($n = 24$) from an area of lower goiter prevalence was also included. Activities of erythrocyte cGPx, SOD, CAT, plasma and erythrocyte selenium and urinary iodine (UI) levels were determined, and found to be significantly lower in the goitrous group than those in both in-region and out-of-region controls. The whole study group was later reclassified into normal, mildly deficient and severely deficient groups according to the degree of iodine deficiency based on UI levels as recommended by the WHO (1997). There were mildly-to-severely iodine-deficient individuals in both goitrous and control groups. Therefore, in order to better understand the effects of iodine deficiency and assess the differences between children with and without goiter, parameters of further classified groups were evaluated. It was found that “severely plus moderately iodine deficient goitrous children” (SMOID-G) had significantly lower selenium levels and AOE activities than “nongoitrous children, with normal UI levels plus mild iodine deficiency” (NMID-C) (Table 51.1). These results indicated the presence of higher oxidative activity in SMOID-G, and suggested the possibility of an enhancement of free radical reactions.

It is known that consistently lower iodine intake ($<50 \mu\text{g}/\text{d}$) usually results in goiter, but not all people with iodine deficiency develop goiter. Adequate adaptation to iodine deficiency has been demonstrated in areas of severe iodine deficiency in the absence of endemic goiter (Delange *et al.*, 1968), and it has been shown that goiter, especially large colloidal goiter, in endemic iodine deficiency represents maladaptation, instead of adaptation, to iodine deficiency (Dumont *et al.*, 1995). The presented study showed that “severely iodine deficient nongoitrous” children existed within the control group (SMOID-C), and as seen in Table 51.1, their AOE and selenium statuses were not different from those of the NMID-C group. Therefore, it seems that goiter development is more likely to occur in individuals having lower status of AOE and selenium. However, whether the high level of oxidant stress encountered in goitrous populations is a cause or a consequence needs to be further elaborated.

We then investigated the levels of modified DNA bases in genomic DNA of peripheral blood in a group of goitrous children and goiter-free control children ($n = 14$ each) selected from the SMOID-G and NMID-C groups by a further simple random technique (Giray and Hincal, 2002). Six modified DNA bases (5-hydroxycytosine

Table 51.1 Thyroid-related parameters of children from an endemic goiter area

Groups	UI ($\mu\text{g}/\text{dl}$)	TT4 (nmol/l)	Plasma selenium ($\mu\text{g}/\text{l}$)	cGPx (U/gHb)	SOD (U/mgHb)	CAT (K/gHb)
Severely plus moderately ID goiter group (SMOIG) (UI < $5\mu\text{g}/\text{dl}$); (n = 45)	2.4 \pm 0.2 ^a (0.43–4.99)	100.5 \pm 21.8 ^a (53.5–150.3)	67.0 \pm 11.1 ^a (47.5–89.9)	14.9 \pm 5.4 ^a (3.0–25.1)	24.3 \pm 3.8 ^a (17.3–34.0)	186.6 \pm 39.0 ^a (102.0–172.3)
Severely plus moderately ID control group (SMOIC) (UI < $5\mu\text{g}/\text{dl}$); (n = 31)	2.6 \pm 1.0 ^a (1.32–4.99)	113.8 \pm 23.8 ^b (67.1–166.5)	75.5 \pm 14.4 ^b (42.0–98.8)	18.9 \pm 5.0 ^b (8.0–28.5)	27.0 \pm 4.3 ^b (18.6–41.1)	216.7 \pm 30.8 ^b (165.0–284.6)
Normal plus mildly ID control group (NMIC) (UI $\geq 5\mu\text{g}/\text{dl}$); (n = 42)	10.9 \pm 6.4 ^b (5.07–31.6)	129.8 \pm 20.0 ^b (91.8–172.6)	74.8 \pm 13.8 ^b (48.6–114.4)	18.5 \pm 5.6 ^b (5.1–29.1)	27.3 \pm 3.4 ^b (20.2–34.5)	207.9 \pm 30.5 ^b (136.1–281.2)

Notes: Values are given as mean \pm SD; data that are normally distributed were analyzed with ANOVA followed by Duncan test; parameters not normally distributed (UI) are compared by Kruskal–Wallis and Mann–Whitney test; values in columns not sharing a common superscript letter differ significantly ($p < 0.05$).

Source: Giray *et al.*, 2001, with kind permission from Springer Science and Business Media.

[5-OH-Cyt], 8-hydroxyguanine [8-OH-Gua], 2,6-diamino-4-hydroxy-5-formamidopyrimidine [FapyGua], 8-hydroxyadenine [8-OH-Ade], 2-hydroxyadenine [2-OH-Ade], and 4,6-diamino-5-formamidopyrimidine [FapyAde]) were identified and quantified. The mean levels of these modified DNA bases are given in Figure 51.2. It was found that, of the six damaged DNA bases quantified, the means of three bases were significantly higher in the goitrous group. In the case of 5-OH-Cyt and 8-OH-Ade, the increases were about 50%, and over 30% increase was determined for 8-OH-Gua. No significant differences were observed for the mean values of 2-OH-Ade, FapyAde and FapyGua between the two groups. Although the group sizes were limited, no statistical differences were detected for levels of modified DNA bases between females and males. Thus, these results further supported the hypothesis that the highly iodine-deficient goitrous children are exposed to oxidative stress, which may lead to greater levels of oxidative damage to DNA. However, it should be added that not only goitrous children, but also our overall study group had borderline, if not deficient, selenium intake. The daily intake of selenium, estimated using an algorithm given by Longnecker *et al.* (1996), was lower than the RDA value ($55\mu\text{g}/\text{d}$) of selenium (Institute of Medicine, 2000) in both goitrous ($39\mu\text{g}/\text{d}$) and control ($46\mu\text{g}/\text{d}$) groups. These findings, therefore, suggested a possible link between increased activities of DNA damage and decreased activities of AOE, as well as lower selenium levels in our goitrous subjects.

Iodine Deficiency and Thyroid Malignancy

Thyroid cancer is a relatively rare form of cancer, with a higher incidence in younger woman (Franceschi *et al.*, 1993; La Vecchia *et al.*, 1992), and its etiology is still under discussion. However, a history of benign thyroid diseases, mostly goiter and nodules (strongly related), and hyperthyroidism and thyroiditis (less strongly related) have been considered as established risk factors for thyroid cancer (D'Avanzo *et al.*, 1995; Galanti *et al.*, 1995; La Vecchia *et al.*, 1992; Mellemegaard *et al.*, 1998; Ron *et al.*, 1987). Long-term residence in regions with iodine imbalance, i.e., high endemic goiter areas (D'Avanzo *et al.*, 1995) or areas with high iodine levels (Goodman *et al.*, 1988; Parkin *et al.*, 2002), and poor nutrition (a diet poor in vegetables and fruits and, hence, in antioxidant nutrients) (D'Avanzo *et al.*, 1997; Franceschi *et al.*, 1991), are other recognized risk factors for thyroid tumors, besides the well-established causal factors of ionizing radiation and radiotherapy (Franceschi *et al.*, 1993; Ron *et al.*, 1987). Various epidemiological data have provided supportive evidence for the promoting effect of iodine deficiency in thyroid cancers, and almost all available data indicate that highly aggressive and prognostically poor types of thyroid cancers (follicular and anaplastic carcinomas) prevail in countries with endemic goiter (Belfiore *et al.*, 1987; Vigneri *et al.*, 1998). In a case control study conducted in northern Italy (Fioretti *et al.*, 1999), about 60% of thyroid cancer

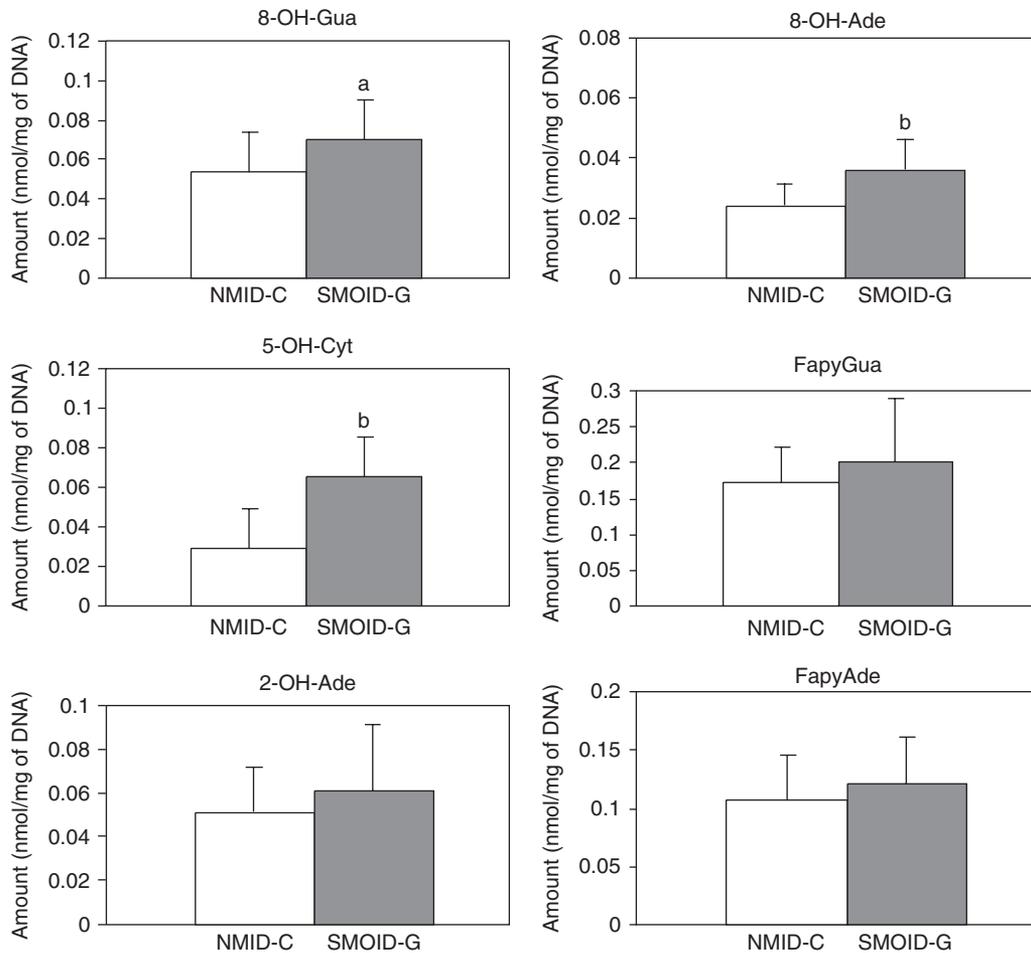


Figure 51.2 The amount of modified DNA bases in total genomic DNA of peripheral blood of goitrous (SMOID-G) and control (NMID-C) children. Source: [Giray and Hincal, 2002](#), with kind permission from Taylor & Francis. 1 nmol/mg of DNA corresponds to approximately 308 lesions/ 10^6 DNA bases. ^a $p < 0.05$, ^b $p < 0.01$ by Student's *t*-test.

cases have been explained by a history of benign thyroid diseases, residence in an endemic goiter area, and a poor diet. A longitudinal study in Sweden ([Galanti *et al.*, 1995](#)) indicated that residence for over 20 years in an endemic goiter area is associated with a higher risk of thyroid cancer (RR: 1.3–1.5), and exposure of females to iodine deficiency during puberty was found to be associated with a 1.9- and 2.5-fold increased risk of follicular carcinoma and papillary carcinoma, respectively. Existing data also indicate that iodine supplementation results in a relative decrease in these tumor types, and the incidence shifts toward a better prognosis, increasing the ratio of papillary cancer to follicular cancer ([Bacher-Stier *et al.*, 1997](#)). In earlier studies, it was also demonstrated that an iodine-deficient diet is able to induce thyroid malignancies in animals ([Ohshima and Ward, 1986](#); [Schaller and Stevenson, 1966](#)). Moreover, when carcinogen-pretreated rats were kept on an iodine-deficient diet, the incidence of thyroid carcinomas increased more than four times, indicating that iodine deficiency is a promoter of tumors initiated by

another carcinogen. In summary, there is experimental, biological, epidemiological and clinical evidence indicating that iodine deficiency acts as a risk factor for thyroid cancer possibly by favoring initiation, promotion and progression of thyroid tumors.

Iodine deficiency leads to diminished thyroid hormone production resulting in cell proliferation; thus, goiter development is related to increased TSH secretion. TSH is a known cell growth factor and certain thyroid tumors are TSH dependent ([Clark and Castner, 1979](#)). However, in chronic iodine deficiency, the thyroid cell is also stimulated to proliferate by various other mechanisms, including increased thyroid cell responsiveness to TSH, decreased TGF- β production, increased thyroid cell EGF-induced proliferation and increased angiogenesis ([Vigneri *et al.*, 1998](#)). Since prolonged cell proliferation is in favor of premalignant transformations, and an increased number of cell divisions enhances the probability of oncogen activation and loss and/or damage of oncosuppressor genes ([Amstad and Cerutti, 1990](#)), the possibility of thyroid

enlargement as a predisposition factor for thyroid malignancies seems to be quite high. In addition, as described above, the highly-stimulated thyrocytes in iodine-deficient thyroid gland produce increased amounts of H_2O_2 for synthesis of thyroid hormones. This may cause more prolonged exposure to oxygen-free radicals, and possibly contributes to degenerative changes in the tissues. The results of a recent study where proteomic analysis was conducted on cold nodules (accounting for up to 85% of thyroid nodules seen in an iodine-deficient area) provided evidence for upregulation of thyroid cell proliferation, turnover of thyroglobulin and H_2O_2 detoxification (Krause *et al.*, 2007). Their findings showed increased 8-oxo-guanidine DNA adduct formation, which also suggested the presence of oxidative stress overriding the antioxidative system.

Moreover, when selenium deficiency is coupled with iodine deficiency, through an increased availability of H_2O_2 and a decreased thyroid GPx activity, the stimulated thyroid gland is possibly exposed to greater levels of H_2O_2 and, in turn, to highly reactive peroxides (Goyens *et al.*, 1987). It is therefore plausible that deficits of antioxidant status may lead to the exposure of thyroid cells to increased oxidative stress, and may eventually contribute to the occurrence of malignant transformations. Since the modified DNA bases we observed in goitrous children were typical products of hydroxyl radical (*OH) attack on DNA, their elevated levels might indicate the participation of *OH in the process. Some of these identified DNA base lesions are known to possess premutagenic properties and may play a role in carcinogenesis (Feig *et al.*, 1994; Purmal *et al.*, 1994; Shibutani *et al.*, 1991; Wood *et al.*, 1990). In fact, pathogenesis of thyroid disease is associated with several genetic alterations (Eng, 1999; Lazzereschi *et al.*, 1997). The RET proto-oncogene encodes a receptor tyrosine kinase, and different forms of RET mutations have been reported to cause different thyroid diseases (Eng, 1999). The TSHR gene and the Gs alpha protein gene become oncogenic through point mutations and are associated with the development of toxic thyroid adenomas (Russo *et al.*, 1999). The *ras* oncogene is implicated in the early stages of development of several thyroid tumor types (Esapa *et al.*, 1999), and mutations in *ras* have been detected in about 20% of thyroid adenomas (Lazzereschi *et al.*, 1997). The p53 gene appears to be involved in the process of transformation to the anaplastic phenotype and the PTEN tumor-suppressor gene in the development of follicular adenomas, but not carcinomas (Lazzereschi *et al.*, 1997). Recently, Hou *et al.*, (2007) collectively examined the major genetic alterations including PIK3CA copy number gain and mutation, *ras* mutation and PTEN mutation, in a large series of primary thyroid tumors. Their data provided strong genetic implications that aberrant activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway plays an extensive role in thyroid tumorigenesis, particularly in follicular thyroid cancer and anaplastic

thyroid cancer, and promotes the progression of benign thyroid adenoma to follicular thyroid cancer and to anaplastic thyroid cancer, as the genetic alterations of this pathway accumulate.

Various previous studies clearly showed elevated levels of typical *OH-modified DNA bases in various cancerous tissues compared to their surrounding normal tissues (Jaruga *et al.*, 1994; Malins *et al.*, 1996; Olinski *et al.*, 1992). There is also evidence of an association between increased levels of modified DNA bases and decreased levels of AOE in human cancerous tissues (Jaruga *et al.*, 1994), including lymphocytes of acute lymphoblastic leukemia patients (Sentürker *et al.*, 1996). Furthermore, human benign prostatic hyperplasia (BPH) tissues have been shown to have higher oxidative DNA base damage and lower AOE than the surrounding normal prostate tissues (Olinski *et al.*, 1995). Thus, the results of our study on DNA base damage (Giray and Hincal, 2002) are in line with the general trends observed in the above-mentioned studies and suggest that goitrous subjects might have a predisposition for thyroid malignancies.

Summary Points

- Iodine deficiency is a worldwide problem and is considered as one of the world's most serious health factors. An estimated 2 billion people of the world have inadequate iodine nutrition, and therefore are at risk of IDD, including goiter and mental retardation.
- Thyroid hormone synthesis requires adequate supply of iodine, as well as continuous production of H_2O_2 as a cofactor of thyroperoxidase. In iodine-deficient thyroid glands, the highly-stimulated thyrocytes synthesize, under TSH control, an increased amount of H_2O_2 , which is toxic to cells and can be the precursor of highly reactive peroxides.
- High levels of H_2O_2 may lead to oxidative stress and in turn LP and oxidative DNA modifications, causing higher mutation rates, although thyroid cells very likely have an effective mechanism, including several forms of selenoenzymes, to regulate antioxidative response that counters the potential threat of oxidant stress.
- When there is selenium deficiency coupled with iodine deficiency, through an increased availability of H_2O_2 and a decrease in thyroid GPx activity, the stimulated thyroid gland is possibly exposed to greater levels of H_2O_2 , and in turn, to highly reactive peroxides.
- Therefore, although the high concentration of intracellular H_2O_2 allows a higher efficiency of thyroid hormone synthesis, the thyroid gland is a source of oxygen radicals in iodine and selenium deficiency.
- Human thyroid contains the highest concentration of selenium compared with all other organs, even in the case of selenium-deficient nutrition, and this indicates

the important role of selenium in thyroid function. However, like iodine, selenium is inadequately available for humans and livestock in many parts of the world.

- Oxidative DNA damage caused by ROS or other DNA-damaging agents has been implicated in mutagenesis, carcinogenesis, reproductive cell death and aging.
- A history of benign thyroid diseases, mostly goiter and nodules, and long-term residency in iodine-deficient areas have now been considered as established risk factors for thyroid cancer. Pathogenesis of thyroid disease is associated with several genetic alterations.
- It is therefore plausible that deficits of antioxidant status, in addition to iodine deficiency, may lead to the exposure of thyroid cells to increased oxidative stress and damage, and may eventually contribute to the occurrence of malignant transformations.

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Iodine Deficiency in Relation to Iron Deficiency and Parasitosis: Effect of Iron Status and Parasites on Iodine Deficiency Disorders

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Abstract

The intrauterine period is vulnerable to long-term neurological and cognitive iodine deficiency. Infants are also susceptible to iodine deficiency disorders (IDD) in conjunction with iron deficiency. As a result, iodine deficiency is the world's leading cause of preventable mental retardation and impaired psychomotor development in young children. This risk is further enhanced when mother and offspring are exposed to iodine deficiency during gestation and the postnatal period. At the beginning of the twenty-first century, salt iodization was the preferred strategy adopted to eliminate IDD through universal iodization. Iron deficiency adversely affects thyroid metabolism and reduces iodine prophylaxis efficacy in areas of endemic goiter. The therapeutic response to oral iodized oil is impaired in goitrous children with iron deficiency anemia. In pregnant women with chronic iodine deficiency, endemic goiter may aggravate anemia, while the severity of subclinical hypothyroidism increases in the presence of anemia. Parasites such as *Ascaris lumbricoides*, *Trichuris trichiuria* and hookworms are the most common, chronic, subclinical infections in childhood. Species of hookworm transmitted in a community influence the burden of iron deficiency anemia. Intestinal parasitic infestations interfere with oral supplementation of iodized ethyl esters, reducing absorption effectiveness. In goitrous children where the prevalence of anemia is high, encapsulated Fe added to iodized salt improves the efficacy of iodine. Deworming and adequate supplements of iron and iodine have potentially beneficial effects on the health of schoolchildren.

Abbreviations

ACC/SCN	Administrative Committee on Coordination/Sub-Committee on Nutrition of the United Nations
AOR	Adjusted odds ratio

CEE/CIS	Central and Eastern Europe/ Commonwealth of Independent States
CI	Confident interval
DAT	Direct agglutination test
DEC	Diethylcarmazine
ELISA	Enzyme-linked immunosorbent assay
FT ₄ and FT ₃	Free thyroid hormones
Hb	Hemoglobin
ICCIDD	International Council for Control of Iodine Deficiency Disorders
ID	Iron deficiency
IDA	Iron deficiency anemia
IDD	Iodine deficiency disorders
IFPRI	International Food Policy Research Institute
IgA and IgM	Immunoglobulin A and M
MCHC	Mean corpuscular hemoglobin concentration
NCHS	National Center for Health Statistics
rT ₃	Reverse triiodothyronine
T ₃	Triiodothyronine
T ₃ RU	Triiodothyronine resin uptake
T ₄	Thyroxine
TPO	Thyroid peroxidase
TSH	Thyrotropin (thyroid-stimulating hormone)
UNICEF	United Nations Children's Fund
WHO	World Health Organization

Introduction

Maternal micronutrient nutrition is an important determinant of fetus size and body composition. Iron (Fe), iodine, calcium, folate, vitamin A and vitamin C all influence the

offspring size. A study by Yajnik (2002), designed to examine the relationship between maternal nutrition, fetal size at birth and postnatal growth, showed that maternal circulating folate and vitamin C concentrations predicted larger offspring size, while higher ferritin levels predicted smaller-sized babies. Nutritional deficiencies of micronutrients can also affect development throughout childhood. Some stages are more vulnerable than others, depending on the particular nutritional deficiency. Due to iodine deficiency during pregnancy, the intrauterine period is the most vulnerable to long-term neurological and cognitive function deficits. The subsequent postnatal period (infants) is also vulnerable to this deficiency, as well as to iron deficiency (ID) (Grantham-McGregor and Ani, 2001). It is well-known that iodine deficiency and ID early in development influence children's present and future quality of life. The shortage of these essential nutrients, its duration and severity may alter the outcome. In less industrialized countries, where the prevalence of these deficiencies is still considerably high, social and economic development is also likely to be affected (Grantham-McGregor and Ani, 2002).

In an extensive revision of micronutrient deficiencies and cognitive functioning, Black (2003) looked for the relationship between four micronutrient deficiencies (iodine, iron, zinc and vitamin B-12) and children's cognitive functioning. He concluded that iodine deficiency during pregnancy has negative and irreversible effects on the developing fetus. Although there is some evidence that postnatal iodine deficiency is associated with cognitive deficits, the findings are controversial. For instance, ID is widespread and has been associated with cognitive deficits, but the results of prevention trials are inconsistent. Among the most vulnerable children, zinc deficiency has also been linked to low activity and depressed motor development. Its relation to cognitive development is less clear, and may be limited to specific neuropsychological processes. In addition, vitamin B-12 deficiency has been linked to cognitive problems among the elderly, but little is known about its effect on children's cognitive functioning. Vitamin B-12 deficiency rates are likely to be high,

because animal products are the only source of vitamin B-12. In conclusion, although micronutrient deficiencies often coexist with poverty, little is known about the impact of multiple micronutrient deficiencies on cognitive development.

However, with the exception of one study (Idjradinata and Pollit, 1993), several reports have shown that children with iron deficiency anemia (IDA) in the first 2 years of life have different cognitive disorders (Table 52.1). It was distressing to find that, in Grantham-McGregor and Ani's revision (2002), anemic children less than 2 years old failed to show benefits in their development when given Fe supplements.

The Challenge

The past decade has seen dramatic progress in the campaign to eliminate iodine deficiency – the world's leading cause of preventable mental retardation and impaired psychomotor development in young children. In its most extreme form, iodine deficiency causes cretinism. It also significantly raises the risks of stillbirth and miscarriage for pregnant women. Most commonly and visibly associated with goiter, iodine deficiency takes its greatest toll in impaired mental growth and development, contributing, in turn, to poor school performance, reduced intellectual ability and impaired work performance (UNICEF, 2006). According to a WHO report in 1993, 1.6 billion people lived in areas of iodine deficiency and 20% of them had goiter (Grantham-McGregor and Ani 2002; WHO/UNICEF/ICCIDD, 1993). Grantham-McGregor and Ani (2002) recognized that enormous progress had been made in combating iodine deficiency; they estimated that, in countries at risk, 68% of the population had access to iodized salt. Many studies comparing children living in iodine-deficient areas with those living in non-deficient areas have shown that iodine-deficient children suffer from impoverished cognition and school performance (Grantham-McGregor and Ani, 2002; Fernald and Grantham-McGregor, 1998; Huda *et al.*, 1999). Although studies of iodine supplementation in pregnant women clearly show that deficiency *in utero* causes long-term neurological and cognitive deficits, it is unclear whether exposure to iodine deficiency after birth has cognitive effects (Grantham-McGregor and Ani, 2002). Globally, about 740 million people are affected by goiter, and more than 2 billion (or over 38% of the population) in 130 countries are likely to be at risk for iodine deficiency disorders (IDD). An estimated 43 million are affected by some degree of brain damage due to inadequate intake before or during infancy and early childhood – the consequence of living in mountainous or flood-plain regions where erosion causes local soil and crops to contain insufficient iodine for healthy thyroid function (UNICEF, 2006; ACC/SNC, 2000).

The preferred strategy being used to eliminate IDD as a public health problem is salt iodization; universal

Table 52.1 Iron deficiency anemia and cognitive development

Cognitive disorders	Other mental deficits
Poor mental and motor development	Between 11 and 14 years
Poor school achievement	Writing
Altered behavior	Reading
Other altered functions	Arithmetic
Preschool skills	Motor skills
Fine and gross integration	Spatial memory
Language	Selective attention
Intelligence quotient	–

Source: Hurtado *et al.* (1999), Lozoff *et al.* (1991), Palti *et al.* (1985), Grantham-McGregor and Ani (2002).

iodization by the year 2005 had been set as a global target. While other foodstuffs can be iodized, salt has the advantage of being widely available and inexpensive to iodize. Salt has been routinely iodized in some industrialized countries since 1920. In the early 1990s only around 20% of households consumed enough iodized salt. Today, in the less industrialized world, more than two-thirds (69%) of households effectively consume iodized salt, and 82 million newborns are now being protected from learning disabilities caused by IDD. The highest levels of iodization are found in Latin America and the Caribbean (86%) and East Asia and the Pacific (85%), whereas the lowest levels are found in Central and Eastern Europe and the Commonwealth of Independent States (CEE/CIS) (47%). In Sub-Saharan Africa almost two-thirds of households consume sufficient iodized salt (64%). Despite such impressive progress in the developing world, there still remain 33 countries where less than half of the households consume adequate salt, and every year 37 million newborns in the developing world are vulnerable to iodine deficiency (UNICEF, 2006).

Iodine Deficiency

Particularly important to human development, iodine, an essential trace element, is a component of the thyroid thyroxine (T_4) and triiodothyronine (T_3) hormones. In the first decade of the twentieth century, iodine deficiency's connection to goiter was defined, and successful goiter prevention with iodine supplementation was recorded in Ohio, USA, and Switzerland (Hetzel, 1991). However, in the last part of the twentieth century, endemic cretinism was rediscovered in remote areas of the world, predominantly mountainous areas such as the Himalayas (India, Nepal, Burma) and the Andes (Peru, Chile, Bolivia, Ecuador), and in Indonesia, New Guinea and Africa (Zaire), as well as in the less developed areas of southern Europe and China. Consequently, the concept of iodine deficiency and its effects has undergone rapid changes in recent years. Originally, the problem was designated "goiter"; more recent research has defined it as the considerable impact of iodine deficiency on brain development and function (Hetzel, 1991). The worldwide consequences of iodine deficiency are fetal and infant mortality, brain damage, irreversible mental and neurological retardation, deafness and reproductive complications: first trimester abortions and gestational hypertension (Dunn, 2005). Today, a major international public health objective (Dunn, 2005) is the use of iodized salt in the correction of IDD. Most iodine-deficient countries have legislation and programs to iodize salt, but they are not as advanced in their iodine nutrition monitoring or in their plans to sustain iodine sufficiency once achieved. However, as happened in Guatemala, complacency after a successful iodization program invites failure (Dunn, 2005).

In Mexico, Villalpando *et al.* (2003) mentioned that information from the 1999 Mexican National Nutrition Survey showed that iodine deficiency prevalence was negligible in both women and children. They also emphasized that abnormal distribution results were so scattered that no observation could be made regarding geographical distribution. On the contrary, in the same survey, ID in preschoolers between 12 and 24 months was as high as 67%, 50% of which were anemia related, whereas in schoolchildren it was around 35%.

Two research studies from the 1990s presented another perspective on iodine deficiency in Mexico. In 1997, the first study was carried out by Vásquez-Garibay *et al.* (2002) in Arandas, a medium-sized town in Jalisco State (northwest of Mexico City). It included 131 children, 1–10 years of age. In this group they found that 29% had iodine deficiency (urinary excretion $<100\mu\text{g I/l}$) and 30% had moderate ($20\text{--}49\mu\text{g I/l}$) to severe (6%) iodine deficiency ($<20\mu\text{g I/l}$); ID was present in 34% (Ferritin $<10\text{ ng/ml}$) and parasitosis in 47% (mainly *Entamoeba histolytica* and *Giardia lamblia*). In 1999, the second study, implemented by Martínez-Salgado *et al.* (2002), evaluated the iodine status of 300 newborns, 300 schoolchildren and 300 pregnant women in three Hidalgo State districts. They also found goiter in a significant number of children and pregnant women from different districts in the state of Hidalgo. Simultaneously, with the same group, 936 interviews showed that only 50% of salt consumption was adequately iodized ($>50\text{ ppm}$). During the same period, Mexico's Secretary of Health's Epidemiological Bulletin informed us that in the 2003 National Survey, 1240 new cases of goiter had been found in the country (Martínez *et al.*, 2005) (Table 52.2).

Feto-Maternal Repercussions of Iodine Deficiency during Pregnancy and the Immediate Postnatal Period

The major change associated with pregnancy in thyroid function is increased thyroid hormone requirements (Glinöer, 1993). This can only be met through a proportional increase in hormone production, which is directly dependent on dietary iodine availability. When iodine intake is adequate, normal "physiological" adaptation occurs. When intake is restricted, physiological adaptation is progressively replaced by pathological changes, corresponding to the degree of iodine deprivation that leads to excessive glandular stimulation, hypothyroxinemia and goiter formation. As a result, pregnancy reveals underlying iodine restriction and gestation results in an iodine-deficient status, even with moderate iodine intake, a condition typical of many European regions. Iodine deficiency during pregnancy has important repercussions for both mother and fetus, namely thyroid malfunction

Table 52.2 Iodine deficiency and goiter in Mexico^a

State and district	Iodine deficiency		Goiter in children ^b (n = 673)		Goiter in pregnant women (n = 300)	
	N	%	N	%	N	%
Jalisco	–	–	–	–	–	–
Arandas ^c	131	–	–	–	–	–
Normal	93	71	–	–	–	–
Deficient	38	29	–	–	–	–
Mild	24	64	–	–	–	–
Moderate	11	30	–	–	–	–
Severe	6	6	–	–	–	–
Total	131	100	–	–	–	–
Hidalgo	–	–	–	–	–	–
Normal	–	–	464	69	27	9
Goiter	–	–	209	31	273	91
Huejutla	–	–	94	14	156	52
Ixmiquilpan	–	–	61	9	60	20
Pachuca	–	–	54	8	57	19
Total	–	–	673	100	300	100

^aSecretary of Health in Mexico, 2003: 1240 new cases of goiter, 50% from five states: Jalisco (273), Mexico City (120), Tabasco (112), Sinaloa (81), and Chihuahua (49) (Martínez *et al.*, 2005).

^b6–14-year-olds.

^c1–10-year-olds.

Table 52.3 Special features of iodine deficiency related to fetal and immediate postnatal growth

Nutritional deficiencies are important determining factors for fetal growth, body composition and childhood development.

Some stages are more vulnerable than others.

The most vulnerable stages may differ according to the particular nutritional deficiencies.

The intrauterine period is the most vulnerable for long-term neurological and cognitive deficit caused by iodine deficiency.

Along with iron deficiency, the immediate postnatal period (infants) is probably the most vulnerable to iodine deficiency.

The risk of abnormal development is further aggravated because mother and offspring are exposed to iodine deficiency during gestation and the postnatal period.

and goitrogenesis. Furthermore, iodine deficiency might be associated with psychoneuro-intellectual alterations in offspring (Table 52.3).

The risk of abnormal child development is greater during gestation and the postnatal period, because mother and offspring have been exposed to iodine deficiency. Because iodine deficiency is still prevalent in many European regions and remains a subject of great concern, investigators have been proposing for years that iodine prophylaxis must be systematically introduced during pregnancy to provide mothers with adequate iodine. In areas with severe iodine deficiency, correcting the lack of iodine has proven to be highly beneficial in preventing mental deficiency disorders. Actions undertaken to eradicate severe iodine deficiency have allowed us to prevent mental retardation in millions of young infants throughout the world. In most public health

programs, iodized salt has been used as the preferred strategy to correct IDD and get iodine supplements into the household. Because it is necessary to limit salt intake, iodizing salt is not the best solution in the case of pregnancy, breastfeeding, or young infants. In our country, careful attention is needed to ensure that pregnant women have an adequate iodine intake and that they be given multivitamin tablets containing iodide supplements (+125 µg/d). Finally, a cause for great concern are the results of a nutritional survey in the United States that recently disclosed that iodine deficiency, which was long thought to have been eradicated, may actually be undergoing a resurgence, particularly in women of childbearing age (Glinoe, 1993). This issue should be seriously considered by the medical community and public health authorities.

Fe Status and Thyroid Metabolism

Wolde-Gebriel *et al.* (1993) studied a total of 14740 school-children for the prevalence of goiter, xerophthalmia and anemia in seven provinces of Shoa's Administrative Region in Central Ethiopia. Hemoglobin (Hb) and packed cell volume were assessed in 966 children in one province, while an in-depth study was conducted on 344 children in the same province along with two others. In the children, goiter, xerophthalmia (Bitot's spots) and clinical anemia were observed in 34.2%, 0.91%, and 18.6%, respectively. Most biochemical variables were within the normal range, while Hb, mean corpuscular Hb concentration (MCHC) and urinary iodine excretion were below the range; mean corpuscular volume, mean corpuscular Hb (MCH) and immunoglobulins G and M were higher. Total and free thyroxine

and T_3 were positively correlated, as were total and free hormone concentrations. Thyrotropin (TSH) was negatively correlated with total and free thyroxine and positively correlated with free triiodothyronine. T_4 and T_3 , in both free and combined forms, were all correlated with T_4 -binding globulin, which in turn was negatively correlated with the triad: retinol, retinol-binding protein and transthyretin. Urinary iodine excretion was positively associated with total thyroxine and negatively associated with TSH. The anemia found was not nutritional in origin, but was due to the effect of intestinal parasite infestation and malaria.

More recently, Eftekhari *et al.* (2006) investigated the relationship between Fe status and thyroid hormone concentration in Fe-deficient adolescent Iranian girls. Considering that extensive data from animal and human studies indicate that ID impairs thyroid metabolism, a stepwise designed random sampling study was carried out in public high schools for girls in Lar and its vicinity in southern Iran. One hundred and three out of 431 Fe-deficient subjects were selected. Urine and serum samples were collected and assayed for urinary iodine and serum ferritin, Fe, total iron binding capacity (TIBC), thyroid-stimulating hormone (TSH), T_4 , T_3 , free thyroid hormones (fT_4 and fT_3), triiodothyronine resin uptake (T_3RU), reverse triiodothyronine (rT_3), selenium and albumin concentrations. Hematological indices for Fe status confirmed that all subjects were Fe deficient. There was a significant correlation between T_4 and ferritin ($r = 0.52$, $P < 0.001$), and between TSH and ferritin ($r = -0.3$, $P < 0.05$). Subjects with low serum ferritin had a higher ratio of T_3/T_4 ($r = -0.42$, $P < 0.01$). Using stepwise regression analysis, only ferritin contributed significantly to rT_3 concentration ($r = -0.35$, $P < 0.01$). Their results seemed to indicate that the degree of ID might affect thyroid hormone status in Fe-deficient adolescent girls.

It has also been mentioned that salt iodization efficacy, the preferred strategy for IDD, might be influenced by ID, because this disorder impairs thyroid metabolism (WHO, 2001, Zimmermann *et al.*, 2000). It has been observed that two initial steps in thyroid hormone synthesis are catalyzed by Fe-dependent thyroid peroxidase (TPO). One Fe-deficiency anemia study in rats showed reduced TPO activity, and decreased plasma T_4 and triiodothyronine (T_3) levels (Hess *et al.*, 2002). IDA may also alter the central nervous system's thyroid metabolism control, and reduce T_4 to T_3 peripheral conversion (Beard *et al.*, 1998), modify nuclear T_3 binding (Smith *et al.*, 1994), and increase circulating thyrotropin (TSH) (Beard *et al.*, 1990). The therapeutic response to oral iodized oil is impaired in children with IDA compared with Fe-sufficient children (Zimmermann *et al.*, 2000).

Yavuz *et al.* (2004) investigated the relationship between Fe status and thyroid hormones in adolescents living in an iodine-deficient area. The purpose was to evaluate the effect of Fe status on the thyroid hormone profile in adolescents living in a mildly iodine-deficient area in Turkey.

Table 52.4 Effects of iron deficiency on iodine deficiency disorders

Iron deficiency and IDA are estimated to affect about half of the world's population, and young children are among the most severely affected.

Iron deficiency adversely affects thyroid metabolism by reducing iodine prophylaxis efficacy in regions with endemic goiter.

The degree of iron deficiency may affect thyroid hormone status.

The therapeutic response in goitrous children to oral iodized oil is weakened when compared to iron-sufficient children.

In anemic pregnant women endemic goiter may aggravate anemia.

Chronic iodine deficiency favors subclinical hypothyroidism where, in the presence of anemia, severity increases, more so if anemia is paralleled by goiter.

They included 330 school-aged children with a mean age of 14. Free triiodothyronine, free tetraiodothyronine and thyrotropin levels, Hb and mean corpuscular volume, Fe and total Fe-binding capacity concentration and ferritin levels were determined. They found that thyroid hormone levels of the children with anemia were not significantly different compared to children without anemia. No significant correlation was found between thyroid hormones and Fe status, and they concluded that the thyroid hormone profile was not significantly affected. As a result, this study showed no correlation between Fe status and thyroid hormone levels.

Under goiter endemic conditions, Zel'tser *et al.* (1994) reviewed thyroid status in anemic pregnant women. They pointed out that while anemia is a highly prevalent condition among pregnant women in the Republic of Kazakhstan, neither its causes nor its resistance to therapy with Fe preparations is clear. Since studies tentatively indicate a relationship between endemic goiter and hypothyroidism, this may explain why many regions in the Republic are foci of endemic goiter. Therefore, in the town of Alma-Ata, a goiter-endemic region, they performed a study aimed at examining thyroid status in pregnant women suffering from anemia. Altogether 120 anemic, pregnant women were examined – 60 with goiter and 60 without. The control group consisted of 20 healthy pregnant women. Clinical and ultrasonic examinations, thyroid puncture biopsy, TSH blood level measurements, total and free T_3 and T_4 , thyroxine-binding globulin, as well as peripheral red blood cell counts, levels of hemoglobin, serum Fe, and total Fe-binding capacity of serum and saturation coefficient were assessed. Healthy pregnant women from a focal point of endemic goiter were found to be at risk for anemia in the third trimester of pregnancy. They concluded that, in anemic pregnant women, endemic goiter aggravates anemia and chronic iodine deficiency promotes subclinical hypothyroidism, while in the presence of anemia its severity increases, more so if anemia is present along with goiter (Table 52.4).

Iodine Deficiency and Parasitosis

(Table 52.5)

One study by Singh *et al.* (1994) in a residential school in the Maharashtra tribal area investigated the association between *Toxoplasma gondii* infections with iodine deficiency. The aim was to determine the sero-prevalence and incidence of toxoplasmosis. They randomly tested 194 serum samples of students 10–18 years old ($n = 178$), their teachers ($n = 10$) and food handlers ($n = 6$) from a residential tribal school situated in Dhule, Maharashtra, west India. Samples were determined for anti-toxoplasma IgG and IgM antibodies using enzyme-linked immunosorbent assay (ELISA). Active infection was confirmed by a stage-specific direct agglutination test (DAT) using *T. gondii* acetone fixed tachyzoites. Their results detected toxoplasma sero-prevalence in 20% of the 10-year-old children. Toxoplasma infection was prevalent in 42.8% of 18-year-old adolescents. The average sero-prevalence toxoplasma infection rate was not significantly different in male or female children. The toxoplasma infection incidence was inversely related to increased age. The school staff, made up of teachers and food handlers (aged 27–45 years), showed a

toxoplasma sero-prevalence rate of 75% ($P < 0.001$). There was also a significant difference ($P < 0.05$) in the prevalence of toxoplasmosis in children with grade II (46.1%) and grade I goiter or no goiter (31.8 and 26.5%, respectively). Consequently a possible relationship between iodine deficiency and toxoplasma infection was found.

Fe Deficiency and Parasitosis

Two of the most common conditions afflicting children in less industrialized countries are ID and helminth infestation. ID and IDA are estimated to affect about half of the world's population (ACC/SNC, 2000), and young children are among the most severely stricken (Stoltzfus *et al.*, 2004). As mentioned above, evidence implicating that IDA adversely affects brain development is mounting (Pinerio *et al.*, 2000; Nelson *et al.*, 1997), and that it has measurable effects on children's behavior, motor development, and cognition (Lozoff *et al.*, 1988; De Andraca *et al.*, 1997; Idjradinata and Pollit, 1993). However, neither the consequences of parasitic infection in young children have been well described, nor has the effectiveness of low

Table 52.5 Principal parasites associated to iodine and/or iron deficiency^{a,b}

Ascaris lumbricoides is the largest nematode (roundworm) parasitizing the human intestine (adult females: 20–35 cm; adult male: 15–30 cm.). It is the most common human helminthic infection with worldwide distribution. Highest prevalence is in tropical and subtropical regions, and in areas with inadequate sanitation.

Hookworms: *Ancylostoma duodenale*; *Necator americanus* The human hookworms include two nematode (roundworm) species – *Ancylostoma duodenale* and *Necator americanus* (adult females: 10–13 mm (*A. duodenale*), 9–11 mm (*N. americanus*); adult males: 8–11 mm (*A. duodenale*), 7–9 mm (*N. americanus*). A smaller group of hookworms infecting animals can invade and parasitize humans (*A. ceylanicum*) or can penetrate the human skin (causing cutaneous larva migrans), but do not develop any further (*A. braziliense*, *A. caninum*, *Uncinaria stenocephala*). Occasionally *A. caninum* larva may migrate to the human intestine causing eosinophilic enteritis; this may happen when larva is ingested rather than through skin invasion. It is the second most common human helminthic infection (after ascariasis). Worldwide distribution, mostly in areas with moist, warm climate. Both *N. americanus* and *A. duodenale* are found in Africa, Asia, and the Americas. *N. americanus* predominates in the Americas and Australia, while only *A. duodenale* is found in the Middle East, North Africa, and southern Europe.

Trichuris trichiuria The nematode (roundworm) *Trichuris trichiura*, also called the human whipworm is the third most common roundworm of humans. Worldwide, with infections more frequent in areas with tropical weather and poor sanitation practices, and among children. It is estimated that 800 million people are infected worldwide.

Entamoeba histolytica Several protozoan species in the genus *Entamoeba* infect humans, but not all of them are associated with disease. *Entamoeba histolytica* is well-recognized as a pathogenic amoeba, associated with intestinal and extraintestinal infections. The other species are important because they may be confused with *E. histolytica* in diagnostic investigations worldwide, with higher incidence of amebiasis in developing countries. In industrialized countries, risk groups include male homosexuals, travelers and recent immigrants and institutionalized populations.

Giardia lamblia *Giardia intestinalis* is a protozoan flagellate (*Diplomonadida*). This protozoan was initially named *Cercomonas intestinalis* by Lambl in 1859 and renamed *Giardia lamblia* by Stiles in 1915, in honor of Professor A. Giard of Paris and Dr. F. Lambl of Prague. However, many consider the name, *Giardia intestinalis*, to be the correct name for this protozoan. The International Commission on Zoological Nomenclature is reviewing this issue worldwide. It is more prevalent in warm climates, and in children.

Schistosomiasis is caused by digenetic blood trematodes. The three main species infecting humans are *Schistosoma haematobium*, *S. japonicum*, and *Schistosoma mansoni*. Two other species, more localized geographically, are *Schistosoma mekongi* and *S. intercalatum*. In addition, other species of schistosomes, which parasitize birds and mammals, can cause cercarial dermatitis in humans. *S. mansoni* is found in parts of South America and the Caribbean, Africa and the Middle East; *S. haematobium* in Africa and the Middle East; and *S. japonicum* in the Far East. *S. mekongi* and *S. intercalatum* are found locally in southeast Asia and central west Africa, respectively.

^aOther parasites related to IDD: *Toxoplasma gondii*, *Taenia* species, *Hymenolepis nana*, Malaria, *Wuchereria bancrofti* microfilaria.

^bhttp://www.dpd.cdc.gov/dpdx/HTML/Search_Choices.htm

doses of Fe supplementation in malaria-endemic settings been well-documented. Infections caused by parasites such as *Ascaris lumbricoides* (Figure 52.1), *Trichuris trichiuria* (Figure 52.2) and hookworms (Figure 52.3) are the most common, chronic, subclinical, childhood infections with widespread global geographic distribution (Stoltzfus *et al.*, 2004). Studies have found that anthelmintic treatment of schoolchildren has improved Fe status, growth and cognition, even though study results were not entirely consistent (Dikson *et al.*, 2000). This inconsistency is not surprising, given the diversity of ages, nutritional risk, helminth species and helminth transmission intensity in the populations studied. The health consequences of helminth infestation in preschool children have been studied less frequently, because the worm burden in young children is not as great as in schoolchildren and it is assumed to be less detrimental. However, young children are at much greater risk for IDA and growth retardation than their school counterparts (Stoltzfus *et al.*, 2004).

In a community-based sample of 459 Zanzibari children, 6–71 months old with Hb > 70 g/l at baseline, Stoltzfus *et al.* (2004) conducted a 12-month randomized, placebo-controlled double-blind trial with 10 mg of daily Fe and/or mebendazole (500 mg) every 3 months. This study was designed to examine the treatment's effect on growth, anemia

and appetite in two subgroups categorized by age. Fe did not affect growth retardation, Hb concentration, mild or moderate anemia (hemoglobin <10 g/l or <90 g/l, respectively), but did significantly improve serum ferritin and erythrocyte protoporphyrin. Mebendazole significantly reduced wasting malnutrition, but only in children <30 months old. The adjusted odds ratios (AORs) for mebendazole in this age group were 0.38 (95% CI: 0.16, 0.90) for weight/height index less than -1 Z-score and 0.29 (0.09, 0.91) for small-arm circumference. In children <24 months old, mebendazole also reduced moderate anemia [AOR: 0.41 (0.18, 0.94)]. According to the mothers' report, both Fe and mebendazole improved children's appetite. Fe's effect on anemia was limited, likely constrained by infection, inflammation and perhaps other nutrient deficiencies. In very young children with light infestations, mebendazole treatment caused unexpected and significant reduction in wasting malnutrition and anemia. Their hypothesis was that the incidence of helminth infestations might stimulate inflammatory immune responses in young children, with deleterious effects on protein metabolism and erythropoiesis (Stoltzfus *et al.*, 2004). There were unexpected benefits of anthelmintic treatment in very young African children, and Fe supplementation prevented severe anemia, but did not reduce mild or moderate anemia. Therefore, anemia control in this population clearly

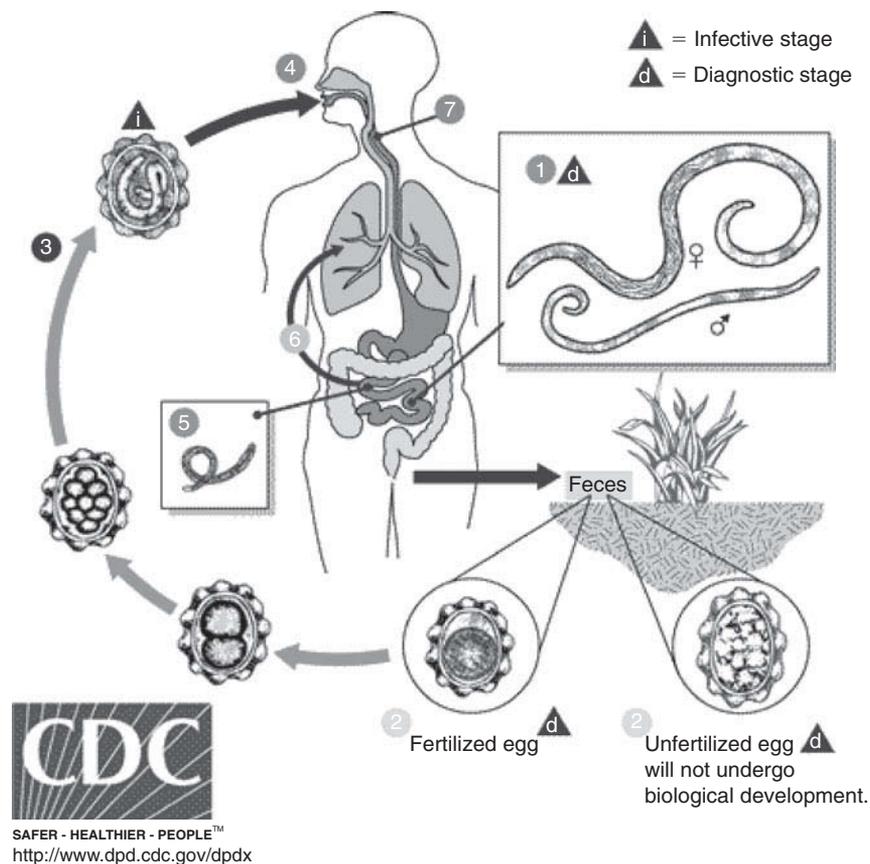


Figure 52.1 Life cycle of *Ascaris lumbricoides*.

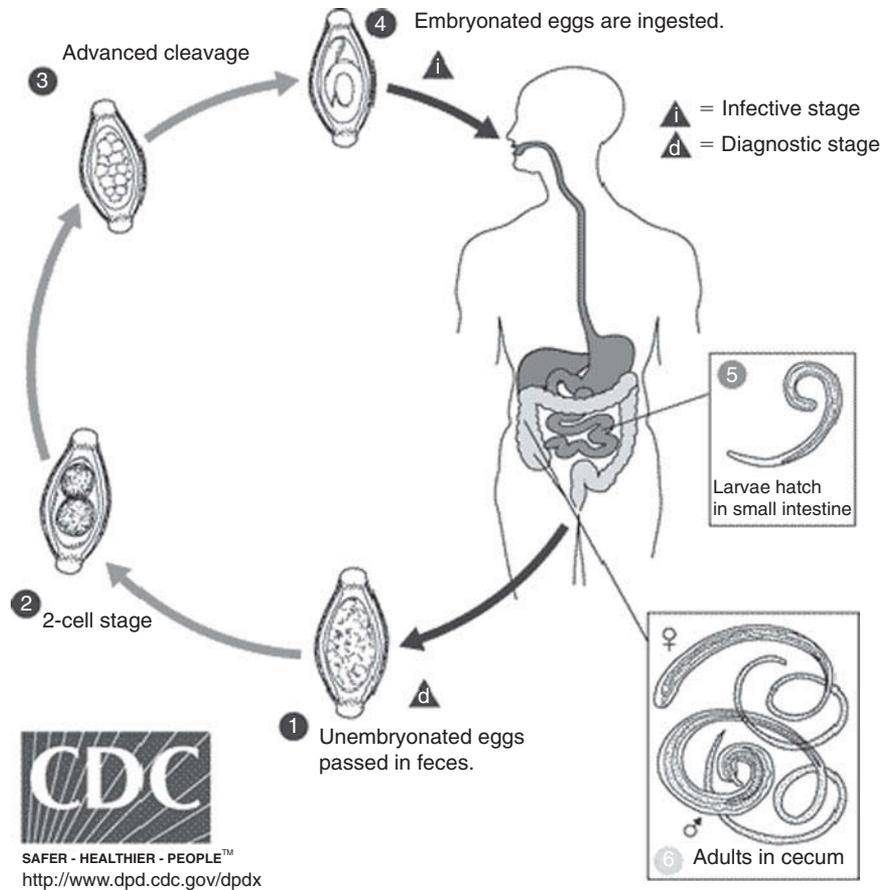


Figure 52.2 Life cycle of *Trichuris trichiura*.

requires a more comprehensive intervention than only a low dose of daily Fe could offer. The reduction of mild wasting malnutrition and anemia that was observed after mebendazole treatment was sizeable and potentially important to the development and survival of these children. If this is true, helminth control programs currently targeting schoolchildren should be extended to include preschool children.

Another study explored the intestinal nematodes' epidemiology in preschool children and the contribution of hookworm infestation to the etiology and severity of anemia. It included 460 preschool children with severe malaria that were part of a larger case-control study in Kilifi on the Kenyan coast (Brooker *et al.*, 1999). Almost one-third (28.7%) of the children studied were infested with hookworm, 20.2% with *A. lumbricoides* and 15.0% with *T. trichiura*. Each species' infestation prevalence rose with age. Serious hookworm infestation occurrence and its mean intensity were markedly age-dependent. One-third (34.3%) of the children were infected by malaria. Overall, 76.3% of children were anemic (hemoglobin <10 g/l), and prevalence decreased with age. Anemia was significantly worse in children with severe hookworm infection (>200 eggs per gram). This relationship, independent of socioeconomic factors, was true for all ages and gender.

Another study (Ulukanligil and Seyrek, 2004) explored the anthropometric status, anemia and intestinal helminthic infections of schoolchildren living in Turkey's Sanliurfa Province. They stratified the Province's urban area based on environmental conditions: shantytowns and apartment areas. Twelve schools in shantytown areas and five in apartment areas were selected, randomly based on probable size. In each school, a third group of students (including 9–10-year-olds) were randomly selected. All children in this class participated in the survey. A total of 806 children took part in the study – 572 of them from shantytown schools and 234 from apartment schools. Height for age, weight for age and weight for height were calculated by New Anthro software using the NCHS/WHO international reference values (2000).

Evidence of chronic ill-health due to malnutrition, anemia and helminthic infections was found in shantytown schools. Both male and female children in these schools had higher stunting rates than those in apartment schools. Being underweight was significantly associated with the child's sex in shantytown schools where, notably, boys weighed less than girls ($p = 0.04$); however, the difference between the sexes was not present in apartment schoolchildren. Wasting was significantly associated with the type of settlement; girls in apartment schools had a significantly

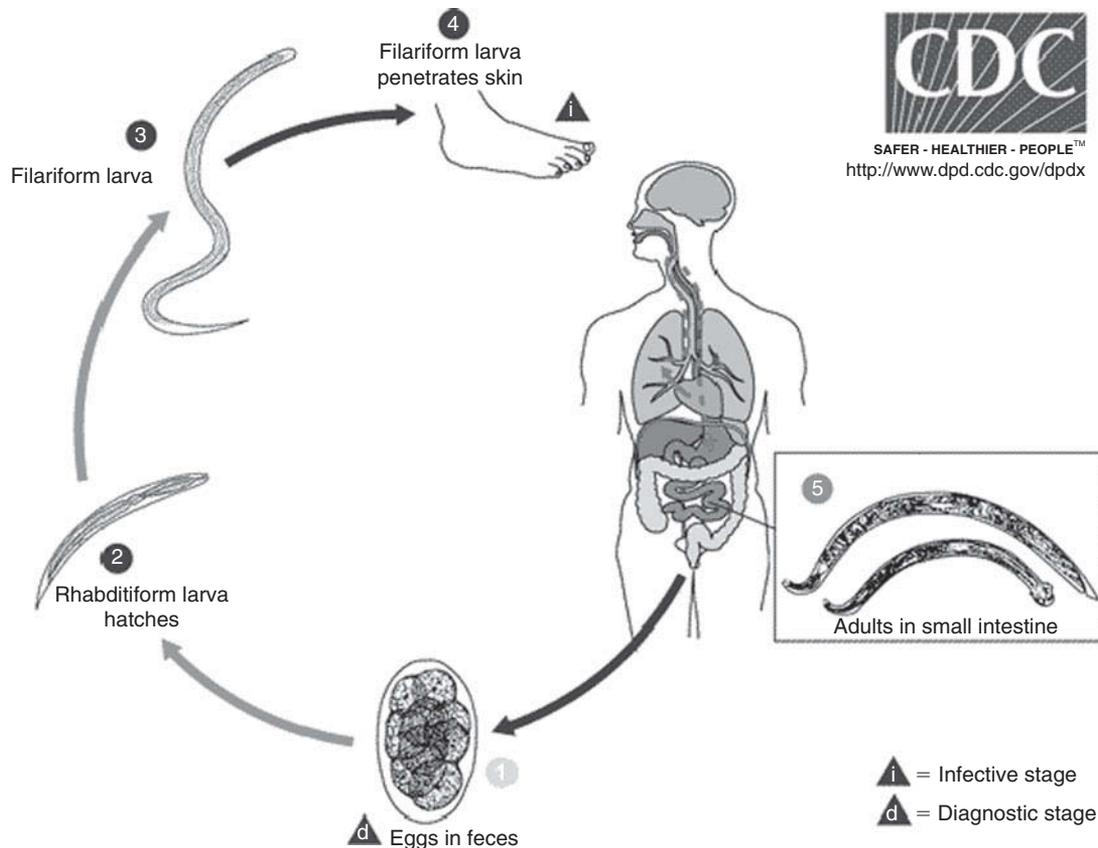


Figure 52.3 Life cycle of hookworms *Ancylostoma duodenale* and *Necator americanus*.

higher wasting rate than those in shantytown schools ($P = 0.02$). The children in shantytown schools had significantly higher anemia rates (45%) than those in apartment schools (15%) ($p = 0.01$). The prevalence of intestinal helminthic infections was significantly higher in shantytown schools (63%) than in apartment schools (37%) ($P < 0.0001$). *A. lumbricoides*, the most prevalent helminth species, was followed by *T. trichiura*, *Hymenolepis nana* and *Taenia* in both study areas. Infestation in children was significantly related to stunting in shantytown schools (multiple $R = 0.147$; $p = 0.005$) and in apartment schools (multiple $R = 0.171$; $p = 0.02$). These results indicate that the prevalence of stunting, anemia and intestinal helminth infections constitutes an important public health problem among shantytown schoolchildren. School health programs that have potentially beneficial effects on the health and education of schoolchildren include health education, deworming, feeding and micronutrient Fe supplements.

Brito *et al.* (2003) studied moderate- to low-intensity coinfections caused by intestinal helminthes and *Schistosoma mansoni* (hookworm, *T. trichiura*, *A. lumbricoides*) dietary Fe intake and anemia in Brazilian children, to determine their role in the prevalence of anemia and their relationship to Fe consumption. In rural Brazil, a cross-sectional study design with 1709 children was completed. All participants were

selected for infestation with one or multiple helminthic parasites; demographic, anthropometrics and dietary intake data were obtained. The prevalence and intensity were as follows: hookworm infestation, 15.7% and 8.6 eggs/g; *T. trichiura*, 74.8% and 190.5 eggs/g; *A. lumbricoides*, 63% and 1905.5 eggs/g; *S. mansoni*, 44.5% and 60.3 eggs/g. There was no increase in odds ratio for anemia with any combination of intestinal helminthes without *S. mansoni* infection. When infected with *S. mansoni* and two intestinal helminthes by means of logistic regression, the odds ratio for having anemia was 1.7 (95% CI, 1.1–2.5), and for *S. mansoni* and three intestinal helminthes, it was 2.4 (95% CI, 1.2–4.6) compared to children with a single parasite species. There were no increased odds of anemia in children with an adequate intake of Fe, which was independent to combined parasite infections.

Albonico *et al.* (1998) mentioned that the hookworms, *Ancylostoma duodenal* and *Necator americanus* caused significant gastrointestinal blood loss. However, they had no evidence that endemic *A. duodenal* infestation had a greater impact on the Fe status of populations than an *N. americanus* infestation. As a result, they decided to do a study with a sample of 525 schoolchildren in Pemba Island, Tanzania, and compare the degree of anemia and ID associated with the two hookworm species at the individual and community

(i.e., school) levels. Multiple regressions were used to control infection intensities and other childhood characteristics. In 492 children with hookworm, positive fecal cultures, Hb and ferritin concentrations decreased with increasing proportions of *A. duodenal*. Among children with only *N. americanus* larvae, the prevalence of anemia was 60.5% and that of ferritin $<2\mu\text{g/l}$ was 33.1%, while in children with $\geq 50\%$ *A. duodenal* larvae, the respective prevalence was 80.6% and 58.9%. When children were grouped by prevalence of *A. duodenal* at the school level, children from high-prevalence ($\geq 20\%$) schools had significantly worse ID and anemia than children from low-prevalence schools. They concluded that the species of hookworm being transmitted in a community influences the burden of Fe deficiency anemia, and should be considered when prioritizing and planning programs for hookworm and anemia control (Albonico *et al.*, 1998).

Khieu *et al.* (2006) have pointed out that according to the WHO, half of the world's children suffer from anemia – a silent, neglected and endemic disease with three major causes: Fe deficiency, intestinal worms and malaria. Consequently, inside a malaria-free area, in a rural primary school in Battambang Province, Cambodia, a 2-month cross-sectional study of anemia was conducted to find causes related to detection and prevalence, and their links to intestinal worms and malnutrition. The main objective of the study was to assess anemia's prevalence and two of

its possible driving factors: intestinal parasites and general malnutrition. A secondary objective was to assess the Hb color scale accuracy, an easy and cheap visual technique compared to using a spectrophotometer reference. Among 168 schoolchildren (average age: 11), moderate and severe anemia prevalence rates were 24%; average Hb was 12.6 g/dl. Anemia was independently associated with *Ancylostoma* carriage ($p = 0.05$) and stunting ($p = 0.01$), with a prevalence of 54% and 40%, respectively; and this occurs in spite of a 500 mg dose of mebendazole that was given 9 months prior to the study as a regular part of a school deworming program. They concluded that, although regular mass deworming in schools does not prevent early *Ancylostoma* reinfestation, it may reduce the severity of anemia, and its effects may be fortified by switching from mebendazole to albendazole. Therefore, according to these studies there are frequent associations among iodine deficiency, Fe deficiency and parasitosis through different means, mechanisms, and treatment and prevention approaches (Table 52.6).

Interaction between Fe Deficiency, Fe Supplementation and Susceptibility to Infection

Interactions between ID and Fe supplementation and their complex susceptibility to infection remain controversial. Malarious and non-malarious regions revised by

Table 52.6 Interactions among parasitosis iron and iodine deficiency disorders

The most common chronic subclinical childhood infections with global dispersion are parasites such as *A. lumbricoides*, *T. trichiuria* and hookworms.

The iron deficiency and helminth infestation are two of the most common conditions afflicting children in less industrialized countries.

Hookworm species being transmitted in a community influence the burden of iron deficiency anemia and should be considered when prioritizing and planning programs for hookworm and anemia control.

By interfering with absorption, intestinal parasitic infestation reduces the efficacy of oral supplementation with iodized ethyl esters.

There is a significant elevation in toxoplasmosis prevalence in children with grade II goiter and a mild elevation in those with grade I goiter.

A possible relationship exists between iodine deficiency and toxoplasma infection.

Chronic iodine deficiency favors subclinical hypothyroidism where, in the presence of anemia, severity increases; more so if anemia is paralleled by goiter.

Table 52.7 Iron deficiency, iron supplementation and susceptibility to infection

Remarks and recommendations

Comprehension about the interaction between iron and infection is incomplete.

Parenteral iron is contraindicated in newborns of both non-malarious and malarious areas

Treatment of anemia in malarious areas:

Oral iron supplementation may carry an increased risk for clinical malaria if given in doses greater than mg/kg/d during times of malaria transmission.

Iron therapy for anemia should be covered or preceded by effective anti-malarial therapy.

Iron treatment should be oral

Lower dosage should be used where risk factors are present

Source: Oppenheimer (2002).

Table 52.8 Magnitude and global prevention approach to iodine deficiency disorders

Globally, about 740 million people are affected by goiter, and more than 2 billion living in 130 countries are estimated to be at risk for iodine deficiency disorders (IDD).

Iodine deficiency is the world's leading cause of preventable mental retardation and impaired psychomotor development in young children. In its extreme form, iodine deficiency causes cretinism.

Iodine deficiency may be associated to alterations in the progeny's psychoneuro-intellectual, developmental prognosis.

Salt iodization is the preferred strategy in eliminating IDD as a public health problem, and universal iodization is the target for the beginning of the twenty-first century.

The addition of encapsulated Fe to iodized salt improves the effectiveness of iodine in goitrous children where the prevalence of anemia is high.

School health programs that include deworming, feeding, giving an adequate supply of iron and iodine supplements, as well as health education, all have potentially beneficial effects on the health and education of schoolchildren.

Oppenheimer (2002) revealed the following conclusions and recommendations described in Table 52.7.

Iodine and Fe Fortification and Parasitosis

Freeman *et al.* (2001) evaluated the effectiveness of salt fortified with diethylcarbamazine (DEC) and iodine to eliminate Bancroftian filariasis and iodine deficiency. During this 1-year period all consenting residents of Mito, Haiti ($n = 1932$) were given salt fortified with 0.25% DEC and 25 ppm of iodine. *Wuchereria bancrofti* microfilaria prevalence and intensity, antigenemia and urinary iodine were measured before and after the first year salt distribution began. To measure the effect of DEC-fortified salt on adult worm motility, 15 microfilaria-positive men were examined with a scrotal area ultrasound. Entomologic surveys were conducted to determine the *W. bancrofti*-infected *Culex quinquefasciatus* proportion. After one year's treatment, the prevalence and intensity of microfilaremia were reduced by more than 95%, while antigenemia levels were reduced by 60%. The motility of adult worms, detected by ultrasound, was not significantly decreased by DEC-fortified salt. The proportion of vector mosquitoes carrying infectious stage larvae decreased significantly from 2.3% in the nine months before the intervention to 0.2% in the last 3-month follow-up period. Iodine deficiency, which had been moderate to severe, was eliminated after one year of iodized salt consumption. The DEC-fortified salt was well accepted by the community and reduced microfilaremia; its low-level transmission had no reported side-effects. Based on their results, they concluded that salt co-fortified with DEC and iodine should be considered as a concurrent intervention for lymphatic filariasis and iodine deficiency elimination programs.

Zimmermann *et al.* (2002) remind us that, in less industrialized countries, children are at high risk for both goiter and anemia, and that ID adversely affects thyroid metabolism, reducing the efficacy of iodine prophylaxis in areas

of endemic goiter. As a result, they carried out a study to verify that cofortification of iodized salt with Fe would improve iodine efficacy in goitrous children with a high prevalence of anemia. This 9-month, randomized, double-blind trial included 6–15-year-old children ($n = 377$). They were given iodized salt (iodine 25 $\mu\text{g/g}$ salt) or dual-fortified salt with iodine (iodine 25 $\mu\text{g/g}$ salt) and Fe (1 mg Fe/g salt, as ferrous sulfate microencapsulated with partially hydrogenated vegetable oil). The dual-fortified salt group's Hb and Fe status improved significantly ($P < 0.05$) when compared to the iodized salt group. At 40 weeks, an ultrasound measured the mean decrease in thyroid volume in the dual-fortified salt group (–38%); it was twice that of the iodized salt group (–18%) ($P < 0.01$). Compared with the iodized salt group, serum T_4 was significantly increased ($P < 0.05$) and in the dual-fortified salt group, the prevalence of hypothyroidism and goiter decreased ($P < 0.01$). They concluded that the addition of encapsulated Fe to iodized salt improves iodine efficacy in goitrous children where the prevalence of anemia is high.

In an intervention study with schoolchildren 8–10 years old in Malawi, Furnee *et al.* (1997) examined the relationship of intestinal parasite treatment and oral iodized oil efficacy. Severely iodine-deficient schoolchildren with a single parasitic infestation, either *A. lumbricoides* ($n = 44$), hookworm ($n = 42$), or *Entamoeba histolytica* ($n = 24$), were randomly allocated to receive or not receive treatment before taking a 1 ml oral supplement (490 mg Iodine) of iodized ethyl esters from poppyseed oil. After supplementation, urinary iodine concentrations were measured regularly, to define time intervals indicating moderate iodine deficiency before urinary iodine concentrations returned to 0.40 mmol/l. Treatment with metronidazole for *E. histolytica* increased the protection period from 2.0 to 21.0 weeks ($P < 0.05$). For all untreated children, the duration effect was 9.2 weeks shorter ($P < 0.001$) than for their treated peers (16.8 weeks). They concluded that, by interfering with absorption, intestinal parasitic infestations reduce the efficacy of oral supplementation with iodized ethyl esters (Table 52.8).

Summary Points

Magnitude

- Iodine deficiency is the world's leading cause of preventable mental retardation and impaired psychomotor development in young children. In its extreme form, iodine deficiency causes cretinism. Globally, about 740 million people are affected by goiter and more than 2 billion living in 130 countries are estimated to be at risk for IDD.

Fetal growth

- Nutritional deficiencies are important determining factors for fetal growth, body composition and childhood development. Some stages are more vulnerable than others, and the most vulnerable stages may differ according to particular nutritional deficiencies. The intrauterine period is the most vulnerable for long-term neurological and cognitive deficit caused by iodine deficiency.

Immediate postnatal period

- Along with Fe deficiency, the immediate postnatal period (infants) is probably the most vulnerable to iodine deficiency. Iodine deficiency may be associated with alterations in the progeny's psychoneuro-intellectual, developmental prognosis. The risk of abnormal development is further enhanced because mother and offspring are exposed to iodine deficiency during gestation and the postnatal period.

Fe deficiency

- ID and IDA are estimated to affect about half of the world's population, and young children are among the most severely affected. ID adversely affects thyroid metabolism by reducing iodine prophylaxis efficacy in regions with endemic goiter. The degree of ID may affect thyroid hormone status. The therapeutic response in goitrous children to oral iodized oil is weakened when compared to Fe-sufficient children. In anemic pregnant women endemic goiter may aggravate anemia. Chronic iodine deficiency favors subclinical hypothyroidism where, in the presence of anemia, severity increases, more so if anemia is paralleled by goiter.

Parasitosis

- The most common chronic subclinical childhood infections with global dispersion are parasites such as *A. lumbricoides*, *T. trichiuria*, and hookworms. ID and helminth infestation are two of the most common conditions afflicting children in less industrialized countries. There is a significant elevation in toxoplasmosis prevalence in children with grade II goiter and a mild elevation in those with grade I goiter. A possible relationship exists between iodine deficiency and toxoplasma infection. Hookworm species being transmitted in a community influence the burden of IDA and should be considered when prioritizing and

planning programs for hookworm and anemia control. By interfering with absorption, intestinal parasitic infestation reduces the efficacy of oral supplementation with iodized ethyl esters.

Prevention

- Salt iodization is the preferred strategy in eliminating IDD as a public health problem, and universal iodization is the target for the beginning of the twenty-first century. The addition of encapsulated Fe to iodized salt improves the effectiveness of iodine in goitrous children where the prevalence of anemia is high. School health programs that include deworming, feeding, giving an adequate supply of Fe and iodine supplements, as well as health education, all have potentially beneficial effects on the health and education of schoolchildren.

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Iodine Deficiency and Thyroid Cancers: Effect of Iodine Prophylaxis on Thyroid Cancer Morphology

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Abstract

Assessment of all possible changes in cancer incidence rates, particularly in sparsely populated iodine-deficient goitrous areas commonly located in remote mountainous regions, is difficult. Factors such as histological criteria for classifying thyroid cancer, pathological techniques, inclusion of papillary microcarcinomas, radioactive fallout, screening programs, and standards of medical care show significant variations with time, particularly in endemic goiter regions, before and after iodine prophylaxis. Papillary carcinoma usually forms the largest group of thyroid malignancies, both before and after iodine prophylaxis, where an increase in the papillary:follicular carcinoma ratio is also noted. Thyroid undifferentiated carcinoma and angiosarcoma decrease in postprophylaxis periods, probably related to lower serum thyrotropin (TSH), as well as to earlier detection of more differentiated precursor tumors, which would explain in part the increasing incidence of papillary carcinoma. Primary thyroid lymphoma tends to show a higher incidence after prophylaxis, probably due to an increased incidence of thyroiditis. The overall increasing incidence of thyroid cancer after iodine prophylaxis is probably related to factors other than iodine itself, one of them being early diagnoses of malignancies due to better access to medical health centers.

Abbreviations

<i>BRAF</i>	Braf oncogene
<i>FAS</i>	Fas gene
MALT	Mucosa-associated lymphoid tissue
MEN2A	Multiple endocrine neoplasia type 2A
MEN2B	Multiple endocrine neoplasia type 2B
MTC	Medullary thyroid carcinoma
<i>PAX8/PPARγ</i>	PAX8/PPAR γ genes translocation
<i>RAS</i>	Ras oncogene

<i>RET</i>	Ret oncogene
<i>TP53</i>	P53 tumor suppressor gene
<i>TRK</i>	Trk oncogene
TSH	Thyrotropin

Introduction

There are many problems in assessing possible changes in cancer incidence rates, particularly in sparsely populated iodine-deficient goitrous areas commonly located in remote mountainous regions. Factors such as standards of medical care, pathological techniques and screening programs to detect familial thyroid carcinoma (Harach, 2001; Lips *et al.*, 1994) are important considerations that may show significant variations with time, particularly in endemic goiter regions before and after iodine prophylaxis.

Pathological criteria for classifying thyroid cancer may show discrepancies in the differentiation between follicular adenoma and minimally invasive follicular carcinoma, and between follicular neoplasia and the follicular variant of papillary carcinoma (Lloyd *et al.*, 2004; Saxen *et al.*, 1978). The inclusion of papillary microcarcinomas (1.0 cm or less in diameter), which are usually incidental findings or detected by the use of ultrasound-guided thyroid fine-needle aspiration cytology (Lin *et al.*, 1997), the existence of radioactive fallout, which may lead to an increase of differentiated thyroid carcinoma and a rise in population age with time, should also be taken into account when analyzing the epidemiology of thyroid cancer.

We will analyze those thyroid cancers that were shown to have undergone changes in their incidence after the implementation of iodine prophylaxis in different endemic goiter areas, probably through the effect of dietary iodine in relation to serum thyrotropin (TSH) levels and increase of thyroiditis, as well as in relation to the standard of medical care. We will also focus on the effect of salt iodization

Table 53.1 Classification of thyroid tumors

Thyroid carcinoma
Papillary carcinoma
Follicular carcinoma
Poorly differentiated carcinoma
Undifferentiated (anaplastic) carcinoma
Squamous cell carcinoma
Mucoepidermoid carcinoma
Sclerosing mucoepidermoid carcinoma with eosinophilia
Mucinous carcinoma
Medullary carcinoma
Mixed medullary and follicular cell carcinoma
Spindle cell tumor with thymus-like differentiation
Carcinoma showing thymus-like differentiation
Thyroid adenoma and related tumors
Follicular adenoma
Hyalinizing trabecular tumor
Other thyroid tumors
Teratoma
Primary lymphoma and plasmacytoma
Ectopic thymoma
Angiosarcoma (malignant haemangioendothelioma)
Smooth muscle tumors
Peripheral nerve sheath tumors
Paraganglioma
Solitary fibrous tumor
Follicular dendritic cell tumor
Langerhans cell histiocytosis
Secondary tumors

Note: Histological classification of thyroid tumors according to the World Health Organization nomenclature (DeLellis and Williams, 2004).

particularly in the population of Salta, Argentina, through a prospective study of 46 years made by the same pathologists, and where some important bias mentioned above did not influence the diagnosis and epidemiological analysis of thyroid cancer before and after salt iodination.

General Features of Thyroid Cancer

Thyroid cancer is the most frequent endocrine gland malignancy, accounting for approximately 1% of all malignant tumors, which predominates in middle-aged females over males in a proportion of 2–4:1 and shows an incidence of 1.9–19.4 females and 0.8–5.0 males/10⁵ population/year (DeLellis and Williams, 2004); its prevalence at autopsy is up to 36%, mainly in the form of papillary microcarcinoma (1 cm or less in diameter) (Harach *et al.*, 1985a). There is no conclusive evidence regarding the existence of either a higher or lower incidence of thyroid cancer in goitrous as compared with nongoitrous areas (Williams, 1985).

The histological classification of thyroid tumors following the World Health Organization nomenclature (DeLellis *et al.*, 2004) is shown in **Table 53.1**. Thyroid cancers that were shown to change their incidence after iodine prophylaxis

Table 53.2 Thyroid cancers and iodine prophylaxis

Thyroid cancer type	Point mutation	Rearrangement
Papillary carcinoma	<i>BRAF</i>	<i>RET</i> , <i>TRK</i>
Follicular carcinoma	<i>zS</i>	<i>PAX8/PPARγ</i>
Undifferentiated carcinoma	<i>TP53</i>	
Angiosarcoma		
Primary thyroid lymphoma	<i>FAS</i>	

Note: Histology and gene mutations of thyroid cancers directly or putatively linked to iodine prophylaxis. *BRAF*, Braf oncogene; *RAS*, Ras oncogene; *FAS*, Fas gene; *TP53*, P53 tumor suppressor gene; *RET*, Ret oncogene; *TRK*, Trk oncogene; *PAX8/PPAR γ* , *PAX8/PPAR γ* genes translocation.

in goitrous regions are shown in **Table 53.2**. Among epithelial cell malignant tumors, carcinomas originating from the follicular cell are more common than those of C-cell origin, mixed tumors and other thyroid epithelial cancers. Familial medullary and nonmedullary thyroid carcinomas occur in about 25% and 5% of thyroid C-cell and follicular cell malignancies, respectively (Harach, 2001). Follicular cell malignancies have a marked variability in biological behavior, ranging from the almost always innocuous papillary microcarcinoma, to clinically significant differentiated (papillary or follicular) carcinoma usually with a good prognosis, to undifferentiated carcinoma that occurs in older people and has a dismal prognosis. Among non-epithelial thyroid malignancies, angiosarcoma (malignant hemangioendothelioma) is more frequently described in goitrous alpine regions, occurs in older people and behaves similarly to undifferentiated carcinoma. Most primary thyroid lymphomas include mucosa-associated lymphoid tissue (MALT) or extranodal marginal zone B-cell lymphoma and diffuse large-cell lymphoma, with areas of transition between the two. The rare plasmacytoma of the thyroid may represent the extreme spectrum of differentiation of these tumors. Most patients are middle-to-older-aged females with a history of Hashimoto's thyroiditis. The tumors generally present with local symptoms, have a tendency to disseminate through the gastrointestinal tract, and have a favorable outcome with appropriate therapy. Prognosis depends on histological grade and staging (Derringer *et al.*, 2000; Isaacson 1997). The pathology and clinical features of this group of thyroid tumors are supported by molecular studies that show the participation of distinct genes, generally with little overlap among the epithelial malignancies (**Table 53.2**) (DeLellis and Williams, 2004). The development of thyroid cancer may be influenced by factors that can be genetically determined, environmental in nature through genotoxic (e.g., radiation) or nongenotoxic (e.g., TSH stimulation) effects, or responsive to other biological mechanisms that would favor sporadic somatic mutation and tumor progression (**Table 53.3**).

Table 53.3 Etiology of thyroid cancer

<i>Aetiological factors</i>	<i>Type of thyroid cancer</i>
High TSH	
Low iodine intake	Follicular, angiosarcoma
Dyshormonogenesis	Follicular
High iodine intake	Papillary
Radiation	Papillary, follicular, undifferentiated, medullary
Inheritance	
Cowden disease	Follicular
Familial adenomatous polyposis	Papillary ^a
Familial papillary carcinoma	Papillary
Familial oxyphil cell carcinoma	Papillary or follicular with cell oxyphilia
Werner syndrome	Papillary, follicular
Carney complex	Papillary, follicular
Familial MTC, MEN2A, MEN2B	Medullary
Precursor follicular cell tumor	
Follicular adenoma	Follicular
Papillary or follicular carcinoma	Undifferentiated
Hypercalcaemia	Medullary
Thyroiditis	Lymphoma

Note: Aetiological factors linked to the development of thyroid cancer. MEN2A, multiple endocrine neoplasia type 2A; MEN2B, multiple endocrine neoplasia type 2B; MTC, medullary thyroid carcinoma.

^aTerm currently used for convenience (DeLellis and Williams, 2004; Harach, 2001).

Table 53.4 Papillary:follicular carcinoma ratio in endemic goiter areas before and/or after iodine prophylaxis

<i>Country</i>	<i>City/province</i>	<i>Before prophylaxis</i>	<i>After prophylaxis</i>
Switzerland	Zürich	0.19:1	1.1–1.5:1
	Berne	0.33:1	0.7:1
	Basel	0.27:1	0.8/0.7:1
Austria	Vaud		2.5:1
	Tyrol	0.2:1	0.5–4.0:1
	Carinthia		2.8–5.4:1
Finland			1.2:1
USA	Rochester		3.9:1
Argentina	Salta	1.7:1	3.1:1
India	New Delhi	1.6	
Columbia	Cali	1.3:1	
Germany	Munich	0.5:1	
	Hamburg	1.6:1	
	Lower Franconia		1.5–3.3:1
Sweden	Iodine-deficient areas		1.2:1
Italy	Northeastern Sicily	1.0:1	

Source: Farahati *et al.*, (2004), Franssila *et al.*, (1981), Gomez Segovia *et al.*, (2004), Harach *et al.*, (2002), Hedinger (1985), Hofstädter (1980), Levi *et al.*, (2002), Lind *et al.*, (2002), Pettersson *et al.*, (1996), Sarda *et al.*, (1988), Williams (1985).

Dietary Iodine and Thyroid Cancer

Papillary and follicular carcinoma

Many publications have shown the incidence of thyroid cancer and its subtypes. Showing the ratio of papillary to follicular carcinoma has some advantages as it avoids the need to have a defined population base, though it is still open to a number of biases mentioned above. The papillary:follicular carcinoma ratio shows differences in different dietary iodide groups: the range before and after iodine prophylaxis is 0.19–1.7:1 and 0.74–4.0:1 (Table 53.4), respectively, while the range in iodine-sufficient regions is 1.6–6.5:1 (Table 53.5). In general, papillary carcinoma is the predominant type of thyroid malignancy in non-endemic areas and shows an increasing incidence in goitrous regions after iodine prophylaxis. In Lower Franconia (Germany), Zürich (Switzerland), Carinthia and Tyrol (Austria), there was a progressive increase in the papillary:follicular carcinoma ratio after salt prophylaxis (Farahati *et al.*, 2004; Gomez Segovia *et al.*, 2004; Hedinger, 1985; Lind *et al.*, 2002) (Table 53.4). This could be related to either the long-term effect of iodine implementation or other factors (e.g., radiation fallout, inclusion of papillary microcarcinomas, better access to medical care, etc.), or both. Indeed, a transition from iodine sufficiency to iodine deficiency was accompanied by an increase of papillary carcinoma in Tasmania (Australia) (Burgess *et al.*, 2000), and in regions from central Italy with moderate iodine deficiency, where salt prophylaxis is as yet not widely implemented (Trimboli *et al.*, 2006). No significant changes

Table 53.5 Papillary:follicular carcinoma ratio in nonendemic goiter regions with or without iodination policy

<i>Country</i>	<i>Ratio</i>
Iceland	6.5:1
USA	
San Francisco Bay Area	6.0:1 ^a
Connecticut	3.8:1
Hawaii	3.6:1
San Francisco	3.4:1
France	3.2:1
Japan	4.5:1
Norway	3.9:1
Italy	
Catania	3.8:1
Scotland	
Aberdeen	3.7:1
Denmark	
West and East	2.1:1
Sweden	
Iodine-sufficient areas	1.8:1
England	
London	1.8:1

Source: Belfiore *et al.*, (1987), Colonna *et al.*, (2002), Ezaki *et al.*, (1992), Franssila *et al.*, (1981), Haber and Lipkovic (1970), Iribarren *et al.*, (2001), Pettersson *et al.*, (1996), Sehested *et al.*, (2006), Williams (1985), Zheng *et al.*, (1996).

^aWhite and total population.

were found in the prevalence of papillary microcarcinoma with regard to iodine intake in Hungary (Kovács *et al.*, 2005). Ras oncogene (*RAS*) mutation was found to be more frequently present in thyroid follicular carcinomas from an iodine-deficient area than in carcinomas from a region with sufficient iodine intake (Shi *et al.*, 1991).

Our hospital provides practically all the thyroid surgical service for the province of Salta, and the few private pathological laboratories that deal with thyroidectomies have also supplied the majority of their cases. The material studied would therefore, cover most cases of thyroid cancer that occurred in this region (Harach *et al.*, 2002). The most recent evaluation of the effect of iodine prophylaxis on the morphology of thyroid cancer in Salta shows that papillary carcinomas form the largest group in both periods, with a significant increase after iodization (Table 53.6). The ratio of papillary to follicular carcinoma rose from 1.7:1 to 3.2:1 (Table 53.4), in accordance with our previous observations (Harach *et al.*, 2002; Harach and Williams, 1995). The number of tumors resected rose from 3.9 per year in the first period to 15.1 in the second, but the overall incidence rate rose to a lesser extent, because of the expansion of the population from the province of Salta (Harach and Williams, 1985; Dirección General de Estadísticas, Gobierno de la Provincia de Salta, <http://www.salta.gov.ar/o/estadisticas>) (Table 53.7).

A rise in papillary carcinoma from most series is accompanied by a decrease in the incidence of follicular carcinoma, which was sometimes not as marked as in Salta (Tables 53.4 and 53.6), probably due to the use of routine systematic capsule sampling from follicular neoplasms from the 1980s leading to more frequent identification of minimally invasive follicular carcinomas (Harach *et al.*, 2002; Harach and Williams, 1995; Lang *et al.*, 1980). If this had occurred, the real change in the papillary to follicular carcinoma ratio would have been greater, but would not have affected the incidence of papillary carcinomas, as microcarcinomas were excluded. Furthermore, an increase

in the mean age of the population (Harach *et al.*, 2002) could be expected to increase the relative proportion of follicular as compared to papillary carcinoma, because of the higher average age of presentation of the former tumor (Table 53.6). No radioactive fallout would be expected in this region and there is no standardized screening for thyroid carcinoma (Harach *et al.*, 2002). Two of the 183 (1%) papillary carcinomas studied in the postprophylaxis period were associated with familial adenomatous polyposis. Aspiration cytology was implemented in 1985 in Salta (Harach, 1989), thus covering the postprophylaxis period studied. This method avoids unnecessary surgery for benign thyroid nodules, but would not influence the increment of thyroidectomies for clinically significant thyroid malignancies, since false negative results also occurred (Harach, 1989; de la Serna Saravia *et al.*, 2006). In any case, the overall incidence of thyroid cancer in the province showed a progressive rise over the last two decades, where the thyroid cancer ratio of females to males remained at about the same range over the last four decades (Table 53.7). This increase in the incidence of thyroid cancer in general, and clinically significant papillary carcinoma in particular, may well be related to better access to health centers, diagnosis of malignancies in earlier stages of differentiation and consequent decrease of undifferentiated carcinomas (Bakiri *et al.*, 1998; Harach *et al.*, 2002; Trimboli *et al.*, 2006). This contention is further supported by the fact that the crude incidence of thyroid cancer was higher in the capital city, as compared with the countryside regions of the province, and increased in both areas in the last decade of the study, as compared with the previous one (Table 53.8).

Undifferentiated (anaplastic) carcinoma

There is enough evidence that undifferentiated carcinoma arises from papillary or follicular carcinoma (Table 53.3)

Table 53.6 Clinically significant thyroid cancer and salt prophylaxis

	Thyroid cancer		Mean age	
	I Nr (%)	II Nr (%)	Females I/II	Males I/II
PC	26 (44.0) ^a	183 (63.5) ^a	37.0/42.4	42.2/45.0
FC	15 (25.4) ^c	57 (19.8) ^c	45.2/47.8	54.6/49.4
UC	10 (16.9) ^b	23 (7.9) ^b	60.0/67.4	62.0/63.7
MC	6 (10.1)	20 (6.9)	33.7/37.2	NA
TL	0	5 (1.7)	NA/74.5	NA
Total	57	345		

Note: PC, papillary carcinoma; FC, follicular carcinoma; UC, undifferentiated carcinoma; MC, medullary carcinomas; TL, thyroid lymphoma; NA, not applicable (TL: none in males and before salt iodination, MC: two and three cases before and after prophylaxis, respectively). Thyroid cancer by type, mean age and gender before (1958–1972) (I); and after (1985–2003) (II); salt iodization in the province of Salta, Argentina.

^a $p < 0.02$.

^b $p < 0.02$.

^c $p < 0.3$.

Table 53.7 Incidence of thyroid cancer in an iodine-deficient region

<i>Census</i>	<i>Population</i>	<i>Mean n of cancers (period)</i>	<i>Incidence</i>	<i>Ratio</i>
<i>Females/males</i>				
1960	201 686/211 168	3.2/1.2 (1958–1962)	1.6/0.6	2.7/1
1970	253 044/256 597	2.7/0.7 (1966–1974)	1.1/0.3	4.0/1
1980	333 023/329 847	3.3/0.7 (1976–1984)	1.0/0.2	5.0/1
1991	436 631/429 522	7.4/1.7 (1987–1995)	1.7/0.4	4.2/1
2001	544 911/531 140	19.8/4.1 (1997–2003)	3.6/0.8	4.2/1

Note: Incidence (n cases/ 10^5 population/year) of thyroid cancer in the province of Salta, Argentina.

Table 53.8 Crude incidence of thyroid cancer in iodine-deficient areas

<i>Census year</i>	<i>Population</i>	<i>Mean n of cancers (period)</i>	<i>Incidence</i>	<i>Ratio</i>
<i>Capital city/countryside</i>				
1991	373 586/492 567	6.4/2.7 (1987–1995)	1.7/0.5	3.4/1
2001	473 267/606 155	15.4/9.1 (1997–2003)	3.2/1.5	2.1/1

Note: Crude incidence (n cases/ 10^5 population/year) of thyroid cancer in the capital city as compared with the countryside regions from the province of Salta, Argentina.

(Heitz *et al.*, 1976; Ibanez *et al.*, 1966), the latter being expected to be a more frequent precursor than papillary carcinoma before iodine prophylaxis (Heitz *et al.*, 1976). Molecular studies suggest progression from papillary cancer to a subset of undifferentiated carcinoma, particularly through Braf oncogene (*BRAF*) rather than Ret oncogene (*RET*) mutation (Quiros *et al.*, 2005), while progression from follicular carcinoma to anaplastic carcinoma through *RAS* point mutation is a matter of debate (Lemoine *et al.*, 1989; Manenti *et al.*, 1994).

Because of the relative infrequency of undifferentiated carcinoma, as compared to differentiated thyroid carcinomas, much of the epidemiological data have commented on the relationship of follicular and papillary cancers. Iodine-deficient regions have a tendency to show higher rates of undifferentiated carcinomas before iodine prophylaxis, as compared with postprophylaxis periods (Bacher-Stier *et al.*, 1997; Bakiri *et al.*, 1998; Belfiore *et al.*, 1987; Cuello *et al.*, 1969; Harach *et al.*, 1985a; Hedinger, 1985; Hofstädter, 1980; Schmid *et al.*, 1986) and with regions with high dietary iodine intake (Bakiri *et al.*, 1998; Ezaki *et al.*, 1992; Jönasson *et al.*, 1989). In Salta, the decrease of undifferentiated carcinoma after iodine prophylaxis is significant, and the peak age and mean age of occurrence, as in Austria for example (Hofstädter, 1980), was delayed (Table 53.6). This change in incidence of undifferentiated carcinoma could reflect a lower TSH-based growth stimulation and also better access to medical care, leading to diagnosis of thyroid malignancies in earlier stages of differentiation (Bakiri *et al.*, 1998; Trimboli *et al.*, 2006).

Angiosarcoma (malignant hemangioendothelioma)

Angiosarcoma of the thyroid is a highly malignant tumor that predominates in alpine goitrous regions; its origin has been a matter of dispute, some regard it as a variety of undifferentiated carcinoma of the thyroid with both epithelial and endothelial cell differentiation, others accept it as a vascular tumor entity when composed purely of endothelial cells, and still others consider thyroid angiosarcoma to be an extreme spectrum of endothelial cell differentiation from undifferentiated carcinoma (Eusebi *et al.*, 1990; Mills *et al.*, 1994; Ruchti *et al.*, 1984; Vollenweider *et al.*, 1989). Thyroid angiosarcoma shows a tendency to decrease in frequency after iodine prophylaxis in Switzerland and Austria, as do undifferentiated carcinoma (Bacher-Stier *et al.*, 1997; Hedinger, 1985). It has been demonstrated that an increase in TSH leads not only to thyroid follicular cell proliferation, but also to endothelial cell proliferation (Many *et al.*, 1984), probably by the release of vascular endothelial growth factor from the thyrocytes (Wang *et al.*, 1998).

Primary thyroid lymphoma

Primary thyroid lymphoma shows marked geographical variation in incidence, but its separation from small cell carcinoma in the 1970s makes one believe that the published figures before that time must be carefully

analyzed or reviewed (Schmid *et al.*, 1986). Thyroid lymphoma is commonly associated with Hashimoto's thyroiditis (Derringer *et al.*, 2000; Isaacson, 1997) and occurs more frequently in populations with a high rather than a low frequency of thyroiditis (Williams *et al.*, 1977), and has a tendency to increase after iodine prophylaxis (Schmid *et al.*, 1986; Harach and Williams, 1995; Harach *et al.*, 2002). In Salta, this type of tumor occurred only after salt prophylaxis (Table 53.6) when thyroiditis also rose (Harach *et al.*, 2002). The number of cases studied, and the increasing age of the population with time, must be taken into account (Harach and Williams, 1996) since this type of neoplasm tends to occur in older people where thyroiditis is also more frequent. Patients with Hashimoto's thyroiditis show a 67–80-fold relative risk for the development of thyroid lymphoma than the control population (Aozasa, 1990; Holm *et al.*, 1985). It would be unlikely that a primary lymphoma would occur in a thyroid without lymphocytic infiltration.

Summary Points

- The ratio of papillary to follicular carcinoma increases after iodine prophylaxis.
- The increase of papillary carcinoma after prophylaxis may be linked to factors other than iodine.
- A decrease of undifferentiated carcinoma after iodine prophylaxis could be linked to better medical care.
- Thyroid angiosarcoma behaves like undifferentiated carcinoma clinically and epidemiologically.
- Higher incidence of thyroid lymphoma after iodine prophylaxis is likely to be related to an increase of lymphocytic thyroiditis.
- It would be unlikely that a primary lymphoma occurred in a thyroid without lymphocytic infiltration.

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Iodine Deficiency in Vegetarian and Vegan Diets: Evidence-Based Review of the World's Literature on Iodine Content in Vegetarian Diets

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Abstract

As the iodine content is generally low in most vegetables and fruits, it is biologically plausible that compliance with strict vegetarian diets would increase risks of iodine deficiency. This chapter responds to these concerns by first considering the prevalence, demography and lifestyle characteristics of vegetarians residing mainly in developed countries and finally, by reviewing the nutrition and epidemiological studies that assessed iodine nutrition in vegetarians. The available data indicate that the number of committed vegetarians in developed countries is very small and, in comparison to the general population, most live healthier lifestyles, have a higher level of education and SES, and better dietary knowledge. Nutritional studies indicate that vegetarian diets need not lead to iodine deficiency; the adequacy of iodine intake in strict vegetarian diets largely depends on the appropriate use of iodine-containing salt and dietary supplements. Paradoxically, some vegetarians risk iodine excess as a result of nonstandardized use of iodine-rich food supplements. On balance, vegetarians and others following restrictive diets should recognize that they may be at increased risk of iodine, as well as other nutritional, deficiencies.

Abbreviations

CSFII	Continuing Survey of Food Intake by Individuals
FDA	Food and Drug Administration, United States
FD&C	Food Drug and Cosmetics
NHANES III	Third National Health and Nutrition Examination Survey
TSH	Thyroid-stimulating hormone
USDA	United States Department of Agriculture
VRG	Vegetarian Resource Group

Introduction

Vegetarianism, the practice of excluding meat, poultry and fish from the diet, has been widely practiced for millennia, mainly for economic or religious reasons. Presently, however, those who adopt vegetarian diets, especially in developed western countries, are often motivated by health concerns and the conviction that vegetarian diets are more likely to promote well-being (Chang-Claude *et al.*, 2005; Dwyer *et al.*, 1974; Jabs *et al.*, 1998; Koebnick *et al.*, 2005; Thorogood, 1995; Waldmann *et al.*, 2003). Other current motivations for such dietary practices include ethical concerns (e.g., animal rights) (Waldmann *et al.*, 2003) and ecological concerns (e.g., global sustainability) (Leitzmann, 2005). In the US, marketing surveys have documented the growing popularity of vegetarian foods and vegetarian diets, which have increasingly gained mainstream acceptance. Similar increases have been noted in the UK, where surveys show that the percentage of vegetarians has increased from 2.1% in 1984 to 4.3% in 1993 (Vegetarian Society, 2007) and 5% in 2002 (Office for National Statistics, 2004).

The nutritional adequacy of vegetarian and vegan diets has been the subject of ongoing scrutiny, focused mainly on intake of protein, n-3 fatty acids, calcium, vitamin B-12, niacin, iron and zinc; deficiencies of one or more have been reported in some vegetarians (Department of Health and Human Services, 2003; Dwyer, 1991; Haddad *et al.*, 1999; Haddad and Tanzman, 2003; Lightowler and Davies, 2000; Miller *et al.*, 1991; Strohle *et al.*, 2006). The adequacy of iodine intake among vegetarians has only rarely been considered. The importance of sufficient iodine intake is that iodine deficiency, one of the four major nutritional deficiency diseases in the world, is the leading cause of intellectual deficiency and mental retardation worldwide (Hollowell and Hannon, 1997). Because vegetarian diets, by definition, exclude in part or wholly the most important food sources of iodine (meat, fish, dairy products and eggs), it is possible that strict vegetarians and vegans might be at increased risk of iodine deficiency.

Only a handful of published studies have directly examined the sufficiency of iodine in vegetarian diets. Unfortunately, the informational value of those studies is limited by small sample size and inadequate study methods. Accordingly, we undertook the following review to better understand the possibility of iodine deficiency in vegetarians and vegans, and the likely magnitude of its public health significance. After reviewing the nomenclature of vegetarian diets, we consider below the prevalence, demography and lifestyle features that characterize vegetarians and vegans, particularly in developed countries. Finally, we review the nutritional and epidemiologic studies relevant to the concerns of iodine nutrition in vegetarians.

Nomenclature, Types and Prevalence of Vegetarians

The risk of iodine deficiency in vegetarians reflects the extent to which iodine-containing foods are excluded from their diets. For example, those who adhere to highly restricted diets are more likely to suffer deficiency than others who consume dairy products, which are generally iodine-rich. Thus, to consider the likely prevalence of iodine deficiency in vegetarians, it is necessary to consider the nature and severity of individual dietary restrictions. However, the scientific and popular literature have adopted an array of variable and sometimes overlapping nomenclature to describe various types of vegetarian diets (Dwyer *et al.*, 1974; Johnston, 2000; Weinsier, 2000). In order to standardize our discussion and enhance comparability between the studies considered below, we have adopted the dietary terms and definitions outlined in **Tables 54.1** and **54.2**, respectively.

The exact number of vegetarians in the United States, Europe and other countries remains unclear, in part because of difficulties in agreeing on who and what is being counted. In most surveys, participants were classified on the basis of self-described dietary habits, rather than objective assessments of actual consumption. As discussed below, survey respondents tend to overestimate their vegetarian status, exaggerating the extent of their dietary restrictions and the consistency of their adherence. Despite that, survey results indicate that the prevalence of consistently practicing vegetarians in the populations of the US and other developed countries is small.

Survey data: US

An important set of US findings derive from the Stanford Five City Project, five cross-sectional, household-based surveys conducted between 1979 and 1990 among participants aged 20–74 years who resided in four cities of California (Frank *et al.*, 1992; White and Frank, 1994). In these surveys, 14.8–16.9% of the participants said

Table 54.1 Types of vegetarian diets, including the foods excluded and included by each

<i>Type of diet</i>	<i>Excluded foods</i>	<i>Included foods</i>
Main vegetarian diets		
Vegetarian	Meat, poultry, fish, seafood	Eggs, milk
Lacto-ovo-vegetarian	Meat, poultry, fish,	Eggs, milk
Lacto-vegetarian	Meat, poultry, fish, eggs	Milk
Vegan	Meat, poultry, fish, eggs, milk	
Pescetarian	Meat, poultry	Fish, seafood
Pollotarian	Meat, fish	Poultry
Macrobiotic	Meat, poultry, eggs, milk	Fish, vegetables, grains, legumes
Raw food diet	Meat, poultry, eggs, milk; exclusions vary by diet type: vegan, lacto-ovo-vegetarian, or omnivorous	Uncooked fruits, vegetables, seeds, nuts, spouted grains
Self-defined vegetarian	No strict exclusions	

Sources: ADA (2003); Appleby (1999); Barr (2002); Dwyer *et al.*, (1974); Freeland-Graves *et al.*, (1986); Fruitarian Foundation (2000); Living Foods (2003); Koebnick *et al.*, (2005); Perry (2002).

they had not eaten “any meat, poultry, fish, egg yolks or cheese (other than low-fat cheeses like ricotta or low-fat cottage cheese) during the past 1–3 days.” When asked about longer periods of dietary restriction, the number of responding participants declined rapidly. For example, 3.0–4.2% had not eaten “any meat, poultry, fish, egg yolks or cheese” during the prior 4–6 days, while only 0.4–1.0% had followed such a diet for 7 days. These data indicate that many people are “occasional vegetarians,” but few adhere rigorously to restricted diets. Moreover, because the smallest proportion (0.4–1.0%) reflected only a 7-day time span and included some individuals who consumed eggs and dairy products, it overestimated the proportion of strict vegans in that population.

The Continuing Survey of Food Intake by Individuals (CSFII), a study conducted by the US Department of Agriculture (USDA), collected food consumption data from representative samples of noninstitutionalized persons in the US (Haddad and Tanzman, 2003; US Department of Agriculture, 2000). Surveys conducted during 1994–1996 and 1998 included 13 341 participants over 6 years of age who were asked about the foods consumed during two nonconsecutive “recall days.” There were 334 participants (2.5%) who described themselves as vegetarians. But, when asked about specific foods consumed on either of the recall days, 97 (29%) of those 334 participants said that they had eaten meat (beef, veal, pork, or lamb) or poultry, while 234 (70%) said that they had eaten meat, poultry, fish, or

Table 54.2 Definitions of vegetarian diet types

Type of Diet	Definition
Omnivorous	An unrestricted diet containing animal and plant foods
Vegetarian	Diet excludes meat, poultry, game, fish, shellfish, crustaceans or their by-products Diet includes grains, pulses, nuts, seeds, vegetables and fruits, with or without dairy products or eggs
Lacto-ovo-vegetarian	Vegetarian diet (defined above) with the addition of dairy products and eggs
Lacto-vegetarian	Vegetarian diet (defined above) with the addition of dairy products
Vegan	Vegetarian diet (defined above) that also excludes eggs and dairy products, and may or may not exclude honey
The following terms describe diets that are not necessarily vegetarian:	
Macrobiotic	Diet mainly consists of whole grains and beans; may also include fish
Raw food diet	Diet mainly consists of fresh and uncooked fruits, vegetables, nuts, seeds, sprouted grains, and beans; may be practiced as an omnivorous (raw meats and fish), vegetarian or vegan diet
Pescetarian	A vegetarian-type diet that includes fish, shellfish and crustaceans
Pollotarian	A vegetarian-type diet that includes poultry and poultry products
Self-defined vegetarian	Diet generally consists of less meat than an omnivorous diet

Sources: Cunningham (2004); Koebnick *et al.*, (2005); USDA (2005); Wikipedia (2007).

seafood. Thus, only 30% of the self-described vegetarians adhered to a vegetarian diet on the two recall days.

In another CSFII survey, 12 634 participants over 6 years of age detailed their food consumption for two “recall days.” The consumption pattern of 43 individuals (0.34%) was consistent with a vegan diet (i.e., no meat, poultry, fish, eggs, or dairy). Of these individuals, only 14 (33%) identified themselves as vegetarians. Because CSFII considered only two specific “recall days,” the reported percentages represent upper bounds on the proportion of respondents (and of the US population) that is actually vegetarian or vegan; the true number is almost certainly smaller. These CSFII data also indicate that only a small proportion adheres to a vegan diet; the number of consistently practicing vegans almost certainly represents significantly less than 0.11–0.34% of the US population.

These studies illustrate the difficulty of characterizing vegetarians on the basis of self-reported dietary practices. Such seemingly inconsistent survey results are likely due to the lack of a universally accepted definition of “vegetarian,” rather than a lack of understanding or intelligence.

Support for that view is found in a survey of US women physicians, a population characterized by both intelligence and knowledge of biology and nutrition (White *et al.*, 1999). When subjects were asked about their diets, about 8% identified themselves as vegetarians; when asked to describe their actual food intake, nearly half of the “vegetarian” physicians admitted to eating meat, poultry, or fish during the prior week.

Over the past decade the Vegetarian Resource Group (VRG), a nonprofit organization dedicated to promoting vegetarianism, sponsored telephone surveys of dietary habits conducted by the Roper Organization (Vegetarian Resource Group, 1994, 1997), Zogby (Vegetarian Resource Group, 2000), and Harris Interactive (Vegetarian Resource Group, 2003, 2006). The VRG surveys found that 4–10% of “the country’s consumers” call themselves vegetarians, but the numbers that actually follow a vegetarian diet decline as dietary restrictions increase. For example, among the 2006 survey respondents aged 18 years or older, 6.7% said that they “never eat meat,” 2.3% said they “never eat meat, poultry, fish/seafood (vegetarian),” and 1.4% said that they “never eat meat, poultry, fish/seafood, dairy products/eggs (vegan, except for possible honey)” (Vegetarian Resource Group, 2006). VRG estimated that vegans comprised about one-half to one-third of the number of vegetarians in the US, while acknowledging that many who call themselves vegetarian do not adhere to such dietary restrictions:

... our theory is that most people who fit the definition of vegetarian (never eat meat, fish, or fowl) are ‘very committed to issues’ and tend to become vegan. Vegans would be a much smaller percentage of those who self-define as vegetarian – that is, the people who think of themselves as vegetarian but may eat meat, fish, or poultry (Vegetarian Resource Group, 2003).

Survey data: Europe

Similar survey results have been reported from other countries. A study by the German Institutes of Food Science and Nutritional Science (Waldmann *et al.*, 2003) reported findings from a 1994 to 1995 cross-sectional survey of diet and lifestyle in German vegans. Subjects, recruited via vegetarian and vegan journals, were included if they were 18 years or older, had maintained a vegan diet for at least the prior 12 months, were not pregnant, and had not given birth during the prior year. A total of 654 respondents “claiming to be vegans” participated in a series of questionnaires, two 9-day food surveys and blood sampling. Only 154 subjects completed all survey segments; 265 self-claimed “vegans” were excluded because they had eaten meat, while 235 others did not complete the survey. Of the 154 “vegans” who completed the survey, 56 (36.4%) said that they regularly consumed small amounts of dairy products and eggs. A small survey of UK “vegans”

found comparable inconsistencies: 21% (8 of 38) regularly consumed at least small amounts of dairy and dairy products (Draper *et al.*, 1993).

One reason for the difficulty in distinguishing “true vegetarians” from intermittent vegetarians is the increasing access to prepared vegetarian meals (Messina and Mangels, 2001). It has become easy to be a “part-time” vegetarian. The National Restaurant Association reports that 80% of US table service restaurants offer vegetarian entrees, many fast food restaurants offer vegetarian fare (e.g., veggie burgers), and vegetarian meals are offered at almost every US college and university (National Restaurant Association, 1999). Likewise, “alternative” foods are increasingly offered to the wider audience of supermarket consumers: approximately 50% of “natural foods” and 75% of soymilk are now sold in supermarkets (Ginsberg and Ostrowski, 2003). Similar patterns of vegetarian food consumption have been documented in the UK (Keynote Publications, 2003). Ease of access, coupled with the positive image of vegetarians (e.g., very committed to issues), may encourage occasional eaters of vegetarian foods to self-describe as “vegetarian.”

In summary, it seems that the number of people who consistently follow strict vegetarian diets is small, probably less than 0.1% of the populations in the US and European countries; there is a lack of data on the numbers who adhere to such diets in developing countries. These are the vegetarians most likely to be iodine-deficient. By contrast, a substantial proportion of self-identified “vegetarians” regularly consume iodine-rich dairy products and a smaller proportion eat meat, poultry, or fish at least occasionally.

Demographic and Lifestyle Characteristics of Vegetarians

Surveys of vegetarians reveal consistent patterns of demographic and lifestyle characteristics. Compared to the general population, for example, self-described vegetarians in the US are more likely to be white females, 20–40 years of age, college-educated, earning relatively high household incomes, and living in east or west coast cities. In the CFSII survey, 67% of self-identified vegetarians were female, 79% were white, and 30% were college graduates (compared to 22% in the general population) (US Department of Agriculture, 2000; White and Frank, 1994). Similar patterns were seen among European vegetarians. In a British study, vegetarians were described as “predominantly from higher social classes, and well-educated” (Draper *et al.*, 1993); they were more than twice as likely to be in the highest socioeconomic category and 51% of vegetarians (compared to 7% of the Greater London population) had a university degree or equivalent. A recent German study of 1225 German vegetarians and 679 “health-conscious” nonvegetarians (Chang-Claude *et al.*, 2005) found both groups “highly-educated,” including a significantly increased proportion of professionals (50%) compared to the general population (18%).

There are also consistent data that vegetarians live healthier lifestyles than omnivores (Key *et al.*, 2006). They are significantly less likely to smoke, drink alcohol, or consume fatty and processed foods, and they are more likely to be physically active (Ellis and Montegriffo, 1970; Chang-Claude *et al.*, 2005; Freeland-Graves *et al.*, 1986; Phillips *et al.*, 1980; Waldmann *et al.*, 2003). Evidence also indicates that the more restrictive the diets, the more likely such patterns will be seen. Such findings strongly suggest that those who adhere to strict vegetarian diets, at least in developed countries, have adopted such diets as a matter of choice, rather than as a result of poverty or ignorance. Moreover, the health-promoting inclinations of vegetarians suggests that they are likely to understand their risks of dietary deficiencies (iodine and otherwise), and would therefore be inclined to adopt appropriate supplement regimens.

Iodine Content of Omnivore and Vegetarian Diets

The iodine content of fruits and vegetables reflects the iodine content of the soil, irrigation waters and fertilizers used in their agricultural production. Likewise, the iodine content of meat, poultry and dairy products reflects both naturally occurring iodine (e.g., drinking water, livestock feed) and the use of iodine-containing supplements. Iodine is often added to feed in order to prevent endemic iodine deficiency and the thyroid-suppressing effects of isoflavone- and thiocyanate-rich feed components (e.g., soy, rapeseed). Also, iodophor sanitizing solutions used to clean cattle udders and milking equipment increase the iodine content of dairy products (Phillips, 1997). Levels in processed foods depend on naturally occurring iodine and the use of iodized salt and additives. For example, potassium and calcium iodate have been used as “dough conditioners” to improve the texture, appearance and shelf-life of baked goods; some breads contain more than 300 µg iodine per slice (Dunn, 2003). Another additive, FD&C Red #3 (erythrosine), a dye used in ready-to-eat cereals and other foods, is 58% iodine by weight (National Academy of Sciences, 2001a; Pennington *et al.*, 1995).

During the 1990s, the US Food and Drug Administration (FDA) analyzed the content of iodine (and other minerals) in 294 “core foods” in the US food supply (Pennington *et al.*, 1995). For prepared foods, all cooking was done with noniodized salt. Multiple analyses (37 analyses per food) were performed to allow calculation of analytical precision. Of those foods, 49 contained more than 20 µg iodine per portion and 32 contained 10–20 µg iodine per portion. Foods with the highest iodine content, 54–450 µg per serving, included a cross-section of typical American cuisine: fruit-flavored cereal, chocolate milk shake, cheese pizza, cod/haddock, chicken pot pie, low-fat plain yogurt,

Table 54.3 Variation in naturally occurring iodine: reported levels in fish

Type of fish	Serving size	Iodine (μg) [mean (range)]	Country	Source
Cod	3 ounces	99	US	Linus Pauling Institute (2003)
		89	Finland	Fineli (2007)
		63 (46–102)	UK	Wenlock <i>et al.</i> , (1982)
		18.7	Slovakia	Krajcovicova-Kudlackova <i>et al.</i> , (2003)
Tuna (canned)	3 ounces (1/2 can)	24	Finland	Varo <i>et al.</i> , (1982)
		17	US	Linus Pauling Institute (2003)
		12	UK	Lee <i>et al.</i> , (1994)

Table 54.4 Variation of iodine supplementation in table salt

Country	Iodine ($\mu\text{g}/\text{g}$)	Source
US	77	Linus Pauling Institute (2003)
Finland	22	Fineli (2007)
Denmark	10–31	Rasmussen <i>et al.</i> , (2007)
Italy	30	Girelli <i>et al.</i> , (2004)
Germany	20	Remer <i>et al.</i> , (2006)

macaroni and cheese, corn grits, homemade lasagna, white rice, pancakes, chocolate milk, chicken noodle casserole, canned spaghetti in tomato sauce, low-fat milk, apple pie, fish sticks, chocolate pudding and mashed potatoes. Iodine levels of prepared foods would have been significantly higher if cooked with iodized salt: about 50–70% of US households use iodized salt, providing about 50 μg iodine per day (American Dietetic Association, 2003; Dunn, 2003; National Academy of Sciences, 2001b). By contrast, the mainstay of vegetarian diets such as vegetables, fruits and nuts, contained essentially no iodine (Pennington *et al.*, 1995).

Generally, the iodine content of foods varies across regions and countries, according to the amounts of naturally occurring iodine and the levels and extent of iodine supplementation in table salt, animal feed and processed foods. This is illustrated in Tables 54.3–54.5, which also reflect differences in reference tables used in various countries to describe the nutritional content of common foods (Krajcovicova-Kudlackova *et al.*, 2003; Lightowler *et al.*, 1996).

Worldwide, milk, fish, eggs and meat have been consistently identified to be the most important natural food sources for iodine (Dahl *et al.*, 2004; Girelli *et al.*, 2004; Remer *et al.*, 2006; Varo *et al.*, 1982; Wenlock *et al.*, 1982). Iodine is also obtained from dietary supplements. About half of prenatal vitamins and many multivitamins are supplanted with 100–200 μg per day, and iodine is often found in “multi-mineral” and “multi-vitamin-and-multi-mineral” preparations (Lee and Roper, 2004). In 1986, the National Health Interview Survey considered the use of vitamin and mineral supplements among a

representative sample of 13 435 US residents (Moss *et al.*, 1989). The survey found that 36% of US adults (excluding pregnant and lactating women) took nonprescription vitamin and mineral supplements. Use was higher in women (41%) than men (31%). These findings were confirmed by the 1988–1994 NHANES III survey, which included 33 905 people comprising a representative sample of the civilian, noninstitutionalized US population (National Center for Health Statistics, 1999). Approximately 42% of adults used one or more supplements; use was greater in women (44%) than men (35%). Among pregnant and lactating women, supplement use was much higher: 76% reported taking at least one dietary supplement daily. Among women of childbearing age (i.e., 20–39 years) who took dietary supplements, about 60% used vitamin/mineral combinations likely to contain iodine, although actual iodine content was not determined. Other non-dietary sources of iodine include “energy” and “protein” shakes (which are often iodine-supplemented), antiseptics and mouthwash.

In light of such evidence, it is not surprising that iodine deficiency is generally not found in the US and most other developed countries. To be iodine-deficient, one must avoid not just meat, fish and poultry, but also many processed foods, most dairy products, flavored cereals, commercial baked goods, iodized salt and iodine-containing vitamins and supplements. In other words, it would require almost deliberate avoidance of iodine. It is precisely that pattern of avoidance, however, that raises concerns that vegetarians and vegans might be iodine-deficient.

Iodine Nutrition in Vegetarians

We identified eight studies worldwide that evaluated iodine nutrition in vegetarians (Abdulla *et al.*, 1981; Draper *et al.*, 1993; Key *et al.*, 1992; Krajcovicova-Kudlackova *et al.*, 2003; Lightowler and Davies, 1998; Rauma *et al.*, 1994; Remer *et al.*, 1999; Waldmann *et al.*, 2003). Those studies are summarized in Table 54.6. The informational value of these studies is limited by small sample size and methodological deficiencies. For example, only four studies included more than 40 vegetarians (Draper *et al.*, 1993;

Table 54.5 The variations in iodine content reported for milk and dairy products, eggs, meat and bread

Food	Serving size	Iodine (μg) [mean (range)]	Country	Sources
Milk	1 cup (~250ml)	116 (88–168)	United States	Pearce <i>et al.</i> , (2004)
		113 (98–130)	Norway	Dahl <i>et al.</i> , (2004)
		67	Italy	Girelli <i>et al.</i> , (2004)
		44.5 (12–165)	Germany	Bader <i>et al.</i> , (2005)
		38 (10–78)	United Kingdom	Lee <i>et al.</i> , (1994)
Diary products		40	Slovakia	Krajcovicova-Kudlackova <i>et al.</i> , (2003)
		116 (88–168)	United States	Pearce <i>et al.</i> , (2004)
Eggs	1 large (~60g)	42	Finland	Fineli (2007)
		31–32	United Kingdom	Wenlock <i>et al.</i> , (1982)
		27 (23–31)	Norway	Dahl <i>et al.</i> , (2004)
		18–29	United States	Linus Pauling Institute (2003), Feinberg School (2006)
MEAT	3 ounces, cooked	6	Slovakia	Krajcovicova-Kudlackova <i>et al.</i> , (2003)
		8	United States	Feinberg School (2006)
		5.1	United Kingdom	Lee <i>et al.</i> , (1994)
		1.7 (<1–5.9)	Norway	Dahl <i>et al.</i> , (2004)
BREAD	1 slice (~25g)			
		8.25 (1.5–53) ^a	United States	Pearce <i>et al.</i> , (2004)
		5.25 (0–11.5)	Denmark	Rasmussen <i>et al.</i> , (2007)
		1.5	Slovakia	Krajcovicova-Kudlackova <i>et al.</i> , (2003)
		1.25–3.75	Finland	Varo <i>et al.</i> , (1982)

^a Three brands of bread with very high iodine content (324–587 $\mu\text{g}/\text{slice}$) were excluded from analysis.

Key *et al.*, 1992; Krajcovicova-Kudlackova *et al.*, 2003; Waldmann *et al.*, 2003), while the others included only 6 (Abdulla *et al.*, 1981; Remer *et al.*, 1999), 9 (Rauma *et al.*, 1994), or 30 (Lightowler and Davies, 1998) subjects. Among the methodological deficiencies was inadequacy or absence of control groups. For example, two studies had no current control or comparison group (Lightowler and Davies, 1998; Waldmann *et al.*, 2003), a third compared vegans from an area known for iodine deficiency and high rates of endemic goiter to omnivores tested years before who had resided in an iodine-sufficient community without endemic goiter (Abdulla *et al.*, 1979, 1981), and two other studies had only incomplete dietary histories for a large proportion of control subjects (Key *et al.*, 1992). Only one study (Remer *et al.*, 1999) used an experimental (cross-over) design to compare vegetarian and omnivore diets.

A second methodological concern was the manner by which iodine intake was measured. Four studies considered daily iodine excretion:

- One study (Lightowler and Davies, 1998) had no control group;
- A second study (Krajcovicova-Kudlackova *et al.*, 2003) reported lower median urine levels among vegans as compared to lacto-ovo-vegetarians and omnivores (71 vs. 177 vs. 210 $\mu\text{g}/\text{day}$), but the ranges of the groups overlapped (9–204 vs. 44–273 vs. 76–423 $\mu\text{g}/\text{day}$);
- A third study (Remer *et al.*, 1999), in an iodine-deficient area of Germany, found low mean urinary levels in both

lacto-vegetarian and omnivore diets (36.6 ± 8.8 vs. $50.2 \pm 14.0 \mu\text{g}/\text{day}$);

- A fourth study (Rauma *et al.*, 1994) used an analytical method that was so insensitive that it could not determine urine concentrations $<150 \mu\text{g}/\text{day}$.

The adequacy of iodine intake can also be assessed by directly measuring iodine in dietary components. But, as the iodine content of different samples of the same food can vary widely, using dietary histories to estimate daily intake is problematic. In its “core foods” study, the FDA found that variability of iodine content was substantially greater than that of any other mineral studied; the average coefficient of variation for iodine content was 158%. Accordingly, data on the iodine content of foods should be used with caution (Pennington *et al.*, 1995). In addition, dietary histories generally ignore food supplements and drinking water, often important, but variable, sources of iodine. A recent study in Denmark, for example, reported that the iodine content of drinking water in 55 locations varied more than 100-fold (Pedersen *et al.*, 1999).

Three of the studies measured dietary iodine content directly by analysis of duplicate food portions:

- An English study (Lightowler and Davies, 1998), which had no control group, found that mean iodine content in vegan diets was 187 $\mu\text{g}/\text{day}$ for women and 137 $\mu\text{g}/\text{day}$ for men;
- A study performed in an iodine-deficient area of Germany (Remer *et al.*, 1999) found that all diets were

Table 54.6 Description of eight studies that evaluated iodine nutrition in vegetarians

Study	Location	Diet types	No. of subjects (males:females)	Urinary iodine	Dietary iodine ($\mu\text{g}/\text{day}$) (males:females)	Thyroid function	
Abdulla <i>et al.</i> , (1981)	Sweden	Vegan	6 (3:3)	ND	82 \pm 29 (δ):58 \pm 12 (δ) ^e	TSH, T ₃ , T ₄	
Draper <i>et al.</i> , (1993)	UK	Vegan	38 (18:20)	ND	89 (δ):62 (f) ^{d,g}	ND	
		Lacto-vegetarian	52 (16:36)	ND	202 (δ):156 (f) ^{d,g}	ND	
		Demi-vegetarian	37 (13:24)	ND	204 (δ):152 (f) ^{d,g}	ND	
Key <i>et al.</i> , (1992)	UK	Vegan	48 (48:0)	ND	ND	TSH	
		Omnivorous	53 (53:0)	ND	ND	TSH	
Krajcovicovi-Kudlackova <i>et al.</i> , (2003)	Vegan	Vegan	15 (6:9)	71 (9–204) $\mu\text{g}/\text{l}$ ^a	ND	ND	
				128 (\pm 8.8) $\mu\text{g}/\text{g cr}$ ^c			
		Lacto-ovo and Lacto-vegetarian	31 (12:19)	177 (44–273) $\mu\text{g}/\text{l}$ ^a	ND	ND	
		Omnivorous	35 (15:20)	226 (\pm 12) $\mu\text{g}/\text{g cr}$ ^c	ND	ND	
Lightowler and Davies (1998)	UK	Vegan		309 (\pm 17) $\mu\text{g}/\text{g cr}$ ^c			
				30 (11:19)	16.8 $\mu\text{g}/\text{l}$ (δ) ^b	137 (\pm 149) (δ) ^{f,h}	ND
					20.5 $\mu\text{g}/\text{l}$ (f) ^b	187 (\pm 346) (f) ^{f,h}	ND
Rauma <i>et al.</i> , (1994)	Finland	Vegan	9	<200–1700 $\mu\text{g}/\text{day}$	29 (\pm 18) ^{d,g}	TSH, T ₄	
		Omnivorous	8	<150–1200 $\mu\text{g}/\text{day}$	222 (\pm 93) ^{d,g}	ND	
Remer <i>et al.</i> , (1999)	Germany	Lacto-vegetarian	6 (3:3)	36.6 (\pm 8.8) $\mu\text{g}/\text{day}$ ^c	15.6 (12–18) ^{e,i}	ND	
		Omnivorous (cross-over)		50.2 (\pm 14) $\mu\text{g}/\text{day}$ ^c	35.2 (25–45) ^{e,i}	ND	
		Omnivorous (protein-rich)		61.0 (\pm 8) $\mu\text{g}/\text{day}$ ^c	44.5 (40–48) ^{e,i}	ND	
Waldmann <i>et al.</i> , (2003)	Germany	Strict Vegan	98 (48:50)	ND	88 (\pm 31) (δ) ^{d,h}	ND	
					82 (\pm 34) (f) ^{d,h}		
		Moderate Vegan	56 (19:37)	ND	94 (\pm 28) (δ) ^{d,h}	ND	
					78 (\pm 26) (f) ^{d,h}		

Note: ND, not done; $\mu\text{g}/\text{day}$, micrograms per day; $\mu\text{g}/\text{g cr}$, micrograms per gram creatinine; $\mu\text{g}/\text{l}$, micrograms per liter; f , female; δ , male.

^aMedian (range).

^bMedian

^cMean (\pm SD).

^dFood diary.

^eAnalysis of duplicate portions.

^fAnalysis of duplicate portions plus dietary supplements.

^gGeometric mean.

^hArithmetic mean (\pm SD).

ⁱArithmetic mean (range).

iodine-deficient; omnivore “controls” provided only 25–48 $\mu\text{g}/\text{day}$;

- A study performed in an iodine-deficient area of Sweden (Abdulla *et al.*, 1981) found the vegan diet to be iodine-deficient, but did not consider the iodine content of an omnivore diet from that iodine-deficient region.

In addition, three of the eight studies (Draper, 1993; Waldmann *et al.*, 2003; Rauma *et al.*, 1994) estimated iodine intake on the basis of personal diaries and standard food tables, while the final two did not quantify iodine intake (Key *et al.*, 1992; Waldmann *et al.*, 2003).

Several studies found large inconsistencies between dietary iodine intake and urinary iodine excretion in vegetarians and vegans (Lightowler and Davies, 1998; Rauma *et al.*, 1994, 1999). The most extreme example was a Finnish study of 9 vegans (Rauma *et al.*, 1994): mean dietary

iodine intake was 29 $\mu\text{g}/\text{day}$, but 4 had urine iodine excretion of 900–1700 μg iodine/day and 2 others excreted more than 200 μg iodine/day. Such findings, attributed to the use of iodine-rich seaweed and kelp supplements, underscore the need to consider total iodine intake, not only that contained in food. They also illustrate the advantage of measured urine excretion, as compared to calculated dietary intake as the measure of iodine sufficiency.

Heterogeneity of vegetarian diets

These studies reflect the marked heterogeneity that can characterize vegetarian and vegan diets. In two studies, subjects were directed to avoid iodized salt, fish, seaweed and kelp, and iodine-containing processed foods, while only iodine-free beverages were provided (Abdulla *et al.*, 1981; Remer *et al.*, 1999). In these individuals, iodine

intake was generally inadequate. By contrast, vegans in three other studies were allowed, but not required, to consume large amounts of seaweed and iodine supplements (Lightowler and Davies, 1998; Rauma *et al.*, 1994). Two of the studies (Lightowler and Davies, 1998; Rauma *et al.*, 1994) indicated that iodine intake was often excessive. In one, urine iodine levels ranged up to 1700 µg/day (Rauma *et al.*, 1994). The second (Lightowler and Davies, 1998) categorized urine iodine levels on the basis of the subjects' use of dietary supplements: those not taking supplements (mean: 87; range: 25–352 µg/day); those taking iodine-containing supplements (mean: 151; range: 67–246 µg/day); and those consuming seaweed (mean: 866; range: 521–1467 µg/day). Even among those not taking supplements, urine iodine levels often indicated adequate iodine intake. The third study (Waldmann *et al.*, 2003) considered only the iodine content of foods, while ignoring the fact that 46% utilized iodine-containing supplements; urinary iodine levels were not reported.

In addition to iodine measurements, three studies (Abdulla *et al.*, 1981; Key *et al.*, 1992; Rauma *et al.*, 1994) performed thyroid function tests as indirect measures of iodine sufficiency. Thyroid function was normal in two studies (Abdulla *et al.*, 1981; Rauma *et al.*, 1994). The third study reported elevated thyroid-stimulating hormone (TSH) levels in 5 of 48 vegans, but did not measure dietary or urinary iodine levels (Key *et al.*, 1992). It is noteworthy that the three highest TSH levels were in vegans who “usually took kelp;” it is possible that these three actually suffered iodine-induced hypothyroidism (Wiersinga and Braverman, 2003) secondary to the consumption of excessive iodine-rich kelp.

These studies confirm that the iodine content of vegan diets is often below recommended levels, but that vegans need not be iodine-deficient. Those vegans who avoid iodized salt and iodine supplements (including seaweed preparations) are at increased risk of iodine deficiency, while those who use such supplements may risk iodine overload. There is less evidence of iodine deficiency in those who follow less restrictive vegetarian diets. Iodine intake was low in lacto-vegetarians directed to avoid iodized salt, seaweed, and iodine-containing processed foods and beverages (Remer *et al.*, 1999), but adequate levels were reported in two other studies of lacto-vegetarians whose diets were not otherwise restricted (Draper *et al.*, 1993; Krajcovicova-Kudlackova *et al.*, 2003). Thus, iodine deficiency seems a concern for vegans, but not for other vegetarians, and risks of deficiency seem to depend on their appropriate use of iodized salt and/or dietary supplements.

Discussion

A conceptual challenge to evaluating the potential for iodine deficiency in vegetarians and vegans is the tendency

to regard “vegetarianism” as a homogeneous practice. That tendency has been continually challenged by nutritionists:

The term vegetarian implies a homogeneity which does not exist among Canadian and American vegetarian women. Their diets and nutritional status vary greatly depending upon the particular regime they follow, other dietary prescriptions they include, their attitudes toward and use of the healthcare system and the presence or absence of other illnesses which compromise nutritional status (Dwyer, 1983).

... there is not one homogeneous group of vegetarians ... the deviating eating habits of vegetarians explain part of the many controversial conclusions about the risks and benefits of vegetarian diets (Leitzmann, 2005).

Such heterogeneity is reflected in the studies discussed above. Among those who describe themselves as “vegetarian,” a substantial proportion eat meat, chicken, or fish. And of those who do avoid meat, chicken and fish, a large majority consume dairy products. Similar observations pertain even to those adhering to the most restrictive of vegetarian diets (e.g., vegans). Thus, it seems that the actual number of consistently practicing vegans is very small, probably less than 0.1% of the general population in developed countries. It is that small group of vegans, not the much larger numbers of lacto-vegetarians and self-defined vegetarians, who might plausibly be at risk of iodine deficiency.

European studies indicate that the iodine content of strict vegan diets is often below recommended levels, but some vegan diets contain adequate iodine and many vegans routinely augment their diets with iodized salt, kelp and other supplements. Because vegans generally have higher-than-average education, greater-than-average income and generally embrace health-promoting lifestyles, it should not be surprising that many understand the limitations of their diets and routinely use nutritional supplements. In studies of European vegans, iodine deficiency was found mainly in those specifically directed to avoid iodized salt, seaweed, supplements and iodine-containing beverages.

A final concern is that because vegetarians and vegans are disproportionately women of childbearing age, their adherence to such restrictive diets might lead to iodine deficiency sufficient to adversely impact fetal development. We have found no data specific to the adequacy of iodine nutrition in pregnant vegetarians or vegans. In principle, such concerns can be addressed by routine use of iodine-containing prenatal vitamin preparations. Most obstetricians and midwives routinely recommend prenatal vitamins to pregnant patients, especially those with restricted diets:

Almost all pregnant women should be able to obtain the Recommended Daily Allowances for minerals and vitamins through their dietary intake. There is no requirement for routine supplementation, with the possible exception of iron. However, daily supplements should be given if the adequacy of a patient's diet is questionable or if she

is at high nutritional risk. The latter category includes ... complete vegans (American Academy of Pediatrics, 1997) (emphasis added).

Historically, “the majority of pregnant and lactating vegetarian women appear to plan their diets with particular care for these physiological events” (Dwyer, 1983), as affirmed recently by the American Dietetic Association and Dietitians of Canada:

well-planned vegan and other types of vegetarian diets are appropriate for all stages of the lifecycle, including during pregnancy, lactation, childhood, and adolescence (American Dietetic Association and Dietitians of Canada, 2003).

It has been recommended that iodine be added to all prenatal vitamins (National Academy of Sciences, 2003) and that women receive 150 µg iodine supplements daily during pregnancy and lactation (Becker *et al.*, 2007; Zimmermann and Delange, 2004).

Thus, the critical issue is not whether there are pregnant vegans, but whether there are any who fail to “plan their diets.” Considering that vegetarians and vegans have generally higher educational, economic and healthy lifestyle characteristics, it can be expected that the great majority would use appropriate prenatal supplements when pregnant. Clinicians who care for women of childbearing age should pay special attention to the possibility of such specific nutritional needs.

Conclusion

Nutritional studies suggest that the iodine content of vegetarian diets may be inadequate, but adherence to a vegetarian diet need not lead to iodine deficiency. As diets become increasingly restrictive, assurance of adequate iodine intake increasingly depends on the appropriate use of iodized salt and other dietary supplements. Fortunately, the actual number of individuals who follow strictly vegan diets is small, and therefore so are public health risks of resulting iodine deficiency.

Summary Points

- The number of people who self-identify as “vegetarian” or “vegan” is significantly larger than the number who adhere to such diets.
- The iodine content of vegetarian diets may be inadequate.
- Vegetarians are generally more educated, have higher socioeconomic status, and more likely to adopt healthy and healthful behaviors.
- Strict vegetarians and vegans are likely to use iodine supplements.

- Pregnant vegetarians and vegans are a population at particular risk of iodine deficiency and should use iodine-containing prenatal vitamins.

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Relationship between Iodine Intake and Thyroid Size

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Abstract

Iodine is necessary for normal thyroid function. Iodine deficiency results in goiter, an increased thyroid gland. An increase in iodine intake can decrease the size of an enlarged thyroid gland. This has been observed in populations where an iodine fortification program has been introduced. When comparing the iodine intake and thyroid size from different populations, an inverse association between iodine intake and thyroid size is found. However, within a population this relationship is not always found. The reason for this is that iodine excretion in a casual urine sample is often used, and this is not a good measure for an individual's habitual iodine intake. A way to determine an individual's habitual iodine intake is to use a food frequency questionnaire. Furthermore, adjustment should be made for the other factors influencing thyroid size, for example, age, sex, thyroid disease in the family, dietary goitrogens, alcohol intake and smoking habits. If this is done, an inverse association between iodine intake and thyroid size can also be found within a population.

Abbreviations

ffq	Food frequency questionnaire
T ₄	Thyroxine
TSH	Thyroid-stimulating hormone

Introduction

The most obvious manifestation of iodine deficiency is goiter, an increased thyroid volume. An assumption about goitrogenesis is that, in iodine deficiency, a fall in the blood level of thyroxine (T₄) leads to increased thyroid-stimulating hormone (TSH) output from the pituitary. TSH increases the uptake of iodide by the thyroid, with increased turnover of iodine associated with hyperplasia of the follicular cells of the thyroid. The size of the gland

increases, with the formation of a goiter. Other factors, such as goitrogens in the diet, may also be responsible for an increased thyroid volume. In some countries, especially some developing countries, goitrogens are important factors for the development of enlarged thyroid glands. This chapter mainly focuses on the relationship between iodine intake and thyroid size in European countries.

Goiter may be defined as a volume above a certain size measured by ultrasonography or, less precise, as a palpable or visible enlarged gland diagnosed by clinical examination. Thyroid size or thyroid volume can be measured by ultrasonography and is commonly calculated as maximal length \times width \times depth \times $\pi/6$ of each lobe. Thyroid enlargement in adults can be defined as a thyroid volume higher than 18 ml for women and 25 ml for men, which corresponds to the mean + 3SD in iodine-sufficient populations (Gutekunst *et al.*, 1988).

Iodine intake can be assessed in various ways. As most iodine is excreted in the urine, the urinary iodine level is a marker of iodine intake. But, since individual urinary iodine varies day by day and within a day, a single-day urinary iodine can only be used for a group of individuals.

Thyroid Size Before and After Introduction of Iodine Fortification

Most countries worldwide have some kind of iodine fortification. However, in few countries has the increase in iodine intake following the introduction of an iodine fortification, and the expected effect on thyroid size, been evaluated in a systematical way. In Austria, goiter incidence in schoolchildren was 45.9% in 1964 (assessed by palpation) (Riccabona, 1993). Iodine fortification of salt was introduced by law in 1963, and by 1980 the goiter incidence had fallen to 12% in schoolchildren. The amount of iodine in salt was doubled from 10 to 20 ppm in 1990, and by 1992 the goiter incidence in schoolchildren was 5% (Riccabona, 1993). The iodine excretion in urine, as a measure of iodine intake, increased from 1964

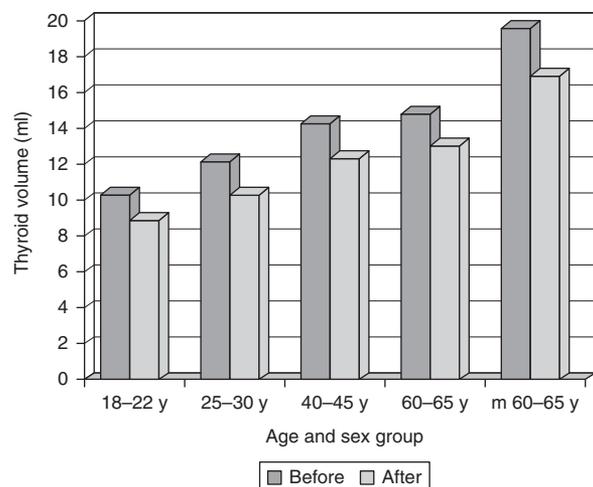


Figure 55.1 Thyroid size before and after iodine fortification in Aalborg, Denmark. Thyroid volume in women in four different age groups and in men 60–65 years of age before and after iodine fortification. Thyroid size decreased significantly in all age groups. The number of subjects is 2101 before fortification and 1693 after fortification. y, year of age; m, men.

to 1980 and a further increase was measured in 1992. Similar findings have been reported from Switzerland (Bürgi *et al.*, 1993). These results show the inverse relationship between iodine intake and the occurrence of goiter in a population.

Voluntary iodine fortification was introduced in Denmark in 1998 and changed to a mandatory fortification in 2000. A monitoring system was set up before iodization began. Thus, a cross-sectional investigation was performed in 1997–1998 and another cross-sectional investigation in 2004–2005 (Laurberg *et al.*, 2006). Similar age and geographical groups were studied in both investigations, and all procedures performed were similar. Furthermore, ultrasonography was performed by the same sonographers in both studies, using the same apparatus. Thyroid size before and after increased iodine intake is shown in Figures 55.1 and 55.2 for the various age groups in two cities, respectively (Vejbjerg *et al.*, 2007). Thyroid size decreased significantly with increased iodine intake in all age groups except the youngest.

Relationship Between Iodine Intake and Mean Thyroid Volume Between Countries/Populations

In populations with even mild iodine deficiency, an increased goiter rate can be seen. Delange *et al.* (1997) have measured thyroid volume and urinary iodine excretion in 5709 children aged 7–15 years in different sites in 12 European countries. All ultrasound examinations and urinary iodine assays were performed by the same investigators. An inverse relationship was found (Figure 55.3).

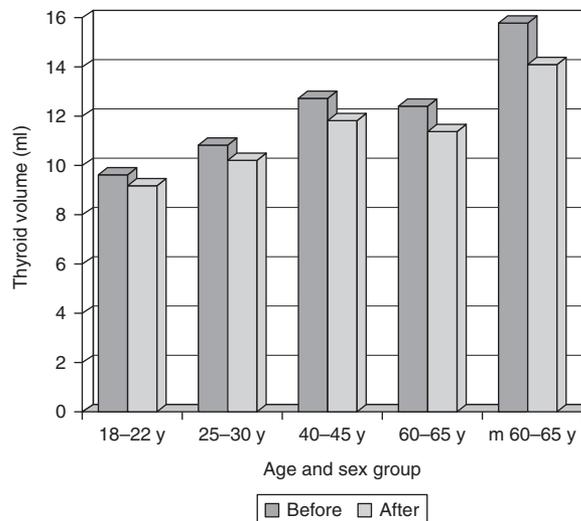


Figure 55.2 Thyroid size before and after iodine fortification in Copenhagen, Denmark. Thyroid volume in women in four different age groups and in men 60–65 years of age before and after iodine fortification. Thyroid size decreased significantly in all age groups except in the youngest. The number of subjects is 2320 before fortification and 1685 after fortification. y, year of age; m, men.

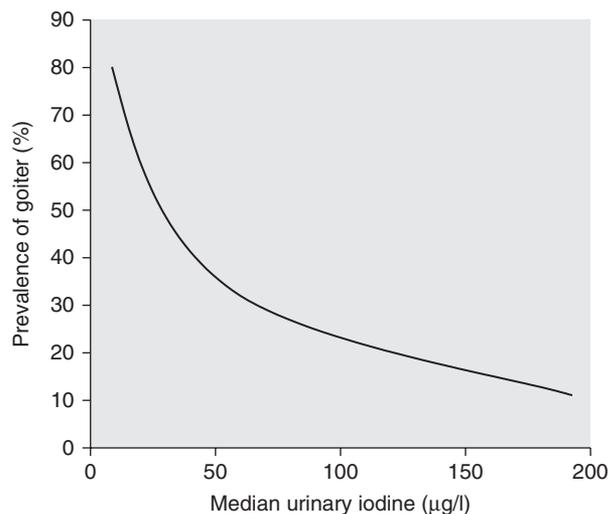


Figure 55.3 Iodine excretion and prevalence of enlarged thyroid gland in European children. The figure shows the inverse relationship between median urinary iodine concentration and the prevalence of goiter in 5709 children aged 7–15 years. Figure adapted from Delange *et al.*, (1997).

A similar study has not been performed in adults. However, one study compared thyroid volume in Sweden and Germany (Gutekunst *et al.*, 1986). Mean thyroid volume was 7.7 ± 4.3 ml in Swedish women and 16.5 ± 12.2 ml in German women. Likewise, mean thyroid volume was 11.1 ± 4.7 ml in Swedish men and 26.9 ± 17.0 ml in German men. Median iodine excretion was $62.6 \mu\text{g iodine/g}$

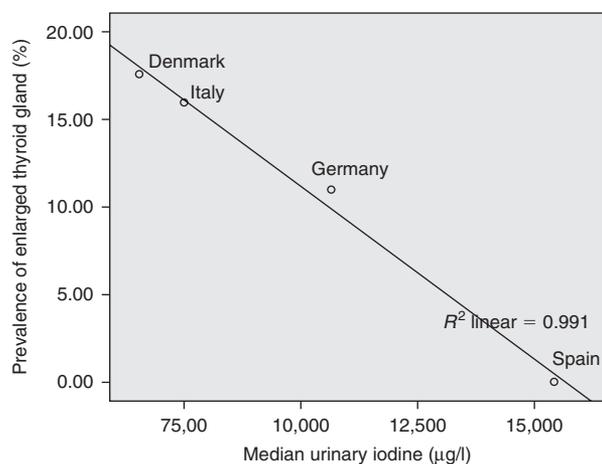


Figure 55.4 Iodine excretion and prevalence of enlarged thyroid gland in four European countries. The figure shows the iodine excretion ($\mu\text{g/l}$) and prevalence of thyroid enlargement determined by ultrasonography in four studies in European adults (Knudsen *et al.*, 2000; Maravall *et al.*, 2004; Valentino *et al.*, 2004; Brauer *et al.*, 2005).

creatinine (Cr) in Germans, whereas it was $141.4 \mu\text{g/g}$ Cr in Swedes.

The relationship between iodine excretion and thyroid size in adults from European countries is shown in Figure 55.4. We included studies if they had published median values for iodine excretion in urine expressed as a concentration and percent of subjects with enlarged thyroid gland measured by ultrasonography. Moreover, the definition for enlarged thyroid gland given in the introduction should be used, and the study population should be adults, not only elderly, and include both genders. A strong inverse relationship was found between thyroid size and iodine intake in these studies.

Relationship Between Iodine Intake and Thyroid Volume Within a Population

As discussed above, a relationship between thyroid size/prevalence of enlarged thyroid gland and mean urinary iodine excretion is found when different geographical areas are compared. When comparing individuals within a geographical area this relationship is rarely found (Table 55.1). The main reason for this may be that urinary iodine excretion in a casual urine sample is not a good measure for an individual's iodine intake, but only applicable at the group level. Thus, a strong relationship between iodine excretion and thyroid parameters cannot be expected, especially when iodine excretion is expressed as a concentration at which the actual dilution of the sample is not corrected. In most of the studies given in Table 55.1, iodine excretion

Table 55.1 Studies in which correlation between urinary iodine excretion and thyroid volume was determined within a population

Country/ population	Age/gender/ number	Correlation	References
Denmark (Holbæk)	15–60 y/w/391	NS	Nygaard <i>et al.</i> , (1993)
Austria	60 ± 14.5/m/ 1018 61 ± 15.6/w/ 1954	NS	Buchinger <i>et al.</i> , (1997)
Denmark (Aalborg)	18–65/m and w/2190	$r = -0.019$, NS	Rasmussen <i>et al.</i> , (2002)
Denmark (Copenhagen)	18–65/m and w/2419	$r = -0.031$, NS	Rasmussen <i>et al.</i> , (2002)
Italy	11–14 y/1730	$r = 0.08$, NS	Busnardo <i>et al.</i> , (2003)
Turkey	Children	$r = -0.45$, $P < 0.01$	Ozkan <i>et al.</i> , (2004)
Germany	? <65/m and w/706	NS	Brauer <i>et al.</i> , (2005)
Turkey	20–76 y/m and w/340	$r = -0.16$, $P < 0.005$	Akarsu <i>et al.</i> , (2005)

Note: The table shows correlation coefficients between urinary iodine excretion and thyroid volume in some populations. Most correlations are not significant. Age, gender, and number of subjects in each study are given. Abbreviations: y, year; w, women; m, men; NS, not significant.

was expressed as a concentration, but in one study, it was expressed as micrograms iodine per gram of Cr ($\mu\text{g I/g}$ Cr). Furthermore, many factors apart from iodine intake may influence thyroid size, for example, age, gender, intake of dietary goitrogens, genetic factors, smoking habits and alcohol intake (Knudsen *et al.*, 2002), and these factors should be corrected for when exploring a relationship between iodine intake and thyroid size.

Relationship Between Various Measures of Iodine Intake and Thyroid Volume

Iodine intake can be measured as urinary iodine excretion in a casual urine sample or, preferably, in a 24-h urinary sample. Iodine excretion in a casual urine sample can be expressed as a concentration, as $\mu\text{g I/g}$ Cr, or as estimated 24-h urinary iodine ($\mu\text{g/day}$) if $\mu\text{g I/g}$ Cr is multiplied by daily expected Cr excretion for the actual age and gender group. However, none of these measures can be used to determine an individual's habitual iodine intake. Iodine intake can also be assessed using dietary intake methods, but like urinary measurements, to determine the habitual iodine intake in an individual, 24-h recalls should be repeated several times or dietary records should be kept for several days (the number of days needed to determine the habitual iodine intake is not known, but is probably more

Table 55.2 Associations between various measures of iodine intake and thyroid volume

Measure of iodine intake	Significance of association (P)
Iodine concentration (n = 4374)	0.40
Estimated 24-h urinary iodine (n = 4422) ^a	<0.001
Iodine intake from diet plus supplements (n = 4135)	<0.001
Iodine intake from diet per kg body weight (n = 4069)	<0.001

Note: The table shows associations between various ways to measure iodine intake and thyroid volume in the same population (Rasmussen *et al.*, 2002). Thyroid volume is included as a dependent variable and a measure of iodine intake as an independent variable in a multiple linear regression model. Each measure of iodine intake is included in a separate model. Other dependent variables in the models include: city, age and gender group, smoking (daily smoker or not daily smoker), drinking (≥ 8 drinks/week or < 8 drinks per week), and thyroid disease in the family. $P < 0.001$ for all these variables. Subjects being treated for thyroid disease (n = 77) were not included in the analyses.

^aEstimated 24-h iodine excretion: iodine-to-creatinine ratio multiplied by the expected daily creatinine excretion for the given individual.

than a week). A food frequency questionnaire (ffq), which asks for the usual intake of food, can give a measure of the individual's habitual intake, and as iodine is found in significant amounts in few foods, the method can be used for the determination of iodine intake. Associations between various measures of iodine intake and thyroid volume when included in a multiple linear regression model are shown in Table 55.2 (Rasmussen *et al.*, 2002). Iodine excretion expressed as a concentration was not related with thyroid volume, whereas estimated 24-h urinary iodine, iodine intake from diet plus supplements (assessed by an ffq), and iodine intake from diet per kilogram body weight were.

Conclusion

The inverse relationship between low iodine intake and enlarged thyroid size, which obviously must be there, is found when urinary iodine excretion and thyroid enlargement are compared between different populations. Within a population, the relationship can only be expected if a measure for an individual's habitual iodine intake is used and the relationship is corrected for other factors that influence thyroid size in the population.

Summary Points

- Iodine deficiency is associated with an increased thyroid gland.
- Introduction of iodine fortification in an iodine-deficient population results in lowering thyroid gland volumes.

- A relationship between iodine intake and thyroid size can be found when comparing different populations.
- A relationship between iodine intake and thyroid size within a population can be found if iodine intake is measured in a way that describes an individual's habitual iodine intake (e.g., food frequency or iodine in more than one 24-h urine sample) and corrected for other factors that influence the thyroid size.

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Thyroid Peroxidase Deficiency and Total Iodide Organification Defect

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Abstract

Thyroid hormone synthesis takes place at the follicular cell level through a number of different steps. The iodination of tyrosine residues or iodide organification, is catalyzed by the thyroperoxidase (TPO). TPO is a membrane-bound hemoprotein located at the apical membrane of the thyroid cell. Besides the binding of iodine to tyrosine, TPO also catalyzes the coupling of iodotyrosines to iodothyronine residues in thyroglobulin (Tg) under oxidative conditions. The human *TPO* gene, which localizes on chromosome 2p25, is divided into 17 exons and covers approximately 150 kilobases (kb) of DNA. Differently sized TPO mRNAs have been recognized in thyroid cells. TPO defects are among the most-frequent cause of inborn abnormalities of thyroid hormone synthesis. Nearly 50 different mutations in the *TPO* gene have been described, mostly single nucleotide substitutions and, in the minority, small deletions/insertions or splicing site mutations. Patients harboring TPO mutations present with total iodide organification defect (TIOD), due to impaired TPO function. This results in congenital hypothyroidism (CH), generally of a severe degree with high plasma thyroid-stimulating hormone (TSH) and Tg concentrations and low undetectable free thyroid hormone levels. A rapid and elevated radioiodine uptake by the thyroid gland is observed, with the complete and immediate release of the accumulated radioiodine from the thyroid after perchlorate (KClO₄) administration, indicating that iodide cannot be bound to proteins. According to the recessive mode of inheritance, affected subjects are homozygous or compound heterozygous for gene mutations. However, cases with a typical TIOD phenotype and a single TPO mutated allele have also been described, the frequency of this phenomenon being unexpectedly high (about 20% of reported families). The etiological diagnosis of CH, including dyshormonogenic defects due to TPO mutations, is based on clinical examination, biochemical tests, thyroid ultrasound and scintigraphy with KClO₄ discharge test.

It is usually performed either at birth, soon after the finding of elevated neonatal TSH levels, at 3–4 years of age after 1 month of L-thyroxine withdrawal, or on administration of RhTSH, which allows a precise diagnosis to be made and thus avoids the hypothyroid state. Finally, the severe hypothyroidism resulting from TPO mutations should be promptly treated with the thyroid hormone, in order to maintain TSH levels at the lower limit of the normal range.

Abbreviations

Bp	Base pair
CH	Congenital hypothyroidism
DIT	Diiodotyrosine
ER	Endoplasmic reticulum
kb	Kilobases
KClO ₄	Perchlorate
MIT	Monoiodotyrosine
NIS	Sodium iodide symporter
PIOD	Partial iodide organification defect
RhTSH	Recombinant human TSH
SSCP	Single-strand conformation polymorphism
T3	Triiodothyronine
T4	Thyroxine
Tg	Thyroglobulin
TIOD	Total iodide organification defect
TPO	Thyroid peroxidase
TSH	Thyroid-stimulating hormone

Introduction

Thyroid hormone synthesis takes place at the follicular cell level through a number of different steps (**Figure 56.1**). The active transport of iodide into the follicular cells is

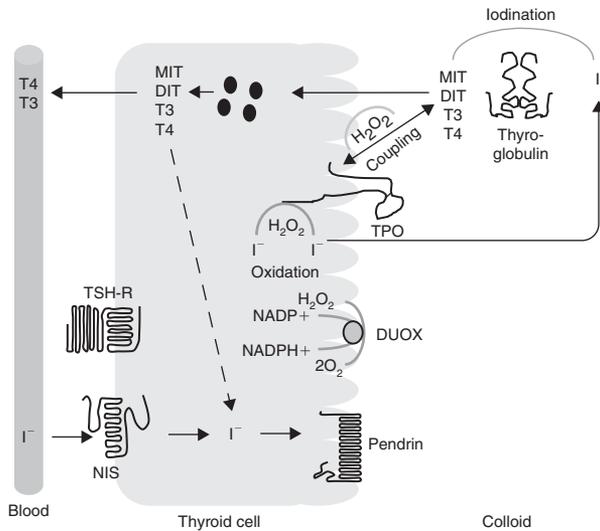


Figure 56.1 This figure gives a schematic representation of the follicular thyroid cell. Thyroid hormone synthesis takes place across a number of different steps, starting from iodide uptake and ending with the release of free thyroid hormones in the bloodstream.

mediated by the sodium iodide symporter (NIS) as the first crucial step in thyroid hormone synthesis (De la Vieja *et al.*, 2000; Dohan and Carrasco, 2003). Several homozygous or compound heterozygous mutations have been identified in individuals with hypothyroidism associated with impaired iodide uptake (De la Vieja *et al.*, 2000). In these patients, a diffuse or nodular goiter with impaired or highly reduced radioiodine uptake is observed. Iodide taken up by the thyrocytes is transported through the apical membrane by the anion transporter, pendrin (SCL26A4) (Yoshida *et al.*, 2002), and most likely by other transport systems not yet identified. Mutations in the *SCL26A4* gene cause Pendred's syndrome, an autosomal recessive disorder characterized by the association of sensorineural hearing loss and partial iodide organification defect (PIOD), with or without goiter and hypothyroidism (Everett *et al.*, 1997; Fugazzola *et al.*, 2002). The oxidation of iodide requires hydrogen peroxide that is synthesized outside the cell at the apical border. The generation of hydrogen peroxide is a crucial step in hormone synthesis, since this element is used by TPO as a substrate in the incorporation of iodine into thyroglobulin (Tg) (Corvilain *et al.*, 1991; Dupuy *et al.*, 1999). Two components of the hydrogen peroxide system, named DUOX1 and DUOX2, have recently been cloned (De Deken *et al.*, 2002) and immunolocalized on the apical membrane of thyrocytes together with the TPO enzyme (Caillou *et al.*, 2001). The genes coding for these two very similar NADPH oxidases are closely linked and located on chromosome 15q15.3. The structure of these proteins includes seven putative transmembrane domains, a NADPH and an FAD-binding site, two Ca²⁺-binding sites (EF hands),

and heme-binding sites. *DUOX2* gene mutations have been reported in patients with either mild congenital hypothyroidism (CH), transient or permanent, and PIOD, or with permanent and severe CH and complete iodide organification defect (Moreno *et al.*, 2002; Vigone *et al.*, 2005).

The iodination of tyrosine residues, or iodide organification, is catalyzed by the membrane-bound thyroperoxidase (TPO). Besides the binding of iodine to tyrosine, TPO also catalyzes the coupling of iodotyrosines to iodothyronine residues in Tg under oxidative conditions. TPO defects are among the most frequent causes of inborn abnormalities of thyroid hormone synthesis. Mutations in the *TPO* gene have been reported in numerous families with an organification defect (see below). Tg, a homodimeric glycoprotein, is another key element in thyroid hormone synthesis and storage. It is encoded by a very large gene, spanning more than 300 kb and containing 48 exons (Mendive *et al.*, 2001). In order to deliver thyroid hormone to the blood circulation, Tg is internalized by endocytosis that can take place by nonselective fluid phase uptake and by receptor-mediated processes (Zheng *et al.*, 1998; Ulianich *et al.*, 1999). Mutations in the Tg gene have been reported in patients typically presenting with goiter, euthyroidism, or hypothyroidism and high radioiodine uptake (van de Graaf *et al.*, 2001). After entering the thyroid cell by endocytosis, thyroxine (T₄), triiodothyronine (T₃), and the iodotyrosine monoiodotyrosine (MIT) and diiodotyrosine (DIT) are released by lysosomal proteolysis. Iodothyronine is secreted into the circulation at the basolateral membrane, noncovalently bound to plasma proteins and transported to target organs. The iodotyrosine molecules are deiodinated and the iodide can be reused as a substrate for the iodinating processes in the thyroid. Patients with leakage of MIT and DIT from the thyroid and urinary secretion of these metabolites have been reported (Medeiros-Neto and Stanbury, 1994).

TPO: Structure and Function

The human *TPO* gene, which localizes on chromosome 2p25, is divided into 17 exons and covers approximately 150 kilobases (kb) of DNA (Kimura *et al.*, 1989; Endo *et al.*, 1995). The 933 amino acid-containing protein is encoded by a *TPO* mRNA of 3 kb (Kimura *et al.*, 1987). TPO is a membrane-bound hemoprotein located at the apical membrane of the thyroid cell, and shows a transmembrane helix and a large N-terminal domain containing a heme group, essential for enzyme activity. Five potential glycosylation sites are present in the extracellular region, and several other heme-binding sites also exist. At least one disulfide bond in the extracellular region exists which creates a closed loop in this region of the protein. TPO is a member of the family of mammalian peroxidases that include myeloperoxidase, lactoperoxidase, eosinophil peroxidase and salivary peroxidase. Mammalian peroxidases

Table 56.1 Human thyroid peroxidase (h-TPO) isoforms

Name	Exons	Protein (amino acids)	mRNA size (kb)	Description	Author
h-TPO-1	1–17	933	3.1	Wild-type	Kimura <i>et al.</i> , (1987)
h-TPO-2	1–9, 11–17	876	2.9	171 bp deletion in exon 10 (1670–1840)	Kimura <i>et al.</i> , (1987)
h-TPO Zanelli	1–15, 17	929	3.1	Lacks exon 16	Zanelli <i>et al.</i> , (1990)
hTPO-I	1–6	225	2.1	Lacks exons 7–17	Nagayama <i>et al.</i> , (1990)
hTPO-II	1–5	174	1.7	Lacks exons 6–17	Nagayama <i>et al.</i> , (1990)

Note: The features of each hTPO isoform are given.

are large proteins (>700 amino acids in length), and most, if not all, contain covalently bound heme (Fenna *et al.* 1995; Andersson *et al.*, 1996). In particular, the amino acid sequence of TPO exhibits a high degree of sequence similarity (42% identity) to myeloperoxidase (Kimura and Ikeda-Saito, 1988). The *TPO* gene is exclusively expressed in the thyroid gland under the influence of the thyroid transcription factors NKX2.1 (TTF1), FKL15 (TTF2) and PAX8 (De Vijlder, 2003). Proper folding and membrane insertion are essential for enzyme activity. TPO is localized at the apical membrane by immunohistochemistry, but it is also abundant in the cytoplasm (Nilsson *et al.*, 1987; Pinchera *et al.*, 1987). Stimulation with thyroid-stimulating hormone (TSH) increases TPO immunoreactivity at the apical membrane and increases enzymatic activity (Bjorkman *et al.*, 1978; Chiovato *et al.*, 1985), suggesting that the TPO is brought to the apical pole through the secretory pathway. TSH stimulates TPO mRNA levels in a dose- and time-dependent manner (Nagayama *et al.*, 1989).

Differently sized TPO mRNAs have been recognized in thyroid cells (Table 56.1). Besides the normal 3.1 kb hTPO mRNA transcript (designated hTPO-1), a TPO mRNA with a 171 bp deletion (hTPO-2) within the 10th exon has been reported in Graves' thyroid tissue (Kimura *et al.*, 1987). hTPO-2 mRNA is also present in normal thyroid tissue and arises by alternate splicing and codes for a protein of 876 amino acids (57 amino acids shorter than the normal protein). There are other hTPO mRNA species present in the thyroid tissue. Zanelli *et al.* (1990) reported an alternate splicing leading to the loss of 130 nucleotides in exon 16 of hTPO. Because of a shift in the reading frame, a carboxyl-terminal extension would lead to a hTPO variant of 929 residues, almost the same length as wild-type TPO. This variant mRNA is highly represented in Graves' thyroid (Zanelli *et al.*, 1990). Moreover, two other transcripts of 2.1 and 1.7 kb (hTPO I and hTPO II, respectively) have been reported in human thyroid cells and tissues (Nagayama *et al.*, 1990). The hTPO I mRNA contains exons 1–6 followed by the 5' end of intron 6. hTPO II contains exons 1–5 and a 558 bp segment at its 3' end. The hTPO I and hTPO II transcripts would code for proteins of 225 and 174 amino acids, respectively. However, these proteins (if expressed) are likely to be nonfunctional because they lack

the functional region coded by exons 8–10 (Kimura and Ikeda-Saito, 1988; Kimura *et al.*, 1989).

TPO: Polymorphisms and Mutations

A number of single nucleotide polymorphisms, located in exons 2, 7, 8, 10, 11, 12 and 15, have been reported for the *TPO* gene. Interestingly, beside exonic variants, a 50 bp repeat in intron 10 has also been described as highly polymorphic, with a number of repeats varying from 9 to 31, but not influencing the alternative splicing of exon 10 (Bikker *et al.*, 1992). No correlation between polymorphisms and TPO dysfunction and/or predisposition to develop benign or malignant thyroid disease has been reported (Pirro *et al.*, 1995).

TPO mutations are among the most frequent cause of inborn abnormalities of thyroid hormone synthesis, as confirmed in a large survey indicating that *TPO* gene defects are the most common cause of severe defects in iodine organification (Bakker *et al.*, 2000).

The first mutation of the *TPO* gene was identified in 1992 (Abramowicz *et al.*, 1992). It was a GGCC duplication in exon 8, at nucleotide position 1227, which is a very common TPO mutation subsequently found in patients originating from different countries. Since then, nearly 50 different mutations have been described, mostly single nucleotide substitutions and, in the minority, small deletions/insertions or splicing site mutations (<http://www.hgmd.cf.ac.uk/ac/index.php>). Although hot spots cannot be identified, most mutations are located in exons 8, 9, and 14 (Figure 56.2).

TPO mutated proteins have been shown, by immunofluorescence studies, to be located in the endoplasmic reticulum (ER) and the nuclear envelope, being absent or only partially expressed at the apical membrane (Kotani *et al.*, 1999; Umeki *et al.*, 2002). Therefore, the abnormal TPO proteins are recognized by the ER "quality control" of the cell because of its improper folding, and cannot continue to move to the membrane.

Patients harboring TPO mutations, in compound heterozygosity or homozygosity, present with a total iodide organification defect (TIOD), due to the impaired TPO function (Abramowicz *et al.*, 1992; Bikker *et al.*, 1994,

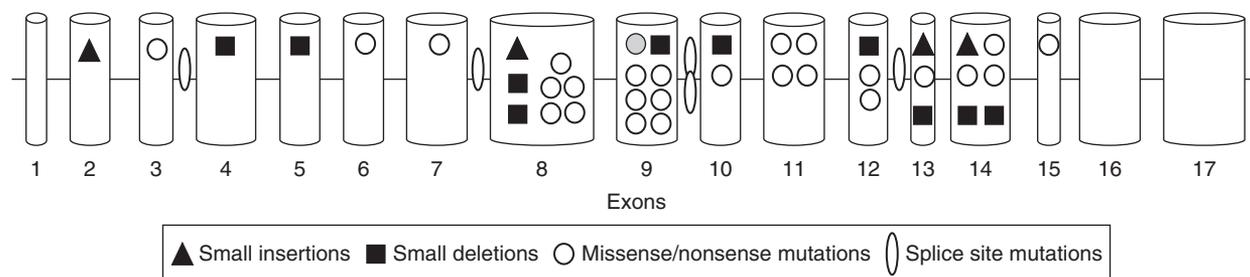


Figure 56.2 In this figure thyroid peroxidase (TPO) mutations up-to-date are described (<http://www.hgmd.cf.ac.uk/ac/index.php>). They are single nucleotide substitutions (missense/nonsense) and, in a minority of cases, small deletions/insertions or splicing site mutations. Most mutations are located at exons 8, 9 and 14. Exons are drawn to scale.

1995, 1997; Gruters *et al.*, 1996; Medeiros-Neto *et al.*, 1998; Bakker *et al.*, 2000; Ambrugger *et al.*, 2001; Fugazzola *et al.*, 2005). This results in CH, generally of a severe degree with high plasma TSH and Tg concentrations. A rapid and elevated radioiodine uptake is observed, with a complete release after perchlorate (KClO_4) administration, consistent with the defect in iodide organification.

According to the recessive mode of inheritance, affected subjects are homozygous or compound heterozygous for gene mutations. However, the literature reports other families from different origins with TIOD phenotype and a single TPO mutated allele (Wu *et al.*, 1990; Gruters *et al.*, 1996; Medeiros-Neto *et al.*, 1998; Santos *et al.*, 1999; Bakker *et al.*, 2000, 2001; Kotani *et al.*, 2001, 2003; Fugazzola *et al.*, 2003a, 2007; Rivolta *et al.*, 2003, 2007; Avbelj *et al.*, 2007). Indeed, more than 10 different TPO mutations (in exons 2, 8, 9, 12, 13 and 14) have been reported to occur in the simple heterozygous state in patients affected with TIOD originating from different countries. Moreover, the percentage of these cases reaches 20% of all cases associated with TPO defects, suggesting that alterations that are not recognized at the *TPO* gene might be rather frequent. The clinical features (including thyroid function parameters and scintigraphic evaluations) reported in these patients do not differ from those observed in patients harboring two mutant TPO alleles. The hypothesis that a single mutated allele could confer the phenotype, already discarded in a large study on 46 Dutch families (Bakker *et al.*, 2000), has recently been tested by a full clinical evaluation, including KClO_4 discharge test, in heterozygous carriers of two different TPO mutations (Fugazzola *et al.*, 2005). The evaluations of these individuals showed a normal thyroid morphology and function, demonstrating *in vivo* that a single wild-type TPO allele is sufficient to maintain required enzymatic activity, consistent with the finding of unaffected obligate heterozygous parents in all the families studied. A normal thyroid function was also demonstrated in a peripubertal heterozygous carrier, suggesting that, contrary to what was

reported for TIOD due to DUOX2 mutations (Moreno *et al.*, 2002), one TPO allele is able to maintain sufficient thyroid hormone production even during a period of increased hormone requirements.

The explanation for TIOD in these single heterozygous cases is presently unknown. One hypothesis is that the mutation in the other allele could be located in the more upstream part of the *TPO* gene, or within the intronic sequences, creating alternative splicing sites. The presence of large gene deletions, not revealed at single-strand conformation polymorphism (SSCP) analysis, has also been advocated (Wu *et al.*, 2002). A definite explanation is available for at least three cases (Figure 56.3). Maternal isodisomy for chromosome 2p was reported in one case (Bakker *et al.*, 2001), and deletion of the paternal *TPO* gene at chromosome 2p25 in another (Kotani *et al.*, 2001). Furthermore, we have recently described one family in which the three children were affected with TIOD, due to the association of a paternal mutation with a defective transcription of the maternal allele (Fugazzola *et al.*, 2003a, b). Indeed, the haplotype of the TPO alleles, performed thanks to the presence of numerous informative polymorphisms both in the coding and uncoding regions, proved that in this family the TIOD phenotype is the result of the monoallelic expression of a mutant allele, due to the nontranscription of the maternal allele at the thyroid tissue level (Figure 56.3). The alteration in the TPO locus (2p25) responsible for the hemizygoty demonstrated at the thyroid tissue level could not be identified. In particular, genomic imprinting of chromosome 2 or *TPO* gene was excluded from the revision of the literature and by appropriate testing (Fugazzola *et al.*, 2003a, b).

It is conceivable that the same defect could be involved in other TIOD cases, with a single TPO mutation reported in the literature. However, this is difficult to prove since thyroid tissue is not often available in the families studied. Indeed, early detection and appropriate treatment of TIOD patients usually prevents the formation of large goiters requiring thyroidectomy. As a consequence, thyroid tissue from these patients is seldom obtainable.

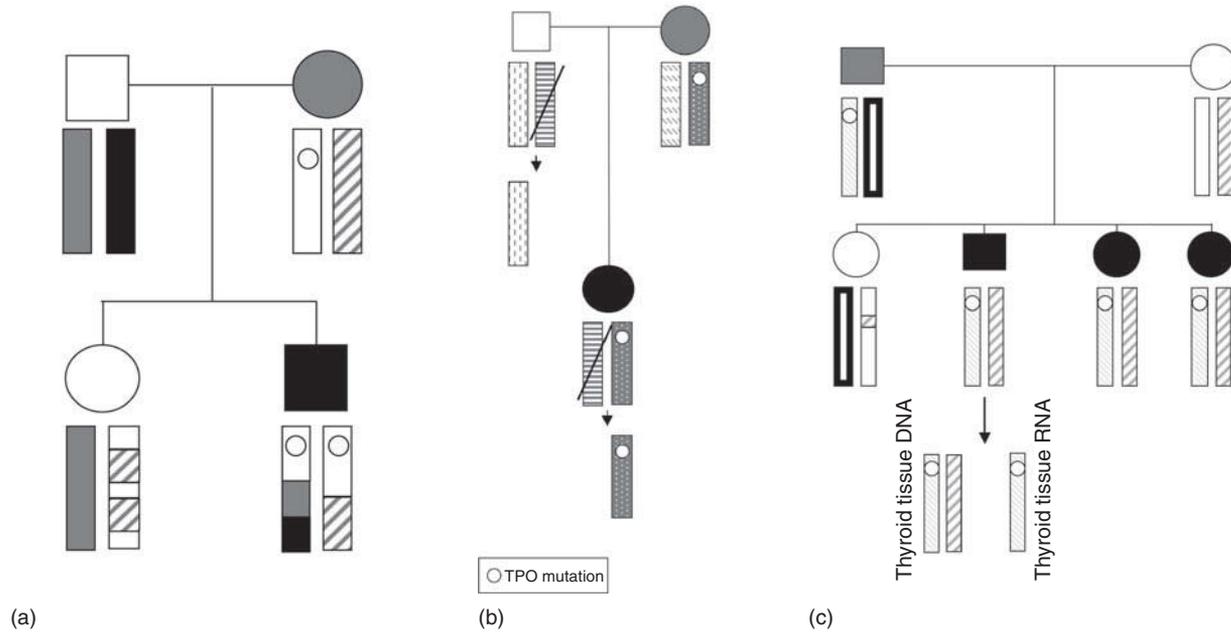


Figure 56.3 The figure shows total iodide organification defect (TIOD) with a single germline TPO mutation. The only three cases with a known genetic basis for the phenomenon are represented. (a) The affected male patient had a homozygous deletion (delT2512) at codon 808 in exon 14. The homozygosity in the patient was due to partial isodisomy of the short arm of chromosome 2 carrying a defective *TPO* gene (Bakker *et al.*, 2001). (b) The affected female had the TPO mutation 2512delT in hemizygosity. This was due to cosegregation of the maternal mutated TPO allele with the 2p25-deleted paternal null allele (Kotani *et al.*, 2001). (c) In the three children, the TIOD phenotype is the result of monoallelic expression of the paternal mutant allele due to the nontranscription of the maternal allele at the thyroid tissue level (Fugazzola *et al.*, 2003a, b). Black symbols indicate affected members, gray symbols unaffected carriers and white symbols noncarriers.

Congenital Hypothyroidism due to TPO Deficiency

Clinical findings

As described above, for thyroid development and for synthesis, storage and secretion of thyroid hormone, a variety of transcription factors and a sequence of precisely tuned events are required. Disorders in one of these factors, or in the intermediate reaction steps, are the molecular bases of abnormalities in thyroid development and/or in thyroid hormonogenesis that result in CH. The incidence of permanent CH is about 1:3000 in newborns (Fisher, 1991; American Academy of Pediatrics & American Thyroid Association, 1993; Toublanc *et al.*, 1999). In most cases, CH is related to developmental defects of the thyroid gland (dysgenesis), which includes agenesis, hypoplasia, or arrested migration of embryonic thyroid cells (ectopy). In approximately 15% of patients, hereditary disorders in thyroid hormonogenesis are found (Devos *et al.*, 1999; Foley, 2000; De Vijlder, 2003). Among these, defects in the *TPO* gene account for almost all the total organification defects, which have an incidence of about 1:60 000 births. Absence of TPO activity indicates the

inability to iodinate tyrosine residues in Tg and to couple these residues to form thyroid hormones.

TPO mutations lead to a typical phenotype characterized by large goiters, a complete discharge of iodide following KClO_4 and severe hypothyroidism (Table 56.2). Other clinical features, such as deafness, language disturbances and low IQ, can also be associated, especially if CH is not treated in the first weeks of life (Fugazzola *et al.*, 2003a, b; Tenenbaum-Rakover *et al.*, 2007). Biochemically, TSH levels are extremely elevated with low or undetectable free thyroid hormones levels. Tg levels are high and, after TSH stimulation, an exaggerated rise in serum Tg reflects an increase in colloid storage capacity. Usually, the Tg is poorly iodinated, and after hydrolysis mostly yields DIT and MIT (Manglabruks *et al.*, 1991). During an ultrasound, the thyroid displays a normal echogenicity and multiple nodules are often present. Thyroid hyperplasia compensates, to some degree, for the lack of adequate iodide organification.

At histology, goiters harbor microfollicular patterns, scant colloid and sometimes aberrant nuclei. The degree of hyperplasia in many areas is high and sometimes suggestive of malignant neoplasia. The association of thyroid cancer has

Table 56.2 Thyroid peroxidase (TPO) deficiency

	Description
Clinics	
Congenital hypothyroidism	Severe
Goiter	Multinodular
Deafness	
Language disturbances	
Low IQ	Nontreated cases
Motor handicaps	
Laboratory findings	
High TSH	
Low undetectable FT4	
High Tg	Poorly iodinated
TPO gene mutations	Germline; homozygosity or compound heterozygosity
Instrumental investigations	
Large multinodular goiter	Ultrasound
Elevated radioiodine uptake	Scintigraphy
Positive perchlorate test	Total iodide organification defect (>90%)

Note: Clinical, laboratory, and instrumental findings in affected patients are reported.

also been described (Medeiros-Neto *et al.*, 1998), probably due to prolonged TSH stimulation. After the administration of radioiodine, a high uptake is observed, and the uptake at 2 h can be higher than that obtained at 24 h, indicating a rapid intraglandular turnover with a spontaneous release of the nonorganified iodide. After the administration of KClO_4 (usually 1 g for adults and 400–500 mg for children), a rapid (within 1 h) and almost complete discharge (>90%, normal values: <15%) is observed, confirming the lack of organification of radioiodine (Figure 56.4).

TPO activity in thyroid tissue of patients with TIOD is not detectable (Bikker *et al.*, 1994; Bikker *et al.*, 1995). Indeed, by assaying TPO in the subcellular fraction of homogenized thyroid tissue, a complete absence, or only a minimal oxidation of iodide induced by the solubilized enzyme, is observed (Medeiros-Neto *et al.*, 1993), confirming the TPO activity defect. This is consistent with the finding of a TIOD in association with TPO mutations. Indeed, in a large Dutch series (Bakker *et al.*, 2000), as well as in the cohorts studied by myself (Fugazzola *et al.*, 2003a, b, 2005, 2007), homozygous or compound heterozygous mutations in the *TPO* gene are invariably associated with the total absence of peroxidase and hormone-producing activities, without variable expression or presentation of the disease. At variance, some authors described a PIOD in association with TPO mutations (Abramowicz *et al.*, 1992; Santos *et al.*, 1999; Tenenbaum-Rakover *et al.*, 2007; Nascimento *et al.*, 2003). The molecular mechanism for PIOD is not completely understood. In most of these cases, only one mutated allele or some polymorphisms have been identified. Thus, it has been hypothesized that minor alterations on the *TPO* gene could lead to improper folding and abnormal apical membrane expression. A further possibility could

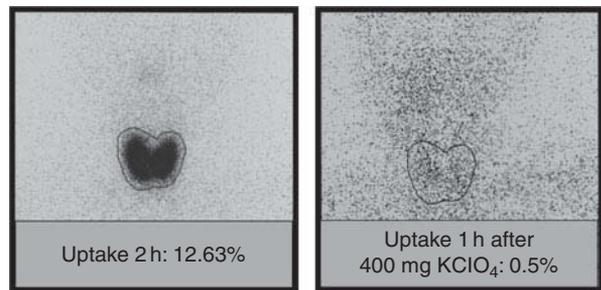


Figure 56.4 The figure shows thyroid scintigraphy with ^{123}I and a perchlorate (KClO_4) testing in a child affected with TIOD due to TPO mutations. A high radioiodine uptake (12.63%, normal values 1–5) is observed at the second hour, and a total discharge 1 h after the administration of 400 mg of KClO_4 is also documented.

be inactivating mutations of the *DUOX 2* gene, previously also described in patients with PIOD (Moreno *et al.*, 2002; Vigone *et al.*, 2005), as well as interindividual or intraindividual factors, such as variable gastrointestinal absorption rates, or epidemiological factors, such as iodine supplementation, that may affect the outcome of the KClO_4 test.

Diagnosis

The differential diagnosis of CH is mainly aimed at distinguishing between transient and permanent hypothyroidism, in order to avoid unnecessary treatment and psychosocial problems due to continuous monitoring. Moreover, an accurate definition of the defect is required prior to undertaking appropriate genetic investigations and counseling. The etiological diagnosis of CH is based on clinical examination, biochemical tests, thyroid ultrasound and scintigraphy (with or without KClO_4 discharge test). It is usually performed at birth, soon after the finding of elevated neonatal TSH levels, or at 3–4 years of age after 1 month of L-T4 withdrawal (Fisher, 1991; Toublanc *et al.*, 1999). Alternatively, the administration of recombinant human TSH (rhTSH) (Thyrogen®, Genzyme Corp., Cambridge, MA, USA) can effectively allow precise diagnosis, avoiding the hypothyroid state deriving from T4 withdrawal. Testing by rhTSH is particularly useful in patients with clinical suspicion of a dyshormonogenic defects. Indeed, the discontinuation of L-thyroxine needed to perform the discharge test is not recommended, since hypothyroidism can induce dramatic thyroid enlargement in these cases. Two different testing protocols have been proposed: (a) two injections (4 $\mu\text{g}/\text{kg}$ each) to children with glands *in situ*; and (b) three injections to patients with neonatal diagnosis of agenesis, in order to enhance testing sensitivity for small thyroid tissue remnants (Fugazzola *et al.*, 2003a, b, 2007). TSH levels peak up to 60 mU/l at 24 h from the last injection, while free thyroid hormones levels remain in the normal range, as do antithyroid auto antibodies. In patients with

TIOD, Tg shows a mean of three-fold elevation after rhTSH stimulus, while blunted Tg responses are found in patients with agenesis or resistance to TSH action. A thyroid scintigraphy (TS) with ^{123}I and a KClO_4 discharge test can be performed 24 h after the last rhTSH injection. The test is always well-tolerated and allows an accurate description of the underlying defect, avoiding L-T4 withdrawal and the untoward effects of hypothyroidism. Moreover, rhTSH testing is cost-effective, since the dose used for each diagnosis is extremely low.

Treatment

As mentioned above, TPO defects lead to a congenital impairment in thyroid hormone organification, associated with severe CH and high TSH levels. L-T4 treatment should thus be promptly started at doses corresponding to about $100\ \mu\text{g}/\text{m}^2$ of body surface area. The dose should be adjusted in order to maintain TSH levels in the normal limit. However, it must be noted that some patients develop goiter, despite precocious treatment with thyroid hormone at replacement doses. Some evidence exists indicating that the early start of L-T4 treatment at doses able to decrease TSH to its lower levels (0.5–1 mU/l) can prevent goiter development in patients with TIOD due to TPO mutations (Fugazzola *et al.*, 2005, 2007).

Summary Points

- Thyroid peroxidase, TPO, is a large heme-containing glycoprotein that plays a key role in the biosynthesis of thyroid hormones. It catalyzes iodide oxidation, iodination of tyrosine residues and coupling of iodotyrosines to generate the iodothyronines T3 and T4.
- Differently sized TPO mRNAs have been recognized in thyroid cells. Besides the normal 3.1 kb hTPO mRNA transcript (hTPO-1), hTPO-2 mRNA caused by alternate splicing and coding for a protein of 876 amino acid has also been described. Other variants include TPO-Zanelli (with a loss of 130 nucleotides in exon 16) and the shorter hTPO I and hTPO II (2.1 and 1.7 kb, respectively).
- Nearly 50 different mutations have been described, mostly single nucleotide substitutions and, in the minority, small deletions/insertions or splicing site mutations. Patients harboring TPO mutations, in compound heterozygosity or homozygosity, present with a TIOD, due to the impaired TPO function, resulting in CH.
- According to the recessive mode of inheritance, affected subjects are homozygous or compound heterozygous for gene mutations. However, about 20% of the reported families with a TIOD phenotype harbor a single TPO mutated allele. The explanation for TIOD in these single heterozygous cases is presently unknown, except for three cases.
- TPO mutations lead to a typical phenotype characterized by large goiters, a complete discharge of iodide following KClO_4 and severe hypothyroidism. TSH levels are extremely elevated, with low or undetectable free thyroid hormones levels and high Tg levels. After the administration of radioiodine, a high uptake is observed and the uptake at 2 h can be higher than that obtained at 24 h, indicating a rapid intraglandular turnover with a spontaneous release of nonorganified iodine. After the administration of KClO_4 , a rapid and almost complete discharge (>90%) is observed, confirming the lack of organification of radioiodine.
- The etiological diagnosis of CH is based on clinical examination, biochemical tests, thyroid ultrasound and scintigraphy (with or without a KClO_4 discharge test). It is usually performed at birth, soon after finding of elevated TSH levels, or at 3–4 years of age after 1 month L-T4 withdrawal. Alternatively, diagnostic tests can be effectively performed after the administration of RhTSH (Thyrogen®, Genzyme Corp., Cambridge, MA).

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Iodine Deficiency, Antioxidant Response and Mutagenesis in the Thyroid Gland: Antioxidant Response and Mutagenesis

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Abstract

The thyroid gland is the organ that is most prominently affected by a lack of regular intake of iodine, a trace mineral, key nutritional factor and indispensable chemical component of thyroid hormones. Because thyroid hormones are essential for the human body, iodine deficiency can cause considerable deviation from normal physical and mental development, including growth. It is the major cause of thyroid enlargement or goiter, which in part is a maladaptation to iodine deficiency. In the long run, goiter is a preferred substrate for the development of thyroid nodules, some of which are thyroid carcinomas. We would therefore, like to summarize scientific knowledge that constitutes a link between iodine deficiency and thyroid physiology to form the molecular basis for increased thyroid mutagenesis, nodular transformation, and tumorigenesis. Tumor-causing events are triggered by the oxidative nature of thyroid hormone synthesis or additional oxidative stress caused by iodine deficiency. If the antioxidant defense is not effective, this oxidative stress causes DNA damage, followed by an increase in spontaneous mutation rate and tumor formation.

Abbreviations

8-OHG8	oxo-Guanosine
8-OHdG	8-oxo-2'-Deoxyguanosine
AFTN	Autonomously functioning thyroid nodules
cAMP	Cyclic adenosine monophosphate
DNA	Deoxyribonucleic acid
dTMP	Deoxythymidine monophosphate
dUMP	Deoxyuridine monophosphate
GPx	Glutathione peroxidase
GthT	Glutathione transferase

OGG1	8-oxoguanine DNA glycosylase
PRDX	Peroxiredoxins
ROS	Reactive oxygen species
SMR	Spontaneous mutation rate
SOD	Superoxide dismutase
T3	L-3,5,3'-Triiodothyronine
T4	L-3,5,3',5'-Tetraiodothyronine
ThOX	Thyroid oxidase
TPO	Thyroid peroxidase
TSH	Thyroid-stimulating hormone
TSHR	TSH receptor

Introduction

The main function of the thyroid gland is to synthesize the thyroid hormones L-3,5,3',5'-tetraiodothyronine (thyroxin, T₄) and L-3,5,3'-triiodothyronine (T₃). The thyroid takes up iodine from the food supply and incorporates it into thyroglobulin, the precursor of the thyroid hormones. Thyroid hormone receptors are essential for the regulation of cellular metabolism and belong to the molecular set-up of every cell in the body. Therefore, iodine deficiency will potentially challenge homeostasis of the body by unsettling the synthesis of thyroid hormones in the thyroid gland. Hence, it is inevitable that mechanisms to balance possible disturbances caused by the lack of iodine have evolved. Clinically, iodine deficiency is most often noticed as thyroid enlargement or goiter, which only in severe cases leads to hypothyroidism, impaired physical and mental development and growth retardation (Delange, 1994). Regions with replete iodine nutrition achieved by iodine prophylaxis or iodized table salt have a much lower incidence of goiter. A moderately enlarged thyroid with increased thyrocyte function that could optimize iodine trapping is the

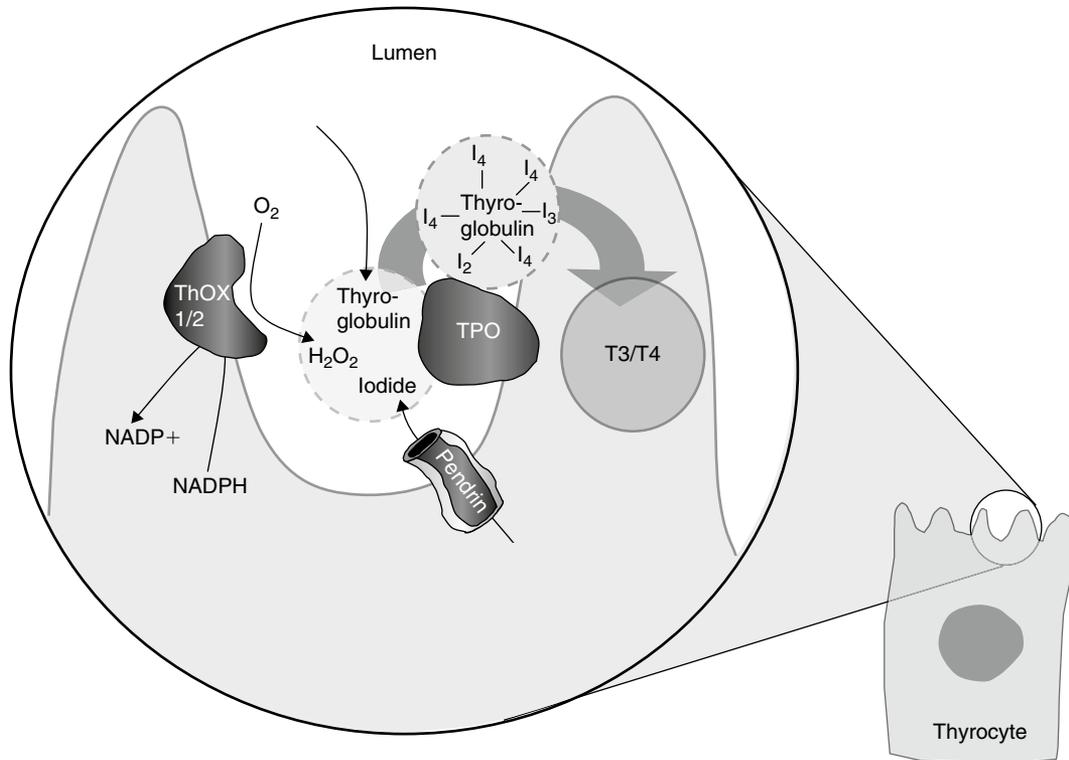


Figure 57.1 The essence of thyroid hormone synthesis. This figure illustrates key steps and proteins of thyroid hormone synthesis. H₂O₂ and iodine are substrates for the iodination of tyrosyl residues on the precursor protein thyroglobulin. The reaction takes place in the follicular lumen near the apical membrane. Thyroid oxidases (ThOX) 1 and 2 reduce O₂ and generate H₂O₂, while oxidizing the reduced form of nicotinic acid dinucleotide phosphate (NADPH). After transport to the lumen by pendrin, iodine is oxidized by thyroid peroxidase (TPO) and transferred to the tyrosyl groups of thyroglobulin. Later, thyroid hormones (T₃, T₄) are proteolytically released from thyroglobulin after internalization and vesicular fusion with endosomes.

most adequate response to iodine deficiency. Large goiters present a maladaptation to iodine deficiency (Dumont *et al.*, 1995); in the long run, goiter serves as a preferred substrate for the development of thyroid nodules. Although many of them are benign thyroid tumors, the risk of malignancy is still a serious diagnostic issue.

Substrates of Thyroid Hormone Metabolism: Iodine and H₂O₂

Thyroid hormone synthesis involves the iodination of tyrosyl residues on the precursor protein thyroglobulin (Figure 57.1). This reaction uses high concentrations of H₂O₂ and oxidized iodine generated by the enzymes thyroid oxidase (ThOX) 1 and 2, and thyroid peroxidase (TPO). Whereas H₂O₂ is available through synthesis, iodine stems from the nutritional supply, is transported into the blood, and is actively taken up by the thyrocytes. Low iodine content in the diet causes iodine deficiency in humans. Three factors contribute to low iodine content of the diet. First, melting of quaternary glaciers washed most of the iodine out of the soil into the sea. Secondly, all

land plants are unable to capture and concentrate iodide or iodine. Thirdly, there is a continued depletion of iodine from the soil because iodide is oxidized by sunlight to iodine, further vaporized into the air, and finally lost into the stratosphere. Iodine deficiency is particularly frequent at higher altitudes, in countries where seafood is not part of a regular diet and table salt is not supplemented with iodide. Currently, it represents a significant public health concern for about 30% of the world's population in 110 countries. It has severe consequences even in developed areas, such as Europe (Vitti *et al.*, 2003). Iodine status worldwide is summarized in the WHO Global Database on Iodine Deficiency (<http://www.who.int/vmnis/iodine/data/database/countries/en/index.html> Delange *et al.*, 2002). One of the most obvious clinical manifestations in geographical areas with iodine deficiency is the thyroid enlargement known as endemic goiter. This enlargement is caused by increased cellular proliferation (Figure 57.2) and supports nodular transformation of the thyroid (Belfiore *et al.*, 2001). However, iodine-deficiency-related thyroid diseases are not restricted to nodules. They typically range from goiter to mild hypothyroidism, and to the severest, but rare, form of cretinism with severe hypothyroidism and

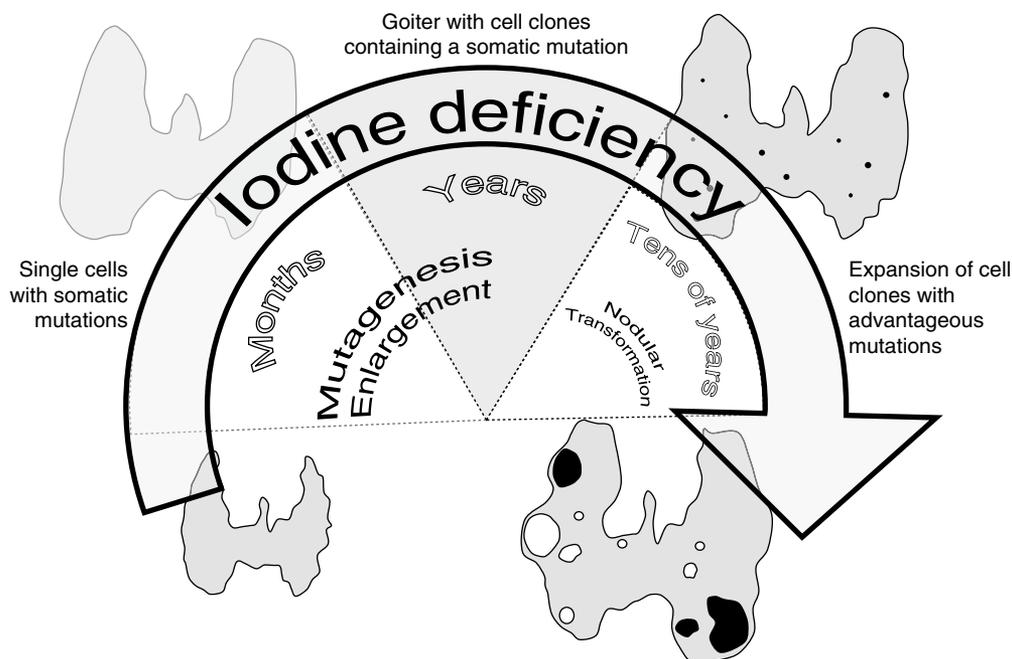


Figure 57.2 Iodine deficiency, thyroid enlargement and tumor growth. The starting point for the development of thyroid nodules is hyperplasia induced by iodine deficiency. Iodine deficiency could increase mutagenesis directly (oxidative stress, DNA damage) or indirectly (proliferation and increased number of cell divisions). Subsequently, ongoing hyperplasia forms cell clones. Some of them contain somatic mutations leading to cold (white) or hot (black) thyroid nodules.

metal retardation. These pathologic manifestations are a direct consequence of iodine deficiency and impairment of thyroid hormone synthesis. In contrast, nodular transformation, as outlined below, is very likely an indirect effect linked to the cosubstrate in thyroid hormone synthesis, H_2O_2 , and its oxidative consequences.

H_2O_2 , one of the most powerful oxidizers, gives rise to free radicals and reactive oxygen species [ROS, e.g., hydroxyl radical (*OH), [Figure 57.3](#)] in a process catalyzed by transition metal ions, typically Fe^{2+} , known as the Haber–Weiss and Fenton reaction ([Shcherbakova et al., 2006](#)). Besides being a substrate in hormone synthesis, H_2O_2 is very harmful to any cell. It has been estimated that the genome of a mammalian cell receives about 10^4 – 10^5 oxidative hits per day ([Beckman and Ames, 1997](#)). Although there are no numbers specifically detailed for thyrocytes, H_2O_2 generated during thyroid hormone biosynthesis adds an extra hazard for the deoxyribonucleic acid (DNA) of thyroid epithelial cells.

To avoid substantial damage and impaired normal function thyroid epithelial cells have a potent defense mechanism to counterbalance radical attack. It has been shown that antioxidant enzymes, such as glutathione peroxidases (GPxs) or TPO, are upregulated during thyroid hormone synthesis ([Howie et al., 1998](#)). Moreover, GPx3 has been suggested to interfere directly with thyroid hormone synthesis by affecting the concentration of H_2O_2

([Howie et al., 1995](#)). Therefore, if antioxidant defense is not effective enough, excessive damage (e.g., peroxidation) should be detectable predominantly not only in DNA, but also in the lipids and proteins of thyroid epithelial cells.

Antioxidant Response during Iodine Deficiency

As outlined above, iodine and H_2O_2 act as cosubstrates in thyroid hormone synthesis. An impairment of thyroid hormone production usually releases the negative endocrine feedback loop via the hypothalamic–pituitary axis. As a consequence, activation of thyrocytes activates iodine uptake and H_2O_2 synthesis. However, since iodine is not sufficiently available, H_2O_2 very likely accumulates near the apical membrane. Although direct evidence for such an outcome is still absent several facts indirectly support this assumption. Generation of H_2O_2 is inhibited by iodide *in vivo* and *in vitro* ([Ohayon et al., 1994](#); [Cardoso et al., 2001](#)). Moreover, H_2O_2 generation, which is mandatory for the organification of iodine, is stimulated by thyroid-stimulating hormone (TSH)/cyclic adenosine monophosphate (cAMP) ([Raspe and Dumont, 1995](#)). Low iodine and markers of increased thyroid functionality suggest activated H_2O_2 generation, which could result in DNA damage and somatic mutation ([Cooke et al., 2003](#)). Protein radical formation and damage to thyroid peroxidase caused by

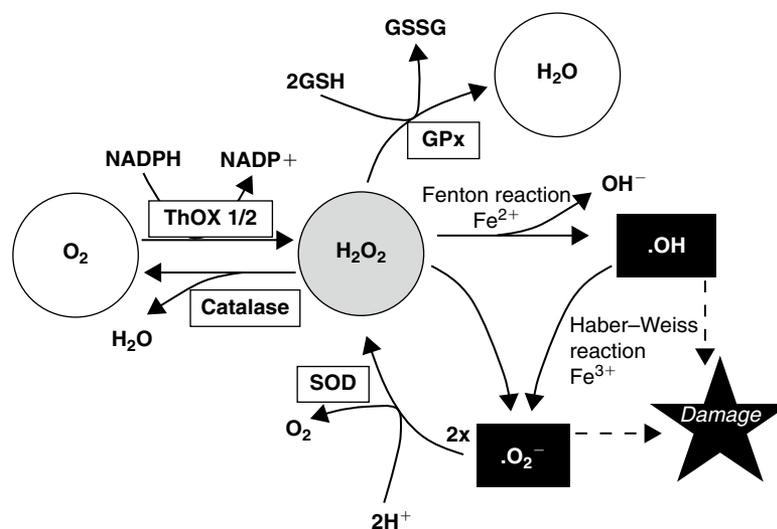


Figure 57.3 Pathways of reactive oxygen radical generation and antioxidant defense. H_2O_2 is very harmful to any cell. Besides being a substrate in thyroid hormone synthesis generated by thyroid oxidases 1 and 2, H_2O_2 is one of the most powerful oxidizers. It gives rise to free radicals and reactive oxygen species like hydroxyl radical ($\cdot\text{OH}$) and the superoxide radical (O_2^-) in a process catalyzed by transition metal ions, typically Fe^{2+} and Fe^{3+} known as the Fenton and Haber–Weiss reaction. These free radicals cause damage to DNA, lipids and proteins. Antioxidant defense is formed by the enzymes catalase, superoxide dismutase (SOD) and different glutathione peroxidases (GPx). Peroxiredoxins work similarly to GPx, but use internal thiol groups instead of the cofactor glutathione.

the substrate H_2O_2 have been reported (Ehrenschaft and Mason, 2006). The extent of radical formation and TPO damage is reduced by the cosubstrate iodine. Low iodine and high H_2O_2 therefore causes oxidative stress, which in turn activates antioxidative defense. This is detectable in the cellular regulation of enzymes involved in the defense against oxidative stress. Enzymes involved in H_2O_2 and superoxide detoxification represent prominent markers for such a scenario. Indeed, a higher mRNA expression of superoxide dismutase (SOD)-3, the extracellular SOD isoform which preferentially acts in the lumen where H_2O_2 is generated, is detected during experimental iodine deficiency in mice (Maier *et al.*, 2007). Low iodine intake increases total SOD enzyme activity in the thyroid (Maier *et al.*, 2007). Moreover, mRNA expression of the glutathione-dependent enzymes (i.e., GPx3, GPx4 and GthT, glutathione transferase) and two peroxiredoxins (i.e., PRDX3 and PRDX5) changes (Maier *et al.*, 2007). PRDX are especially related to H_2O_2 detoxification (Kim *et al.*, 2000), and PRDX5 shows a strongly increased immunolabeling in goitrous rats fed a low iodine diet. Interestingly, for PRDX2 and PRDX6 upregulation has been shown in cold thyroid nodules (Krause *et al.*, 2007), which have a status of intranodular iodine deficiency (Eszlinger *et al.*, 2001).

Markers of Oxidative DNA Damage

Although the chemical compound H_2O_2 itself is almost inert toward DNA, the H_2O_2 -derived $\cdot\text{OH}$ radicals are actively involved in the oxidation of DNA. $\cdot\text{OH}$ -induced

DNA oxidation comprises five kinds of damage, including oxidized bases (Bjelland and Seeberg, 2003), abasic sites, DNA–DNA intrastrand adducts (Randerath *et al.*, 1996; Lloyd *et al.*, 1997), DNA strand breaks and DNA–protein cross-links (Cadet, 1994; Cadet *et al.*, 1997). Owing to their significance for survival, cells have developed mechanisms to detect the presence of ROS to regulate metabolism and antioxidant responses (Demelash *et al.*, 2004). ROS-derived signals are used by cells to regulate growth or proliferation (Burdon, 1995), cell differentiation (Hansberg and Aguirre, 1990; Toledo *et al.*, 1994), and death (Buttke and Sandstrom, 1994; Riou *et al.*, 1999; Demelash *et al.*, 2004). In thyrocytes, the H_2O_2 -mediated cytotoxicity appears to be dose-dependent, requiring only low concentrations to result in thyroid cell apoptosis rather than in necrosis (Riou *et al.*, 1999; Demelash *et al.*, 2004). Furthermore, findings in the thyroid from male Wistar rats suggest that the predominant cytotoxic response to oxidative stress might differ, depending on the functional state of the gland (Mutaku *et al.*, 2002; Demelash *et al.*, 2004).

Of the more than 20 DNA base products and intermediates identified, which are caused by reactive oxygen and free radical species (Table 57.1), 8-oxo-2'-deoxyguanosine (8-OHdG) is the most frequently investigated DNA adduct (Halliwell, 1998). Because of its mutagenic character and the high sensitivity of its immunological detection, 8-OHdG serves as the ultimate marker for oxidative DNA damage. In thyroid tissue sections, staining with an antibody to 8-oxoguanine to detect 8-OHdG or 8-oxo-guanosine (8-OHG) (the modified RNA nucleotide) is most prominent in the follicular cells near the lumen where

Table 57.1 Known oxidative DNA modifications and their consequences for mutations

DNA modification	Mutation (base change)	Species/cell line	References
5-Formyluracil	C → T	<i>E. coli</i>	Anensen <i>et al.</i> , 2001; Cooke <i>et al.</i> , 2003
	G → T	<i>E. coli</i>	Anensen <i>et al.</i> , 2001; Cooke <i>et al.</i> , 2003
	T → C	<i>E. coli</i>	Anensen <i>et al.</i> , 2001; Miyabe <i>et al.</i> , 2001; Zhang, 2001; Cooke <i>et al.</i> , 2003
	T → A	<i>E. coli</i>	Anensen <i>et al.</i> , 2001; Miyabe <i>et al.</i> , 2001; Zhang, 2001; Cooke <i>et al.</i> , 2003
	T → G	<i>E. coli</i>	Zhang <i>et al.</i> , 1997; Cooke <i>et al.</i> , 2003
5-Hydroxyuracil	C → T	<i>E. coli</i>	Kasprzak <i>et al.</i> , 1997; Kreutzer and Essigmann, 1998; Cooke <i>et al.</i> , 2003; Evans <i>et al.</i> , 2004
5,6-Dihydrouracil	G → A	<i>In vitro</i> transcription	Liu <i>et al.</i> , 1995; Cooke <i>et al.</i> , 2003
5,6-Dihydroxyuracil			Kasprzak <i>et al.</i> , 1997; Cooke <i>et al.</i> , 2003
5-Hydroxy-6-hydrouracil			Cooke <i>et al.</i> , 2003
5-Hydroxymethyluracil	C → T	<i>E. coli</i>	Cannon-Carlson <i>et al.</i> , 1989; Kasprzak <i>et al.</i> , 1997; Hori <i>et al.</i> , 2003; Cooke <i>et al.</i> , 2003
Uracil glycol	C → T	<i>E. coli</i>	Kreutzer and Essigmann, 1998; Cooke <i>et al.</i> , 2003; Evans <i>et al.</i> , 2004
5-Hydroxymethylcytosine	C → T	Bacteriophage T4	Baltz <i>et al.</i> , 1976; Hori <i>et al.</i> , 2003
5-Hydroxycytosine	C → T	<i>E. coli</i>	Kasprzak <i>et al.</i> , 1997; Kreutzer and Essigmann, 1998; Cooke <i>et al.</i> , 2003; Evans <i>et al.</i> , 2004
5,6-Dihydroxycytosine			Cooke <i>et al.</i> , 2003
5-Hydroxy-6-hydroxycytosine			Cooke <i>et al.</i> , 2003
5-Formylcytosine	C → T	Hypothetical	Karino <i>et al.</i> , 2001; Evans <i>et al.</i> , 2004
	C → A	Hypothetical	Karino <i>et al.</i> , 2001; Evans <i>et al.</i> , 2004
Cytosine glycol			Cooke <i>et al.</i> , 2003
8-Hydroxyguanine	G → T	NIH3T, COS-7	Kasprzak <i>et al.</i> , 1997; Tan <i>et al.</i> , 1999; Zhang, 2001; Cooke <i>et al.</i> , 2003; Evans <i>et al.</i> , 2004; Kamiya, 2004
	G → C	NIH3T, COS-7	Tan <i>et al.</i> , 1999; Zhang, 2001; Cooke <i>et al.</i> , 2003; Kamiya, 2004
	G → A	NIH3T, COS-7	Tan <i>et al.</i> , 1999; Kamiya, 2004
	A → C	<i>E. coli</i>	Cheng <i>et al.</i> , 1992; Evans <i>et al.</i> , 2004
8-Hydroxyadenine	A → G	COS	Tan <i>et al.</i> , 1999; Tuo <i>et al.</i> , 2003; Cooke <i>et al.</i> , 2003
	A → C	COS	Tan <i>et al.</i> , 1999; Tuo <i>et al.</i> , 2003
2-Hydroxyadenine	A → G	<i>E. coli</i> ; COS-7	Cooke <i>et al.</i> , 2003; Evans <i>et al.</i> , 2004; Kamiya, 2004
	A → T	<i>E. coli</i> ; COS-7	Evans <i>et al.</i> , 2004; Kamiya, 2004
	A → C	<i>E. coli</i>	Evans <i>et al.</i> , 2004; Kamiya, 2004
5-Hydroxy-6-hydrothymine			Cooke <i>et al.</i> , 2003
Thymine glycol	T → C	<i>E. coli</i>	Basu <i>et al.</i> , 1989; Cooke <i>et al.</i> , 2003; Evans <i>et al.</i> , 2004
5,6-Dihydrothymine			Cooke <i>et al.</i> , 2003
5-Hydroxy-5-methylhydantoin			Kasprzak <i>et al.</i> , 1997; Cooke <i>et al.</i> , 2003
<i>Trans</i> -1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine			Cooke <i>et al.</i> , 2003
5-Hydroxyhydantoin			Kasprzak <i>et al.</i> , 1997; Cooke <i>et al.</i> , 2003
Alloxan			Cooke <i>et al.</i> , 2003
4,6-Diamino-5-formamidopyrimidine (FapyA)			Kasprzak <i>et al.</i> , 1997; Delaney <i>et al.</i> , 2002; Cooke <i>et al.</i> , 2003
2,6-Diamino-4-hydroxy-5-formamidopyrimidine (FapyG)			Kasprzak <i>et al.</i> , 1997; Wiederholt and Greenberg, 2002; Cooke <i>et al.</i> , 2003
Oxazolone	G → T	Hypothetical	Duarte <i>et al.</i> , 2000; Cooke <i>et al.</i> , 2003; Evans <i>et al.</i> , 2004

Notes: Oxidative attack of DNA leads to base modification. Due to altered binding properties of modified bases non-Watson-Crick pairing of these bases can occur and could lead to mutations during replication if not repaired.

H₂O₂ is generated (Maier *et al.*, 2006), which might indicate a higher load of oxidatively modified DNA, possibly due to high H₂O₂ concentrations. A similar conclusion is supported by the data of single-base DNA modifications detected with the comet assay. In principle, this assay is based on the ability of denatured, cleaved DNA fragments to migrate out of the nucleus if subjected to an electric field, thereby forming a tail. Migration and hence the extent of the tail depends on the loss of DNA integrity (e.g., through strand breaks and abasic sites). Optional *in vitro* treatment with repair enzymes causes formation of abasic sites and strand breaks at sites with specific DNA modifications, in addition to the standard comet assay (Cadet *et al.*, 2000). For the repair enzyme protocols that detect the differences in the oxidative attack on DNA between thyroid and liver, lung and spleen are most prominent (Maier *et al.*, 2006). In addition, 8-OHdG/8-OHG staining perfectly agrees with the results of the modified comet assay that includes detection of 8-OHdG (Maier *et al.*, 2006). Our hypothesis of excessive oxidative DNA damage in the thyroid is further supported by the data on mRNA expression of 8-oxoguanine DNA glycosylase (OGG1), the enzyme that would repair 8-oxoguanine modifications (Rosenquist *et al.*, 1997). OGG1 is highly expressed in the lung, an organ with pronounced oxygen exposure. However, a very similar level of mRNA expression of OGG1 is detected in the thyroid, much higher than that in spleen or liver (Maier *et al.*, 2006). In addition, preliminary data suggest a reduced expression of OGG1 in follicular thyroid cancer and higher expression in Graves' disease (Karger *et al.*, personal communication). Such a distribution would again correlate with the functional activity of thyroid cells, and could indicate a higher load of ROS during hormone synthesis. Because many of the DNA modifications lead to non-Watson-Crick pairing of the affected bases, they are potentially mutagenic. For example, 8-OHdG pairs preferentially with A rather than with C; this generates a G → T transversion (Moriya and Grollman, 1993), which is predominantly found in tumor-relevant genes (DeMarini *et al.*, 2001; Keohavong *et al.*, 2003; Hong *et al.*, 2007). Many oxidative base lesions are mutagenic, no matter whether they are formed *in situ* or misincorporated from the deoxynucleotide pool during replication. It is therefore, very likely that the thyroid might constitute a mutagenic environment that is related to oxidative stress. Such a hypothesis has been tested in studies on TSH receptor (TSHR) (Farid *et al.*, 2000) and p53 mutations (Shahedian *et al.*, 2001; Farid, 2004). Strikingly, rates of silent mutations in p53 indicate hypermutability in human thyroid tumors compared to tumors in general (Shahedian *et al.*, 2001). We followed this line of thinking by analyzing the spectrum of base exchanges in somatic mutations of thyroid tumors causing constitutive activation of TSHR (Maier *et al.*, 2006). Among the generally high frequency of transitions previously noted in mutation

Table 57.2 Frequency of base exchanges in human somatic TSHR mutations

Wild-type base	Base exchange	Frequency (%)
A	C	3
	G	8
	T	4
C	A	8
	G	9
	T	32
G	A	4
	C	4
	T	14
T	A	4
	C	11
	G	1

Notes: 184 cases of hyperfunctioning thyroid adenoma, adenomatous nodules, or carcinomas listed in the TSHR Mutation Database II (<http://www.uni-leipzig.de/~innere/tsh/>) were studied for their base exchanges.

hot spots (Farid *et al.*, 2000) the predominance of C → T transitions in somatic mutations (Table 57.2) compared to the spectrum of germline TSHR mutations and higher frequencies of G → T and T → C base exchanges further suggest a mechanism that is very likely caused by oxidized base adducts (McBride *et al.*, 1991; Kreutzer and Essigmann, 1998).

Spontaneous Mutation in the Thyroid

The studies summarized above elucidate an oxidative fingerprint in the databases of TSHR and p53 mutations. Proliferation is very important in the context of mutagenesis. DNA replication during cell division leads to a fixation of spontaneous mutations into the genome, causing a certain mutation load for dividing cells. However, thyroid tissue is only slowly proliferating. In dog and human adult thyroid the mitotic index for cells in the S phase are 39.5×10^{-5} and 13.4×10^{-5} , respectively (Coclet *et al.*, 1989). This translates into an estimated time between cell divisions of thyrocytes of about 8.5 years in humans and 2.3 years in dogs (Coclet *et al.*, 1989), meaning that thyroid cells divide about five times during adulthood. Although mitotic rates in rat thyroid are higher than those in dogs and humans, the number of thyrocyte divisions during adulthood is slightly lower (Conde *et al.*, 1992). Hence, compared to highly proliferating and therefore tumor-prone tissues such as the colon, endometrium, skin, prostate, or breast, at a similar mutation rate the number of mutations in the thyroid should be low. It is possible to determine the spontaneous mutation rate (SMR) with the help of mice transgenic for a lacZ reporter construct (Gossen *et al.*, 1993) that acts as a reporter for spontaneous mutations. The value is much higher in the thyroid than in other tissues (Maier *et al.*, 2006).

With an 8–10-fold higher number compared to liver, the thyroid stands out from many other tissues (Cole and Skopek, 1994). Indeed, SMR in mice thyroids without any experimental mutagenic challenge shows values that are usually only found in animals treated with mutagens, such as ethyl nitrosourea or benzo[*a*]pyrene (van Steeg *et al.*, 2000).

DNA Damage in the Iodine-Deficient Thyroid

As outlined above, differential expression of antioxidant enzymes marks increased oxidative stress in the thyroid that could cause DNA damage. In the studies of iodine deficiency, the comet assay is also a valuable tool that reveals a significant increase of uracil and oxidized purine/pyrimidine adducts in thyroid DNA during iodine deficiency (Maier *et al.*, 2007). The increase of uracil modifications under iodine restriction could be the reason for the high frequency of C → T base changes in TSHR mutations (Table 57.2), which are found in autonomously functioning thyroid nodules (AFTN) in iodine-deficient areas (Maier *et al.*, 2007). Uracil in DNA may arise from spontaneous hydrolytic or enzymatic deamination of cytosine or from misincorporation of deoxyuridine monophosphate (dUMP) instead of deoxythymidine monophosphate (dTTP) during DNA replication (Lindahl, 1993; Shen *et al.*, 1994; Samaranyake *et al.*, 2006). Whereas deamination of cytosine generates G:U mismatches that cause G:C to A:T transitions (Beletskii and Bhagwat, 1996; Nilsen *et al.*, 2001; An *et al.*, 2005) misincorporation of the substrate dUMP during DNA replication would not cause a base change. Our theory of an increased oxidative burden in the thyroid gland through iodine deficiency is also supported by the results of the comet assay with repair enzyme protocols to detect oxidative DNA damage (Maier *et al.*, 2006). For example, one of these modifications (i.e., 5-formyl-2'-deoxyuridine) induces T → C and C → T transition, as well as G → T transversions (Anensen *et al.*, 2001), which are also very prominent in the spectrum of somatic activating TSHR mutations found in AFTNs (Maier *et al.*, 2006). Furthermore, C → T transitions are also the result of another set of cytosine modifications (i.e., 5-hydroxycytosine, 5-hydroxyuracil, and uracil glycol) detected with the modified assay (Kreutzer and Essigmann, 1998; Cadet *et al.*, 2000).

In normal thyroid DNA, we could show a correlation of DNA damage and SMR. While SMR is very high in the normal thyroid gland it remains unchanged in experimental iodine deficiency. This could either be a weakness of the experimental model, which has been discussed in detail elsewhere (Maier *et al.*, 2007), or might indicate a more effective repair of DNA damage in response to iodine deficiency. So far no data concerning DNA repair are available for the thyroid.

Consequences of a High Somatic Mutation Rate in the Thyroid

In general any external factor (e.g., iodine deficiency) that would cause oxidative stress, DNA damage, or increase in the SMR should aggravate the chances for tumorigenesis. Indeed, the prevalence of multinodular goiter and thyroid nodules is much higher in geographical regions with iodine deficiency (Belfiore *et al.*, 1987; Laurberg *et al.*, 1991). If most nodular lesions are benign tumors that prevalently arise from a single lesioned progenitor cell, tumorigenesis is very frequent in the thyroid gland. Traditionally, only thyroid adenomas are considered true tumors based on an exclusive histologic definition: by the presence of a capsule and a growth pattern that is different from the surrounding normal parenchyma in an otherwise normal thyroid gland. However, pure histologic criteria are difficult in a frequent background of goiter or thyroiditis. Therefore, the biological basis for separating thyroid nodules from true tumors also depends on their clonality (Chan *et al.*, 2004). In the light of many thyroid nodules without a capsule (adenomatous nodules), which are monoclonal, a mixed functional and molecular definition of true thyroid tumors appears more objective and consistent. A molecular definition of nodular lesions being tumors is based on several lines of evidence. First of all, constitutively activating somatic mutation in the TSHR or Gs alpha protein is the major cause of AFTN (Paschke and Ludgate, 1997; Davies *et al.*, 2005). Secondly, a frequent monoclonal origin is suggested from the studies of thyroid adenomas or adenomatous nodules diagnosed as single lesion or within multinodular goiters in endemic areas (Krohn *et al.*, 2005). A monoclonal origin often suggests a somatic genetic change (e.g., point mutation or rearrangement) transferred from the founder cell of the clone. Thirdly, somatic mutations already occur in microscopic areas comprising only a few thyroid follicles in the sections of euthyroid goiters (Krohn *et al.*, 2000).

The frequency of thyroid nodules/tumors is high. Studies that use sensitive ultrasound methods detect a frequency of about 50% (Castro and Gharib, 2005; AACE/AME Task Force on Thyroid Nodules, 2006). A similar number is detected in postmortem evaluations (Tan and Gharib, 1997). Thyroid nodules occur as solitary nodules or multinodular thyroid disease. In terms of functional activity, as tested by radioactive iodine or technetium uptake on scintiscan, hyperfunctional, normofunctional, or hypofunctional entities can be differentiated. There is consensus that the percentage of malignant neoplasias among thyroid nodules is in the order of 5% for patients in thyroid clinics (Gharib, 1997). Moreover, papillary microcarcinomas are a frequent finding in autopsy studies (Piersanti *et al.*, 2003).

Owing to the slow proliferation rate of thyroid epithelial cells a long period (tens of years) between the initiation

of the tumor and the nodular appearance is predicted (Figure 57.2). In this regard, the frequency of thyroid tumors is a paradox that could only be explained by frequent tumor initiation and/or by enhanced thyroid epithelial cell proliferation. Therefore, tumor formation in the thyroid could be caused by a natural or induced high mutation rate and aberrant growth stimulation of the thyroid. Iodine deficiency very likely increases both mutation and proliferation, and is therefore the most important endogenous growth factor for nodular transformation of enlarged thyroids.

Conclusion

Starting from iodine deficiency and the characteristics of thyroid hormone metabolism, we propose a sequence of molecular events that include oxidative stress, DNA damage and mutagenesis to explain the frequent nodular and tumor transformation in the thyroid (Figure 57.4). Although also detectable in the normal thyroid gland the oxidative burden linked to hormone synthesis and H_2O_2 production is exaggerated by lack of iodine in the diet. This in turn causes a higher frequency of thyroid tumors in iodine-deficient regions. Despite being predominantly benign tumors, the risk of malignancy is a frequent diagnostic

issue. Moreover, the current medical treatment options for benign nodules are of limited efficiency. Therefore, understanding the molecular etiology of thyroid tumors and the main factors that cause them is an ongoing issue in endocrinological research.

Summary Points

- The thyroid gland is most prominently affected by a lack of iodine in the diet, because iodine is a precursor for the synthesis of thyroid hormones.
- Iodine deficiency leads to thyroid enlargement and goiter. In the long run, this supports the development of thyroid nodules, some of which are thyroid carcinomas.
- At the molecular level, iodine deficiency leads to an increased expression of antioxidant genes as a response to increased oxidative stress.
- Increased oxidative attack is also detectable in the form of modified DNA, which could lead to mutations.
- These mutations are the prerequisite for development of thyroid nodules, which are predominantly benign thyroid tumors, but could also constitute malignant lesions.

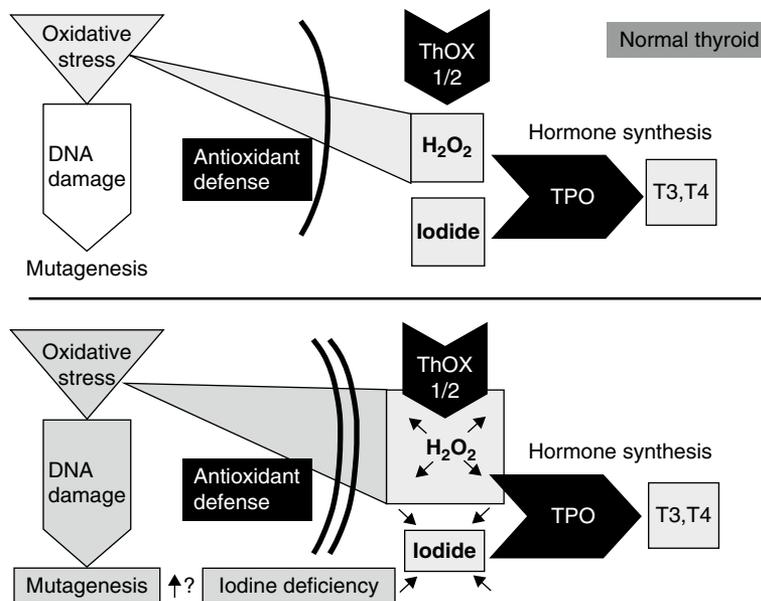


Figure 57.4 The possible link between thyroid hormone synthesis, oxidative stress and mutagenesis in the thyroid gland. This figure illustrates the mechanism, the sequence of steps and the key molecules that link thyroid hormone synthesis, iodine deficiency, oxidative stress, DNA damage and possible mutagenesis. ThOX, thyroid oxidase; TPO, thyroid peroxidase; T3, T4, thyroid hormones.

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Adaptation to Iodine Deficiency: Experimental Aspects: T4 and T3 in Plasma and Different Tissues

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Abstract

Human populations in areas where iodine intake is lower than necessary to prevent depletion of iodine-containing compounds in the thyroid gland present with a great variety of manifestations, usually included in the term iodine deficiency disorders (IDD). Some of the manifestations, especially overt hypothyroidism, might not be caused by iodine deficiency (ID) alone ("pure" ID), but by concomitant factors, such as the intake of goitrogens. For years, experimental models were being developed to define the consequences of "pure" ID, and the corresponding literature is abundant. However, the iodine content of diets was not always the only variable. We present a study here where five groups of rats received the same low iodine diet (LID), with additions that resulted in an almost 250-fold range of iodine availability to the gland. This study stresses the importance of TSH-independent autoregulatory responses that are rapidly activated by mild ID, including an increase in thyroid weight and activation of the preferential synthesis of T3 over T4, resulting in the maintenance of elevated, or normal, circulating T3, even in severe ID. Very severe ID is needed to decrease the circulating T3 below normal values. To answer the question of whether or not animals (including man) with "pure" ID are hypothyroid, T3 concentrations were measured in many tissues. It was found that the question could not be resolved, as it is clearly tissue-specific at all degrees of ID, where elevated, normal and low concentrations of T3 are found in different tissues of the same animal.

Abbreviations

BAT	Brown adipose tissue
BW	Body weight
C	Control
D1	Type 1 iodothyronine deiodinase
D2	Type 2 iodothyronine deiodinase
ID	Iodine deficiency

IDD	Iodine deficiency disorders
LID	Low iodine diet
LID'	LID with trace amounts of potassium perchlorate
T3	3,5,3'-Triiodo-L-thyronine
T4	Thyroxine
TSH	Thyroid-stimulating hormone, thyrotropin

Introduction

A large proportion of terrestrial animals, including man, are presently living in areas where the supply of iodine is insufficient for optimal health. The degree of deficiency may vary considerably, and may result in qualitatively and quantitatively different negative biological consequences. In man, developmental defects that have been attributed to iodine deficiency (ID), termed iodine deficiency disorders (IDD), may be different, not only due to different degrees of ID, but also due to the developmental period when ID occurred (Hetzel, 1983, 2005). The manifestations in different areas where ID has been identified as an important causal factor resulting in different IDD are also complicated by factors other than "pure" ID, such as the simultaneous ingestion of goitrogens and selenium deficiency (Contempré *et al.*, 2004).

One of the most frequent questions asked has been whether or not "pure ID" results in hypothyroidism (Morreale de Escobar *et al.*, 1997). The answer to this is especially difficult, because there are few quantitative indices of thyroid hormone action in different tissues. For this reason, we have focused on concentrations of 3,5,3'-triiodo-L-thyronine (T3), which was taken as an index of possible thyroid hormone effectiveness at the individual tissue level, as discussed elsewhere (Escobar-Morreale *et al.*, 1995). As will be seen, the thyroid status of rats with ID cannot be defined for the animal as a whole, because it is eminently tissue specific: At all grades of ID, elevated,

normal and low concentrations of T3 are found in different tissues of the same animal.

Finding answers to the above questions regarding the consequences of “pure” ID, namely, differences in iodine intake as a single variable, soon prompted experiments in animals (Studer *et al.*, 1974), where this condition could be met. Here we will focus on experimental aspects in rodents, as findings in other animal species, such as sheep and marmosets, have been recently reviewed elsewhere (Morreale de Escobar *et al.*, 2007). The general methodology of such experimental approaches was to limit iodine intake, without introducing additional variables. The aim of a single variable – iodine content – has rarely been achieved, as very often there were other differences between the composition of diets ingested by animals on low a iodine diet (LID) and their controls (Riesco *et al.*, 1976). Even in experiments where such variables were avoided, answers as to whether or not iodine deficiency results in hypothyroidism were usually obtained for a single degree of ID, with few biological endpoints related to the thyroid status being studied (Riesco *et al.*, 1977; Santisteban *et al.*, 1982; Obregón *et al.*, 1984, 1991; Escobar del Rey *et al.*, 1987; Martínez-Galán *et al.*, 1997). The pioneering studies by Heninger and Albright (1966, 1975) clearly pointed out that the answer is tissue specific. More recent studies (Peeters *et al.*, 2001) involving the study of iodothyronine deiodinases as biological endpoints of thyroid hormone action in different regions of the central nervous system in response to ID also indicate the tissue specificity of the effects.

Experimental approach

Young adult female rats were subjected to five different degrees of ID, using the same LID plus KI added in different amounts, covering a more than 250-fold difference in iodine availability. Table 58.1 summarizes the amounts of iodine likely to be available to rats of the five different groups (Pedraza *et al.*, 2006). Animals were then sacrificed at a single time point (3 months) after the start of different iodine-supplemented diets, and concentrations of T4 and thyroxine (T4) in the thyroid gland, in plasma and in 12 different tissues, and circulating thyroid-stimulating hormone (TSH), were measured.

Results

Some of the most unexpected and interesting results are briefly summarized below.

General Response As far as could be assessed from the increment in body weight (BW), the decrease in iodine availability did not affect the general condition of the animals until it became severe (LID and LID with trace amounts of potassium perchlorate [LID'] groups). Despite

Table 58.1 Degrees of iodine deficiency (ID)

Group	Degree of ID	Diet + supplements	Iodine (μg/day/rat)
LID'	Very severe ID	LID'	0.021
LID	Severe ID	LID	0.052
LID + 0.5	Moderate ID	LID + 0.5 μg I/20g	0.50
LID + 1.0	Mild ID	LID + 1.0 μg I/20g	1.0
Controls (LID + 5.0)	No ID	LID + 5.0 μg I/20g	5.0

Notes: Estimation of the amounts of iodine available daily to the thyroid of rats from different experimental groups fed low iodine diet (LID) and supplements. Pedraza *et al.*, (2006).

smaller increases in their BW, even the animals of the LID' group did not show “clinical” signs of hypothyroidism comparable to those of animals that were thyroidectomized and stopped growing within a few weeks. This is in agreement with previous results from this laboratory using the same strain of animals and types of LID and LID' (Escobar del Rey *et al.*, 1987; Lavado-Autric *et al.*, 2003).

Thyroid Glands The content of T4 and T3 in the thyroid were measured separately in two different fractions which we refer to here as the “Free” T4 and T3 pools and the “Total” T4 and T3 pools. When applied to the thyroid, the adjectives “Free” and “Total” have an entirely different meaning than for plasma. In the thyroid, “Free” T4 and T3 correspond to the iodothyronines present in the gland as amino acids, no longer incorporated into proteins by peptidic bonds, and presumably available for secretion as hormones into the bloodstream. “Total” T4 and T3 correspond to the iodothyronine residues still incorporated by peptidic bonds into thyroglobulin and other proteins. The concentrations of “Free” T4 and T3 were obtained using methanol extracts of the thyroid homogenates, then processed as other tissue extracts. The thyroid pools of “Total” T4 and T3 were measured in methanol extracts of proteolytic hydrolysates of the pellets remaining after the initial methanol extraction (Calvo *et al.*, 1997).

As summarized in Figure 58.1 the iodine concentration fell markedly and progressively with decreasing iodine availability. The “Total” T4 and T3 decreased rapidly and progressively with a five-fold decrease in iodine intake between the LID+5.0 and the LID+1.0 group. The “Free” T4 decreased even more markedly, whereas the “Free” T3 was still 25% of the control (C) values in the group with the lowest iodine availability.

The most unexpected finding was the doubling of the weight of the gland in the LID+1.0 group as compared to the LID+5.0 group, further increases being comparatively rather small. This was quite difficult to understand in view of the changes in circulating TSH, shown in Figure 58.2: There was no statistically significant increase in TSH when iodine intake decreased five-fold, from the LID+5.0 group to the LID + 1.0 group, despite which the thyroid

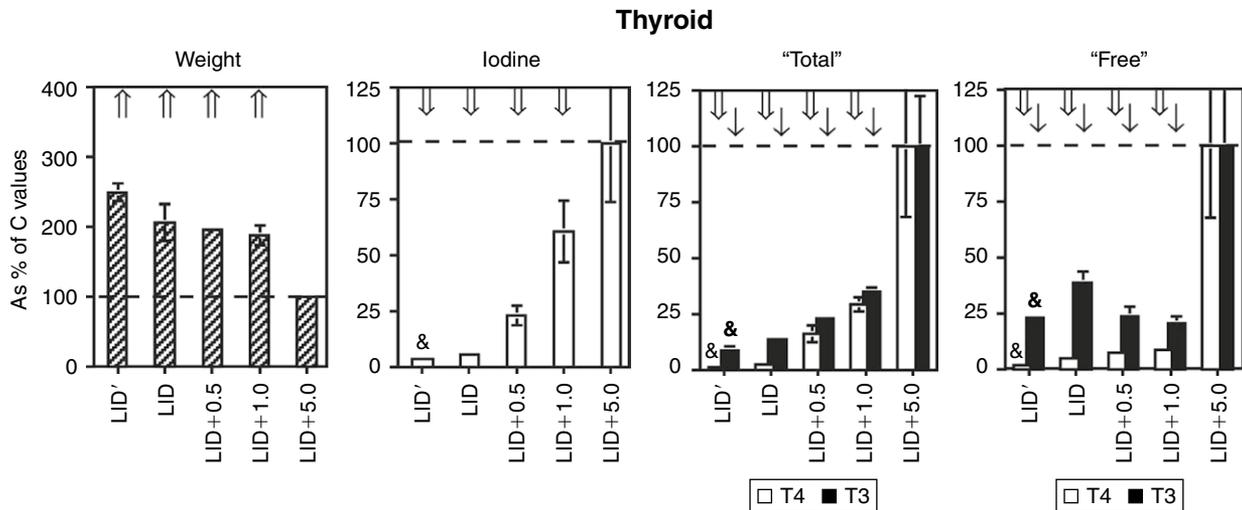


Figure 58.1 A summary of the changes in thyroid weight and total iodine, T4 and T3 content in groups of rats with a decreasing iodine intake. “Total” T4 and T3 correspond to residues incorporated into thyroglobulin and other proteins by peptidic bonds, whereas “Free” T4 and T3 are present in the gland as iodoamino acids, available for secretion as hormones into the bloodstream. All values are “normalized” by taking as 100% the mean value of the corresponding variable in the C group (LID+5.0) of animals; weight: 23 ± 1.2 mg; total iodine: 3.19 ± 0.85 μg/gland; “Total” T4 and T3: 4053 ± 1297 and 212 ± 47 ng/gland; and “Free” T4 and T3: 139 ± 46 and 15.7 ± 4.3 ng/gland. Open arrows identify statistically significant increases, or decreases, versus the C group (LID+5.0); & and & identify statistically significant differences between the mean values for animals on LID' and LID. The black arrows and & correspond to the variables shown as black bars, while the white arrows and & correspond to those represented by white bars.

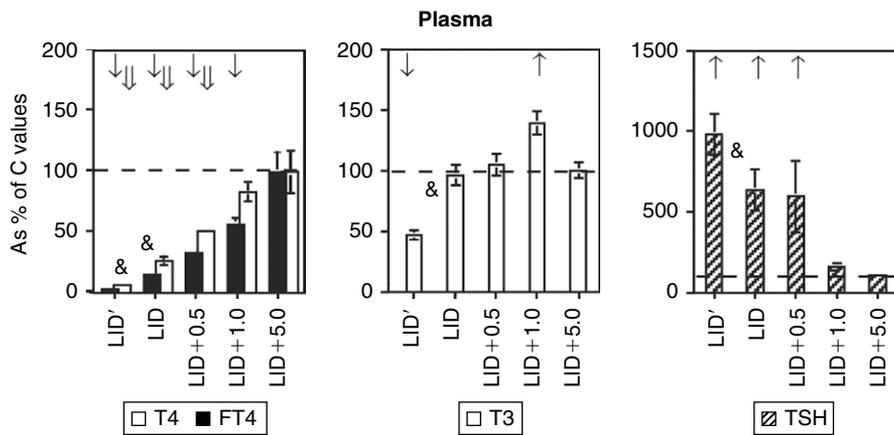


Figure 58.2 Changes in plasma T4, free T4 and T3, and in circulating TSH, in groups of rats with decreasing iodine availability. All values are “normalized” by taking as 100% the mean value of the corresponding variable in the C group (LID+5.0) of animals; T4: 22.9 ± 3.9 ng/ml; free T4: 29.6 ± 0.4 pg/ml; T3: 0.31 ± 0.02 ng/ml; TSH: 1.10 ± 0.05 ng rTSH RP-3/ml. The meaning of symbols is the same as in Figure 58.1.

weight doubled. Circulating TSH then increased seven-fold as iodine availability decreased further (LID group), whereas the weight of the gland hardly changed.

Plasma Figure 58.2 shows not only the unexpected pattern of changes in the circulating TSH, but also those in the total and free T4, and in T3. Both total and free T4 decreased progressively and markedly with decreasing iodine availability, with a pattern reminiscent of the decrease in total iodine content of the thyroid gland (see Figure 58.1). Circulating T3 actually increased significantly in the LID+1.0 group as compared with the

LID+5.0 group (C group), and then decreased to the values in the LID+0.5 and LID groups. Serum T3 was still 50% of the C value in the LID' animals in whom iodine available to the thyroid gland was more than 200-fold less than that available to the C rats.

Extrathyroidal Tissues Figures 58.3–58.6 summarize the main results concerning the concentrations of T4 and T3 in different tissues, as compared to those in the plasma. In practically all of them, the changes in the concentration of T4 decreased progressively with decreasing iodine availability, following a pattern reminiscent of the total T4 in plasma. The

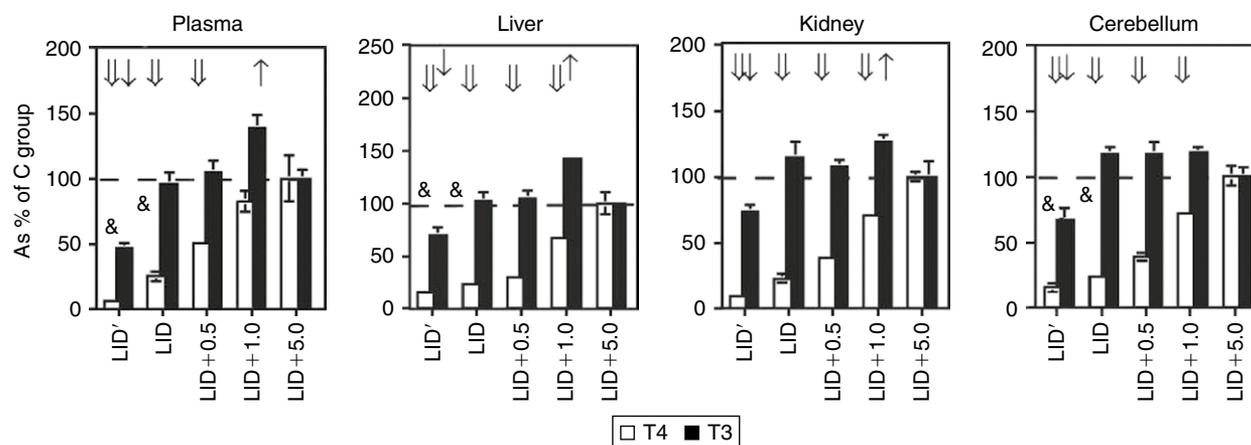


Figure 58.3 Changes, with decreasing iodine availability, in the concentrations of T4 and T3 plasma, liver, kidney and cerebellum. The values corresponding to C rats (LID+5.0 group) were T4: 22.9 ± 3.9 ng/ml and T3: 0.31 ± 0.02 ng/ml for plasma; liver: 40.00 ± 4.23 ng T4/g, 3.59 ± 0.12 ng T3/g; kidney: 20.70 ± 0.80 ng T4/g and 7.08 ± 0.79 ng T3/g; cerebellum: 3.98 ± 0.31 ng T4/g and 2.18 ± 0.14 ng T3/g. The meaning of symbols is the same as in [Figure 58.1](#).

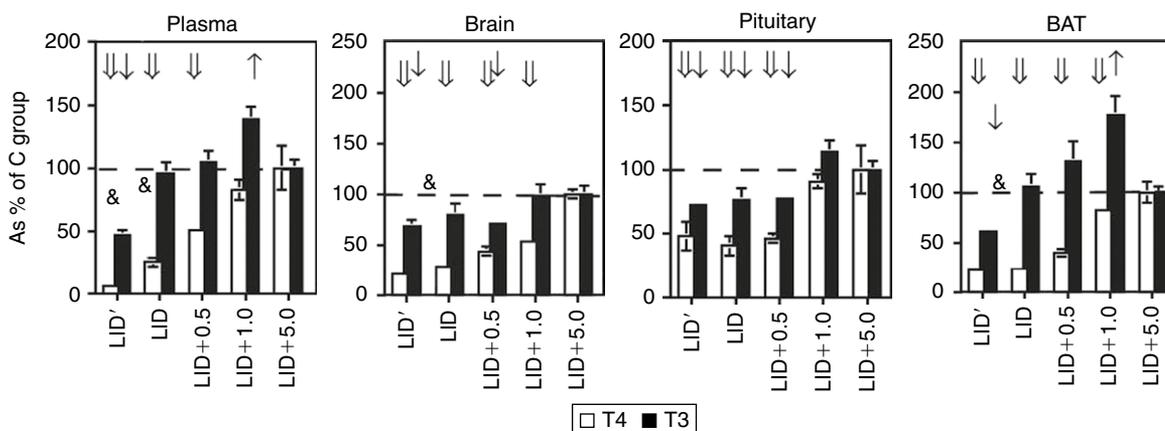


Figure 58.4 Changes, with decreasing iodine availability, in the concentrations of T4 and T3 plasma, brain, pituitary and BAT. The values corresponding to C rats (LID+5.0 group) were T4: 22.9 ± 3.9 ng/ml and T3: 0.31 ± 0.02 ng/ml for plasma; brain: 1.81 ± 0.09 ng T4/g and 2.44 ± 0.20 ng T3/g; pituitary: 54 ± 10 pg T4, 93 ± 6 pg T3; BAT: 2.04 ± 0.21 ng T4/g, 3.02 ± 0.18 ng T3/g. The values shown for the pituitary are the total T4 and T3 contents, as the recorded weights were considered unreliable. The meaning of symbols is the same as in [Figure 58.1](#).

findings regarding changes in the concentration of T3 were markedly variable for different tissues. As indicated above, plasma T3 actually increased, and then returned to C values with increasing ID, and was still 50% of the C value in the LID' animals. Similar patterns were found for the concentrations of T3 in liver, kidney and cerebellum ([Figure 58.3](#)) and in brain, pituitary and brown adipose tissue (BAT) ([Figure 58.4](#)), although in most of them the concentration of T3 in the LID' group was still above 50% of the C values. In lung, heart and muscle ([Figure 58.5](#)), the concentrations of T3 did not decrease with decreasing iodine availability. The pattern of changes in T3 in the ovary and adrenal glands ([Figure 58.6](#)) stand out as remarkably different from those of other tissues. T3 in the ovary was still above C values in the LID' animals,

whereas T3 in the adrenal glands decreased with the same pattern as T4, and more markedly than plasma T3.

Discussion

Mild iodine deficiency: LID+1.0 group ([Table 58.1](#))

Intrathyroidal response mechanisms predominate when the daily iodine intake is reduced from 5 to $1 \mu\text{g}$, an amount which is no longer sufficient to compensate for daily requirements. This affords an explanation for the most unexpected finding reported above, namely the doubling of thyroid weight, without a concordant increase in circulating TSH ([Figures 58.1 and 58.2](#)). This finding

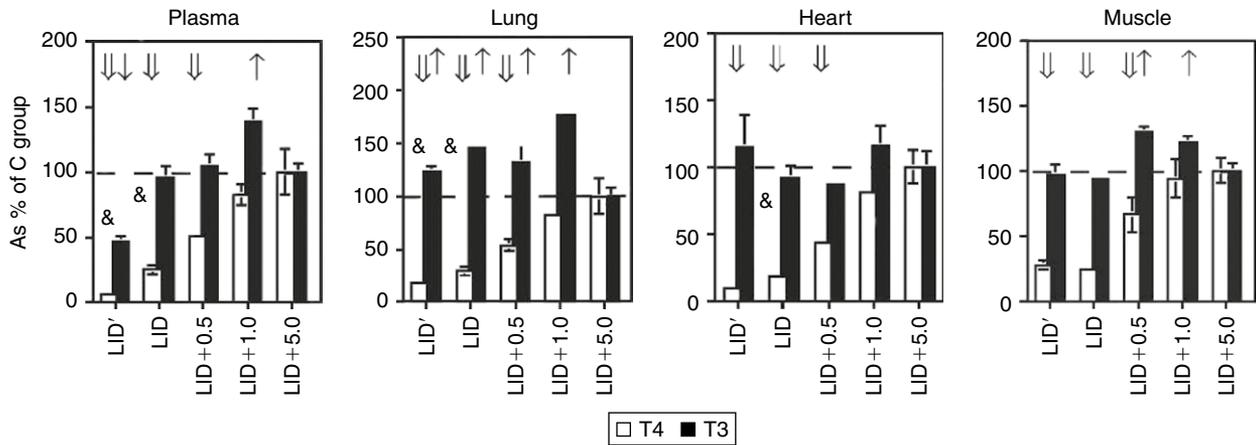


Figure 58.5 Changes, with decreasing iodine availability, in the concentrations of T4 and T3 plasma, lung, heart and muscle. The values corresponding to C rats (LID+5.0 group) were T4: 22.9 ± 3.9ng/ml and T3: 0.31 ± 0.02ng/ml for plasma; lung: 8.08 ± 1.35ng T4/g, 1.71 ± 0.15ng T3/g; heart: 3.82 ± 0.49ng T4/g, 1.54 ± 0.18ng T3/g; muscle: 1.91 ± 0.19ng T4/g and 1.18 ± 0.06ng T3/g. The meaning of symbols is the same as in **Figure 58.1**.

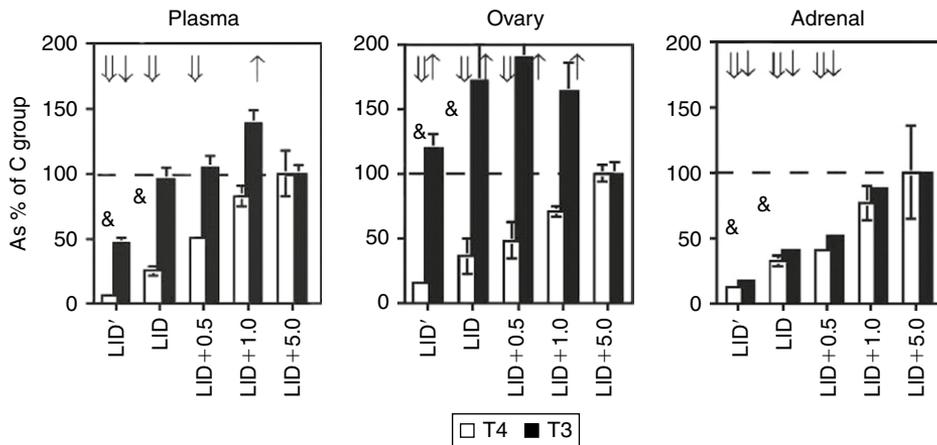


Figure 58.6 Changes, with decreasing iodine availability, in the concentrations of T4 and T3 plasma, ovary and adrenal. The values corresponding to C rats (LID+5.0 group) were T4: 22.9 ± 3.9ng/ml and T3: 0.31 ± 0.02ng/ml for plasma; ovary: 1.18 ± 0.07ng T4 and 0.15 ± 0.01ng T3; adrenal: 0.30 ± 0.04ng T4 and 0.20 ± 0.07ng T3. The values shown for the ovary and adrenal are the total T4 and T3 contents, as the recorded weights were considered unreliable. The meaning of symbols is the same as in **Figure 58.1**.

Table 58.2 Autoregulatory response of the thyroid to iodine deficiency

Animals	Iodine intake	Average weight (mg)	Average acinar cell height (µm)
Intact	Iodine supplement	13.0	6.0
Intact	Low iodine diet	27.5 ^a	10.7 ^a
Hypophysectomized	Iodine supplement	6.9	1.5
Hypophysectomized	Low iodine diet	9.3 ^a	2.6 ^a

Notes: Experimental results showing the increase in weight of thyroids and increase in acinar cell height in response to a decrease in iodine intake, even in hypophysectomized rats (Chapman, 1941).

^aIdentifies a statistically significant difference with respect to the corresponding control group on iodine supplements.

initially surprised us, until we reviewed earlier studies in rats on an iodine intake similar to that of our LID+1.0 group (Pedraza *et al.*, 2006), where an increase in vascularity, blood flow, iodine trapping, acinar cell height (Chapman, 1941) (Table 58.2), and hyperplasia of the gland occurred immediately without any significant increase in circulating TSH, even in hypophysectomized rats. The same occurred with the changes in intrathyroid iodine metabolism, which lead to a preferential synthesis and secretion of T3 over T4, and an increased T3/T4 ratio in the circulation (Greer and Rockie, 1969). The permanence time of intrathyroidal iodine was also markedly increased, at least in man, thus enhancing intraglandular reutilization of the decreasing iodine supply (Stanbury *et al.*, 1954; Delange, 2000). These TSH-independent autoregulatory changes are correlated to the degree of iodine depletion, and are the opposite of

the TSH-independent response of thyroid exposed to a sudden iodine excess (Nagataki and Yokohama, 1996). Our present data in the LID+1.0 group, therefore, confirm the unique role of thyroid autoregulatory responses in efficient adaptation to a mild degree of ID.

Extrathyroidal response mechanisms involved in these mildly iodine-deficient rats are less evident than the intrathyroidal autoregulatory ones that result in the higher plasma T3/T4 ratio, predominantly accounted for by the higher plasma T3 resulting from its preferential synthesis and secretion by the gland. The patterns of changes in the concentration of T4 and T3 in the tissues studied here however, appear to be tissue-specific and not easily predictable from the change in circulating T4 and T3. A similar conclusion was reached years ago by Heninger and Albright (1965, 1975), who measured the concentration of T4 and T3 in several tissues of rats on a diet with an iodine content similar to that of the present LID+1.0 group. They also found that T3 increased in plasma and in many tissues, but not in all tissues (i.e., the brain), and that the degree of change was tissue specific.

In summary, both intra- and extrathyroidal mechanisms are involved in the adaptation of the rat to mild iodine deficiency: the former are autoregulatory and very effective in avoiding T3 deficiency in most tissues; and the latter occur in tissues in which type 2 iodothyronine deiodinase (D2) plays an important role for the local generation of T3. In mild ID, hypothyroidism, as inferred from the concentrations of T3, is avoided in all tissues studied. The question remains as to whether or not tissues with elevated T3 concentrations may actually be hyperthyroid. As far as we know, this question cannot yet be answered because tissue-specific thyroid hormone-sensitive biological endpoints have not been measured in mildly iodine-deficient rats with increased circulating T3.

Moderate iodine deficiency: LID+0.5 group

There is a further decrease in the iodine, "Total" T4, and "Total" T3 contents of the thyroid to about 25% of C values. There is also a marked increase in circulating TSH. In contrast, thyroid weight is hardly affected by the increase in TSH, a finding consistent with the concept that thyroid growth is mainly determined by autoregulatory processes not only in mild, but also in moderate, ID.

Plasma T3 was no longer elevated in animals with moderate ID, but remained normal despite a 50% decrease in T4. T3 deficiency was prevented in tissues that derive T3 mostly from plasma, and also in BAT and cerebellum. Unexpectedly, despite normal T3 and very low T4 in plasma, some tissues maintained high T3 levels, most notably the lung, ovary and muscle. The underlying mechanisms have not been identified: in the lung, for instance, an increase in type 1 iodothyronine deiodinase (D1) activity

was not involved. In the brain, T3 decreased, despite the increased D2 activity, and so did pituitary T3. As already noted in the mild ID group, T3 decreased in the adrenal glands in parallel with T4.

In summary, in the case of moderate ID, intra- and extrathyroidal responses are still adequate to prevent low T3 levels in plasma and most tissues, despite a reduction in the iodine intake to 25% of that of controls. Some tissues even maintain elevated T3 concentrations, whereas others are markedly (adrenal) or moderately (brain and pituitary) T3 deficient.

Severe iodine deficiency: LID group

The intrathyroidal response mechanisms operative in previous groups continue to minimize the effects of a further marked decrease in iodine intake, and circulating T3 remains normal. Despite a major decrease in plasma T4 to 25% of C values, T3 concentration remained normal not only in plasma, but also in most tissues. Again, the most unexpected and striking results are those obtained for the concentration of T3 in the ovary and lung, where it is much higher, and in the adrenal gland, where it is much lower, than expected from the normal circulating T3.

In summary, despite a 100-fold decrease in iodine availability, a combination of intra- and extrathyroidal adaptive mechanism still mitigates T3 deficiency, and presumably hypothyroidism, in most, but not all, tissues.

Very severe iodine deficiency: LID' group

Intrathyroidal adaptive mechanisms are no longer sufficient in LID' rats to ensure normal T3 levels, which decreases in plasma to 45% of C values, and also in many tissues that depend on plasma-derived T3, including the liver. The brain, cerebellum, pituitary and BAT tissues that obtain most of their T3 by local generation from T4, are T3 deficient, probably because of the very low availability of plasma T4, which decreases to 5% of normal values. We have previously shown that the brain, pituitary and liver of such animals are indeed hypothyroid, as assessed from several biological endpoints of thyroid hormone action (Santisteban *et al.*, 1982). Tissue T3, however, decreases less than would be expected from the circulating T3 level (adrenal glands again excepted), and some tissues continue to have normal (muscle, heart) or even elevated (lung, ovary) T3 concentrations.

In summary, the threshold iodine availability below which most tissues are T3 deficient appears to be reached when the intra- and extrathyroidal adaptive mechanisms are no longer capable of ensuring normal circulating T3 levels. But even then, adaptive mechanisms become operative and protect most tissues, and especially the lung, heart, muscle, and ovary, from the degree of T3 deficiency

(and hypothyroidism), which would be expected from the decrease in plasma T3.

Conclusion

Rats with ID are endowed with numerous and very efficient adaptive mechanisms, most of which require a fully-functioning normal thyroid gland, and are thus lost in animals with primary thyroid failure. Rats with ID are often considered to be either hypothyroid, because of their low circulating T4 and increased TSH, or euthyroid, because of their normal (or increased) plasma T3, but present results stress that neither assumption is correct: thyroid hormone status is not only related to the degree of depletion of iodine available to the thyroid, but is also eminently tissue-specific. As discussed elsewhere (Escobar-Morreale *et al.*, 1995), few tissue-specific direct effects of thyroid hormone action are available, and we have measured the concentration of T3 in the tissues as a first step in assessing their thyroid hormone status. With a moderate, or even severe, ID most tissues depending on plasma T3 would be euthyroid, or even slightly hyperthyroid, most notably the ovary and the lung. However, tissues that depend to an appreciable extent on T4 for the local generation of T3 are protected from T3 deficiency to a lesser degree. As a consequence, in the latter type of tissues, thyroid hormone-sensitive functions are more likely to be affected than those characteristic of tissues depending on plasma-derived T3. Such is the case of the brain and pituitary, for instance, and cerebral functions may be impaired in mild ID, because brain T3 may already be decreased despite the normal plasma TSH. Present results also indicate that the degree of increase in D2 activity in different cerebral structures of rats with ID does not permit *per se* conclusions to be drawn regarding their protection from T3 deficiency, as the latter also involves the amount of T4 available in each area (Peeters *et al.*, 2001).

Circulating T3 has to decrease before many T3-dependent tissues become hypothyroid. This appears to occur when circulating T4 decreases from 25% to 5% of normal values. But even under such conditions, the many known and as yet undefined intra- and extrathyroidal adaptive mechanisms are efficient enough to maintain euthyroidism in muscle and heart, and even slightly elevated T3 in lung and ovary. The findings in the ovary may underlie the observation that even very severely iodine-deficient animals are easily mated, do not show decreased fertility, and bear litters of normal size (Escobar del Rey *et al.*, 1987; Lavado-Autric *et al.*, 2003), in contrast to thyroidectomized- or goitrogen-treated hypothyroid females.

Possible Implications for Man

As already pointed out in the introduction, the present study is only relevant for inhabitants of areas where ID

is the sole cause of goiter, but not of areas where other environmental factors may lead to destruction of the gland, and therefore to clinical hypothyroidism. The present findings in very severe ID (LID' group) are probably less relevant for man, because it is unlikely that hypothyroxinemic human populations with a significant decrease in circulating T3 would survive for long: when both T4 and T3 are low, fetal loss is the outcome (McMichael *et al.*, 1980; Dunn and Delange, 2001) and the population would eventually disappear from the region. We believe that present results in mild and moderate grades of decreased iodine availability are especially relevant for man, because such conditions are still widespread in western industrialized countries (Vitti *et al.*, 2003).

Many of the findings in rats, described here and by others, have also been described for people from areas with an adequate iodine intake, who change to an iodine-deficient diet, or for inhabitants of the areas of ID defined above. Thus, for instance, the gland responds within a few days with a striking increase in blood flow, occurring before any change is detected in plasma T4 or TSH (Arntzenius *et al.*, 1991). Increased iodine trapping and circulating T3/T4 ratios, and prolonged half life of intrathyroidal stores, have also been shown (Delange, 2000). In simple sporadic goiter, the increase in thyroid volume occurs without a necessary increase in circulating TSH (Barakat and Ingbar, 1965; Vagenakis *et al.*, 1973; Patel *et al.*, 1973; Pharoah *et al.*, 1973). Even in very severe ID, the increase in the circulating TSH is markedly blunted as compared to that usually observed in hypothyroid patients. Missler *et al.* (1994) reported that in 304 children from an area of ID, 60% had an enlarged gland, but only 9% had TSH > 4.5 mU/l. In the seminal studies by Glinoe (1997) on thyroid function in pregnant women from a population with mild to moderate ID, TSH levels above the normal reference range were not found, even among women with the lowest first-trimester free T4 levels. The same was observed in pregnant women from an area with very mild ID (Morreale de Escobar and Escobar del Rey, 2003).

Regulation of thyroid function through the hypothalamic-pituitary-thyroid negative feedback is so ingrained in western clinical practice that low T4 is automatically associated with high TSH, a prerequisite for the classification of both primary overt and subclinical hypothyroidism. Thus, the definition of hypothyroxinemia itself – a decreased T4 without an increase in TSH above normal – is instinctively rejected. The very efficient autoregulatory mechanisms controlling thyroid function, which do not involve the negative feedback system, are overlooked by most physicians, unless an iodine excess is involved. The present experimental data obtained with mild-to-moderate grades of ID stress the primary importance of thyroid autoregulatory mechanisms that permit the normal thyroid to protect many tissues from overt hypothyroidism and that do not require triggering of the hypothalamic-pituitary-thyroid negative

feedback mechanism. Even in very severe ID, people are able to sustain heavy physical work and have normal cardiac function, observations that might be related to the present findings in muscle and heart, which maintain normal T3 concentrations even in the LID' group.

As discussed elsewhere in more detail (Morreale de Escobar *et al.*, 2004), it is inaccurate to assume, which has been very frequently done, that inhabitants of areas of ID are clinically hypothyroid individuals. The present experimental model supports the epidemiological findings that inhabitants of areas of ID are not clinically hypothyroid individuals, as their normal circulating T3 ensures euthyroidism of most tissues by extrathyroid adaptive mechanisms known to be operative in man when iodine availability decreases. But, as shown experimentally here, this does not avoid *selective* hypothyroidism of tissues, such as the brain, that depend mostly on T4 for their intracellular T3 supply. This selective hypothyroidism is already present in conditions of mildly decreased iodine availability, and may already negatively affect mental functions (Delange, 2001; Vitti *et al.*, 2003; Vermiglio *et al.*, 2004). Indeed, inhabitants of areas of ID are often described as "dull." Whole populations appear to "wake up" when their ID – and the consequent hypothyroxinemia – are corrected (Dunn, 1992).

It is often assumed that eradication of severe ID is enough to avoid the most important IDD, including those affecting mental processes. Present experimental results stress that this is not so, and countries with areas of mild-to-moderate ID, should actively correct it: an important proportion of their inhabitants, and their future progeny, may still suffer from easily preventable impairment of mental functions and the consequent socioeconomic implications (Morreale de Escobar *et al.*, 2004), as well as the increased incidence of thyroid disorders accompanying thyroid hyperplasia.

Summary Points

- Experimental models of ID have afforded important relevant information for man.
- Any degree of ID, even if mild, ought to be avoided because it rapidly activates TSH-independent autoregulatory mechanisms that result in goiter, and an increased incidence of thyroid disorders accompanying thyroid hyperplasia.
- A combination of intra- and extrathyroidal mechanisms of adaptation leads to the preferential synthesis and secretion of T3 over T4, as a consequence of which circulating T3 levels are normal, or even increased, at the expense of decreasing circulating T4 concentrations. Circulating T3 only decreases in situations of very severe ID.
- Extrathyroidal consequences of the changes in circulating T4 and T3 are not the same for all tissues. At all degrees

of ID, indices of thyroid hormone status are clearly tissue specific: elevated, normal, and low concentrations of T3 are found in different tissues of the same animal.

- Animals, including man, living in conditions of "pure" ID are not necessarily hypothyroid, although some tissues (i.e., the brain) might be selectively so.
- Promote actively and worldwide all policies leading to universal salt iodization.
- Eradicate once and for all the major cause, after starvation, of preventable mental deficiencies.

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Exercise and Iodine Deficiency

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Abstract

Although it is standard practice to replace fluid and electrolytes lost during vigorous exercise, replacement requirements seldom exist for minerals such as iodine. Iodine is an essential component for the production of thyroid hormones; hence, inadequate dietary intake leads to diminished thyroid hormone levels. On the basis of the published sweat iodine content of 35–40 µg/l and a potential sweat loss of 4–5 liters following vigorous exercise, daily iodine losses in sweat equivalent to the WHO-recommended adult daily intake (150 µg) might be expected. When added to urinary iodine excretion, such losses would result in a significant diminution in the body's iodine stores. Previous reports have suggested that sweat iodine content is independent of dietary intake, but data indicate that this may only apply with replete iodine status. Administration of KI to subjects living in an area of borderline iodine intake showed an increase in sweat iodine. Under such conditions it is postulated that sweat iodine may increase until it reaches an optimal level and plateau thereafter. In the absence of definitive evidence that iodine loss through excessive sweating can induce a relative iodine deficiency with consequences for thyroid hormone formation, there is not yet a case for iodine supplementation of those involved in vigorous exercise. However, the calculated levels of potential iodine loss through excessive sweating in the absence of adequate replacement at least raise the question of the implications of exercise-induced iodine loss for thyroid status and possibly consequential athletic performance.

Abbreviations

TSH Thyroid-stimulating hormone
WHO World Health Organization

Introduction

It is well-established that losses of fluid and electrolytes during vigorous exercise require replacement, not only

of fluids, but also of a range of minerals. This has led to the consumption of a wide range of isotonic drinks during and after the exercise. Relatively less attention has been paid to the losses of less abundant trace elements, since they can be readily replenished from the normal diet. One element that may escape this criterion is iodine, which continues to be in short supply in diets consumed in many areas of the world. An adequate supply of iodine in the diet is essential as a raw material for the synthesis of the thyroid hormones, thyroxine and triiodothyronine, in the thyroid gland (De Groot and Niepomniszcze, 1977). The major sources of bioavailable iodine are shown in Table 59.1. Although seawater is not particularly iodine rich (approximately 60 µg/l), its sheer volume makes the sea and its flora and fauna a major dietary source of iodine in mammals. Another important source of dietary iodine is iodized salt, in which NaCl is fortified with potassium iodide (KI) or potassium iodate (KIO₃) to a concentration of 20–40 mg/l (WHO, 2001).

Utilization of iodized table salt, together with its use in processed food should theoretically provide for dietary requirements. Except in countries who have adopted a policy of universal salt iodization (USI) the availability and

Table 59.1 Sources of iodine

Source	Concentration (ppm)
–	Naturally occurring
Seawater	0.1
Chilean caliche (nitrates and iodates)	150
Seaweed	100–700
–	Dietary
Iodized salt	20–40
Sea fish	~1 (much lost in cooking)
Dairy milk (most important source in northern Europe)	Variable (50–1000 µg/l)
Food additives	Variable
Medications (radioopaque contrast media, cough bottles)	Variable (may contain mg quantities)
Amiodarone	75 mg/200 mg tablet

Source: Data taken from Smyth and Duntas (2005) with permission.

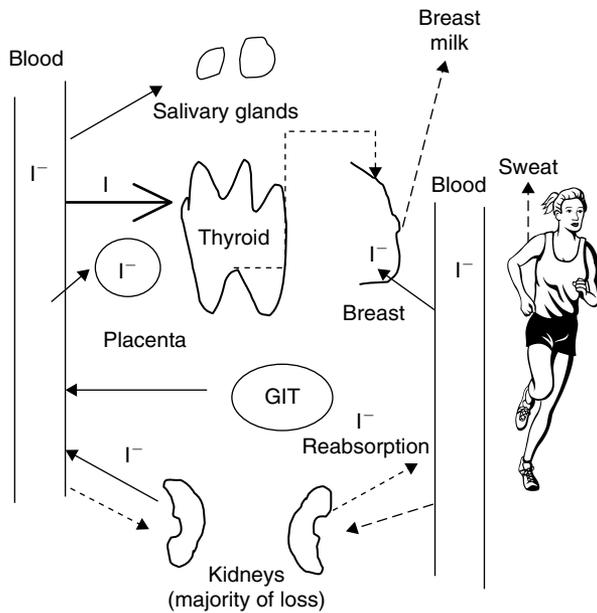


Figure 59.1 *In vivo* iodide (I^-) uptake \longrightarrow and efflux \dashrightarrow pathways. Data taken from Smyth and Duntas (2005). © Georg Thieme Verlag Stuttgart, New York.

the use of iodine varies from country to country (Delange *et al.*, 2001). Iodide in food substances is absorbed from the gut into the bloodstream, where it is taken up from by a number of organs, as shown in Figure 59.1. The most iodide avid organ, the thyroid gland, utilizes iodine to form thyroid hormones, which are deiodinated at peripheral tissues through the action of specific deiodinases. Some of the iodide from this deiodination is conserved for reabsorption by the renal tubules, with the remaining loss accounted for by urinary and fecal excretion (Vought *et al.*, 1964). By far the most important pathway of iodine loss (approximately 90%) is through urinary excretion, but the loss through exhalation and the skin, by sweating, are also important.

Such losses may be negligible in those parts of the world where diets are iodine-replete and intrathyroidal iodine and thyroid hormone stores abundant. However, in areas of iodine deficiency, overt or borderline, the additional stress of iodine loss in sweat may have relevance to thyroid status (Smyth and Duntas, 2005). It is this possibility which is the subject of the present study.

Iodide Loss in Sweat

Profuse sweating, e.g., during vigorous exercise, leads to significant losses of minerals and electrolytes (Mao *et al.*, 2001; Maughan *et al.*, 2004). This loss is obviously greater at high temperatures or high levels of humidity. Although the need to replace electrolytes lost during sweating is well-established, little attention has been paid to iodine losses in sweat. An early report (Consolazio *et al.*, 1966) examined nitrogen, calcium and iodine loss in sweat from volunteers

Table 59.2 Iodine in sweat measured over time in volunteers maintained under control conditions at a temperature of 38.5°C and relative humidity of 30%

Sweat weight (g)	Time (h)	Iodine conc. ($\mu\text{g/l}$)	Total iodine loss (μg)
1235	3.5	26.7	46.2
2817	7.0	37.8	74.5
3559	12 (awake)	37.6	94.5
2017	12 (sleep)	38.8	52.0
5576	24	38.0	146.5

Source: Data taken from Consolazio *et al.* (1966), with permission.

maintained under control conditions at a temperature of 38.5°C and a relative humidity of 30%. The amounts of sweat produced related to iodine concentration at 3.5, 7.0h and during 12 waking and 12 sleeping hours, are shown in Table 59.2. Over a 24h period losses amounted to 5576g of sweat with a daily (24h) mean iodine loss of $146 \pm 31 \mu\text{g}$ of iodine. This would approximate to an iodine concentration of 38 $\mu\text{g/l}$ of sweat.

Interestingly, these sweat iodine levels have remarkable agreement with sweat iodine excretion studied in exercising and sedentary Japanese male university students by Suzuki and Tamura (1985), who showed that the iodine content of sweat was approximately 37 $\mu\text{g/l}$ and appeared independent of dietary iodide intake. Similar sweat iodine concentrations (37 $\mu\text{g/l}$) were reported by Mao *et al.*, 1990, who showed little variation in individual sweat iodine loss; the level of sweat iodine was found to be more stable than that of urinary iodine. The same workers (Mao *et al.*, 2001) further demonstrated the potential for iodine loss in sweat from athletes undergoing vigorous exercise over sustained periods. In suggesting that such losses would produce an iodine-deficiency state they demonstrated that 46% of members of a soccer team in training had thyroid enlargement compared to 2% of sedentary controls.

Effect of Iodine Supplementation

Studies by one of the authors in Ireland, an area of borderline dietary iodine intake, consistently showed a much lower level of iodine in sweat ($19 \pm 8 \mu\text{g/l}$). The percentage distribution of individual values is shown in histogram form in Figure 59.2. It can be seen that the majority of individual values (64.8%) lie between 20 and 30 $\mu\text{g/l}$, with a median value of 20 $\mu\text{g/l}$. This is difficult to explain in the context of the apparent independence of sweat iodine content from dietary iodine intake reported by Mao *et al.* (1990). In order to study this phenomenon further volunteers were given 400 μg of KI orally before exercise and sweat samples were collected within one hour. As shown in Figure 59.3, this resulted in a doubling of sweat iodine from 20 to 40 $\mu\text{g/l}$ after 1h, which returned to baseline 24 $\mu\text{g/l}$ after 24h and increased to 32 $\mu\text{g/l}$ at 48h.

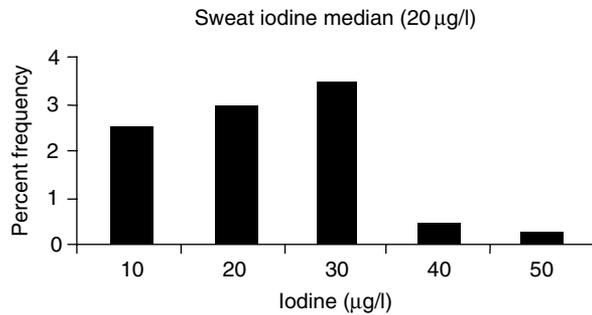


Figure 59.2 Percent frequency distribution of sweat iodine levels ($N = 43$).

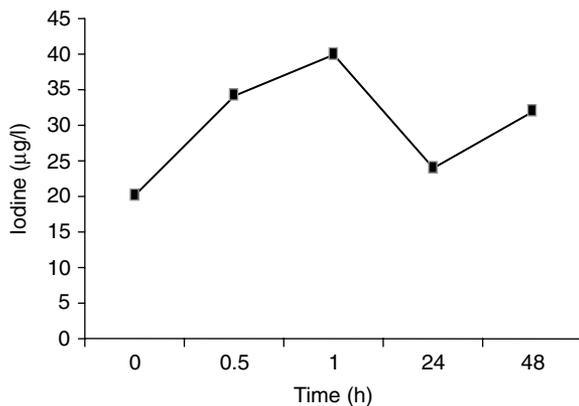


Figure 59.3 Sweat iodine value following ingestion of KI ($400\mu\text{g}$).

Magnitude of Iodine Loss

In examining the significance of iodine loss in sweat, [Mao et al., 2001](#) based their calculations on an average sweat loss of 400–600 ml through normal perspiration. On this basis, using a sweat iodine concentration of $37\mu\text{g/l}$, iodine loss would amount to $22\mu\text{g}$. However, in the case of an elite athlete sweating up to 3–5 liters daily, loss could amount to 111–185 μg . This loss may be modulated by a decreased urinary output, but would nonetheless produce a significant increase in daily iodine losses, which in the absence of iodine replacement could result in an iodine-deficient state ([Suzuki and Tamura, 1985](#)). A roughly similar daily iodine loss (146 μg) was calculated by [Consolazio et al. \(1966\)](#). Weight loss by soccer players recorded by [Mao et al. \(2001\)](#) in the course of a one-hour game was $1.54 \pm 0.57\text{kg}$, which is consistent with $1.1 \pm 0.43\text{kg}$ recorded in the United Kingdom by [Maughan et al. \(2004\)](#). Such losses would, of course, be dependent on temperature and humidity.

Sweat loss

In the case of the volunteers maintained in a controlled atmosphere ([Consolazio et al., 1966](#)), the amount of sweat

Table 59.3 Iodine loss (urine + sweat) in training athletes and controls

Iodine content	Sedentary control	Training athlete
Thyroid	10000 μg	10000 μg
Daily iodine intake	150 μg	150 μg
Urinary iodine	100 $\mu\text{g/l}$	100 $\mu\text{g/l}$
Daily iodine loss (urine)	150 μg	150 μg
Sweat iodine (approx)	40 $\mu\text{g/l}$	40 $\mu\text{g/l}$
Sweat volume (approx)	500 ml	4000 ml
Daily iodine loss (sweat)	20 μg	140–160 μg
Weekly iodine loss (urine + sweat)	–	–
Training 4 days per week (7 days urine + 4 days sweat)	1130 μg	~1700 μg
Weekly excess compared to sedentary controls	–	~570 μg (50.0%)

Source: Data derived from [Mao et al. \(2001\)](#), with permission.

produced over a period of time was estimated and losses in a 24 h period approximated to 5.5 liters with an iodine content of approximately 146 μg . Using the findings reported by [Consolazio et al. \(1966\)](#), [Suzuki and Tamura \(1985\)](#), and [Mao et al. \(2001\)](#) it was possible to construct a rough mathematical model of projected iodine losses in training athletes compared to sedentary controls. [Table 59.3](#) shows such a projection. For the purposes of this exercise thyroidal iodine content was assumed to be 10 mg. Daily iodine intake was set at the WHO-recommended value of 150 μg ([WHO, 2001](#)). Urinary iodine concentration was assumed to be constant at 100 $\mu\text{g/l}$ and daily urine volume at 1.5 liters. Sweat iodine was set at 40 $\mu\text{g/l}$, and sweat volumes were as suggested by [Mao et al. \(2001\)](#). Athletes were assumed to train for 4 days per week. As shown in [Table 59.3](#), under these conditions the excess loss in athletes over sedentary controls was calculated as 570 g (~50%) per week. It can be seen from these, admittedly crude, calculations that over a 10-week period a training athlete could lose approximately an extra 5 mg of iodine, severely depleting the iodine stores.

Sweat versus urinary iodine loss

Examination of urinary iodine excretion values in soccer players versus sedentary controls show that total iodine loss in both was of the same order of magnitude – sweat 11.6–99.8 μg , urine 10.2–178.4 $\mu\text{g/g}$ creatinine ([Mao et al., 2001](#)) – although this relationship could be altered dramatically if the subjects were consuming high-iodine foodstuffs such as seaweeds ([Suzuki and Tamura, 1985](#)). Expressing such sweat iodine loss as a percentage of urinary loss showed a range of 11.1–367.0%, with 38% of players having a mean loss for a one-hour game exceeding the mean urinary iodine excretion ([Mao et al., 2001](#)).

Sweating and iodine loss

Excessive sweating can occur not only in those engaged in vigorous exercise, but also in the general population residing in areas with a warm climate and high humidity. The reports reviewed in this study demonstrate that, under the above conditions, daily iodine loss through sweating could be of the same order of magnitude as urinary iodine loss. Such losses would be of no significance in the case of subjects taking occasional physical exercise. Similarly, in situations where dietary iodine intake is adequate or super-optimal, any additional sweat loss could be readily tolerated. However, in the case of an elite athlete or competitor pursuing a program of frequent exercise the continuous loss of iodine in sweat, if not replaced, would eventually result in an iodine deficiency state with the possibility of thyroid hypofunction (Vanderpump and Tunbridge, 2002). Replacement of electrolytes lost during vigorous exercise is a well-established practice. However, the possible need for iodine replacement has received little attention. Sweating, and therefore iodine loss, would be more severe in hot climates (Maresh *et al.*, 2004) and may require pre-exercise hydration.

Role of dietary iodine intake

Seasonal factors could also play a part, as it has been reported that dietary iodine intake shows a seasonal variation, at least in northern European countries (Phillips *et al.*, 1988; Hetherington and Smyth, 1993). Milk and dairy products are a major source of iodine intake, but their iodine content shows seasonal variation, having a higher iodine content in winter as cattle are brought into winter housing where they are fed dietary supplements including iodine. Therefore, lower iodine intake would coincide with a period when the majority of sporting activity and highest temperatures occur. An attempt to calculate the probable excess iodine losses resulting from vigorous exercise revealed an excess of approximately 50% loss, which over a 10-week training period could amount to some 5 mg of iodine. Undoubtedly, increased thyroidal uptake of ingested iodine would make up some of this loss, but in the absence of iodine supplementation, it is very likely that a state of negative iodine balance would arise. It must be stressed that the calculations used in this review are extremely crude; they neither attempt to reproduce criteria such as used in sweat prediction equations (Cheuvront *et al.*, 2007), nor do they address possible gender differences in sweat response (Hazelhurst and Claassen, 2006), which could have relevance in terms of iodine loss in view of the reported increased iodine excretion in pregnancy described below. The reason for the variability of iodine shown in the Irish study compared to that reported in the United States, Taiwan, or Japan may reflect the lower

dietary iodine intake in the Irish population (Smyth *et al.*, 1993, 2006) or temporal effects on the appearance of administered iodine in sweat. Any contribution or relationship of intrathyroidal iodine stores to iodine in sweat remains a matter of conjecture, but the demonstration that sweat iodine may not be independent of dietary intake at least raises the question of the significance of iodine distribution between sweat and other body iodine pools (Brown-Grant, 1961; Wolff, 1980).

Iodine loss and thyroid function

Although long-term iodine deficiency is known to impact on thyroid function, any significant effect of iodine loss through excessive sweating without replacement remains to be established. Indeed, most reports show that changes in thyroid function, if any, following vigorous exercise in both humans and animals are transient (Kanaka-Gantenbein, 2005; Graves *et al.*, 2006; Hackney and Zack, 2006). However, Kilic *et al.* (2006) reported that exercise-induced exhaustion was associated with lower levels of thyroid hormones. A stimulatory effect on both serum thyroid-stimulating hormone (TSH) and parathyroid hormone (unrelated to dermal calcium loss) has been demonstrated following intense exercise (Brabant *et al.*, 2005; Barry and Kohrt, 2007), but any metabolic consequences arising from these changes remain unclear.

Consequences of sweat iodine loss

The possible consequences of sweat-related iodine loss in terms of thyroid function in subjects undergoing vigorous exercise remain speculative. However, the report of Mao *et al.* (2001) of thyroid enlargement being observed in 46% of members of a soccer team in training, compared to 2% of sedentary controls, suggests that the iodine loss was at least producing a form of thyroid stress (Glinoe, 1997). An analogous situation exists during pregnancy where increased iodine requirements coupled with increased iodine excretion can lead to thyroid enlargement, the so-called pregnancy goiter (Smyth *et al.*, 1997; Delange, 2005), and can result in a negative iodine balance. Thyroid enlargement and other biochemical features of pregnancy-induced thyroid stress can be eliminated by the administration of iodine supplements (Glinoe, 1997).

Conclusions

In the absence of definitive evidence that iodine loss through excessive sweating can induce a relative iodine deficiency with consequences for thyroid hormone formation, there is not as yet a case for iodine supplementation of those involved in vigorous exercise. However, the

calculated levels of potential iodine loss through excessive sweating in the absence of adequate replacement at least raise the question of the implications of exercise-induced iodine loss for thyroid status and possibly consequential athletic performance.

Summary Points

- Sweating in the course of vigorous exercise involves loss of not only fluid and electrolytes, but also minerals such as iodine.
- An adequate supply of dietary iodine is essential to produce thyroid hormones and control metabolic processes whose optimal function is essential for the top-performing athlete.
- In the case of iodine such losses may, if not adequately replaced, eventually result in diminished thyroid hormone production.
- As a published sweat iodine concentration of 35–40 µg/l sweat losses of 4–5 liters would involve iodine losses equivalent to the WHO-recommended adult daily iodine intake (~150 µg).
- Iodine losses in sweat may not be independent of dietary iodine intake.
- Over a prolonged period of exercise, sweat iodine losses would result in a significant depletion of thyroidal iodine stores.

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Antibodies to Thyroid Peroxidase and Thyroglobulin in Iodine Deficiencies

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Abstract

Autoimmune thyroiditis (AITD) is one of the most common autoimmune disorders. The etiology is unknown, but both genetic and environmental factors may be involved, including iodine intake. Nearly all patients with chronic AITD have high concentrations of thyroglobulin antibodies (Tg-Ab) and thyroperoxidase antibodies (TPO-Ab) in serum. Circulating thyroid autoantibodies are also common in population studies of apparently healthy subjects. Epidemiological studies on thyroid autoimmunity are difficult to compare, due to differences in the kinds of biochemical and epidemiological methods applied. The assays for measuring TPO-Ab and Tg-Ab have improved recently, but no reference ranges exist and different cutoff values are often chosen. A sudden increase in iodine intake by an iodine-deficient population may induce enhanced thyroid autoimmunity, although the findings are ambiguous. The exact mechanism behind such an increase in autoimmunity is unknown, but damage to thyroid tissue by free radicals and enhanced antigenicity of thyroglobulin may be involved. TPO-Ab and Tg-Ab are common both in populations with a stable high iodine intake and those with mild and moderate iodine deficiency (ID). The mechanisms behind the development of thyroid antibodies in ID may be different from the mechanisms involved in high iodine intake. It is possible that antibodies in some cases may develop secondarily to goiter formation, with exposure or release of Tg and other antigens from the thyroid. Further studies are needed to obtain information on the interplay between iodine intake and thyroid autoimmunity, but adjustment of population iodine intake to around the recommended level may be optimal for the prevention of thyroid disease.

Abbreviations

AITD	Autoimmune thyroiditis
ELISA	Enzyme-linked immunosorbent assays

ICCIDD	International Council for Control of Iodine Deficiency Disorders
ID	Iodine deficiency
IRMA	Immunoradiometric assays
Mic	Microsomal
MRC	Medical Research Council
RIA	Radioimmunoassays
Tg	Thyroglobulin
Tg-Ab	Thyroglobulin antibody
TPO	Thyroid peroxidase
TPO-Ab	Thyroid peroxidase antibody
UNICEF	United Nations Children's Fund
WHO	World Health Organization

Introduction

Autoimmune diseases are a poorly-understood group of disorders, which have been defined as clinical syndromes caused by activation of T or B lymphocytes or both, in the absence of ongoing infections or other discernible causes (Davidson and Daimond, 2001). Regulatory malfunction of the immune system is supposed to be secondary to a genetic predisposition currently thought to be multigenetic (Davidson and Daimond, 2001). However, even in a genetically predisposed person, a triggering event is probably required for frank autoreactivity, although knowledge of the nature of this trigger is often limited or unknown (Safran *et al.*, 1987; Tomer and Davies, 1993).

Autoimmune thyroiditis (AITD) is one of the most common autoimmune disorders. The humoral immune response is dominant in Graves' hyperthyroidism, whereas cellular immune response is more dominant in hypothyroidism caused by chronic AITD (Marcocci and Chiovato, 2000). Thyroid autoantibodies are proteins manufactured by the immune system that are directed against proteins in the thyroid. Although nearly all patients with chronic

AITD have high concentrations of circulating thyroid autoantibodies (Arai *et al.*, 2000; Carle *et al.*, 2006; Feldt-Rasmussen, 1996), for the most part the disorder appears to be the consequence of tissue damage initiated by T lymphocytes (Weetman and McGregor, 1994). Measurement of autoantibodies against thyroid peroxidase (TPO-Ab) and thyroglobulin (Tg-Ab) has for many years been a major tool in the diagnosis of autoimmune thyroid diseases, such as Hashimoto's thyroiditis, primary myxedema and postpartum thyroiditis (Feldt-Rasmussen *et al.*, 1991; Feldt-Rasmussen, 1996).

Among the many environmental factors that have been suggested to take part in the development of thyroid autoimmunity, iodine intake may be the most important (Prummel *et al.*, 2004).

Thyroglobulin and thyroglobulin antibody

Thyroglobulin (Tg) is a large 660 kDa dimeric glycoprotein composed of two identical polypeptide chains, and is unique in its content of iodinated amino acids. Most iodinated amino acids in Tg are iodotyrosines, which serve as precursors of the biologically active thyroid hormones, thyroxine and triiodothyronine (Tomer, 1997). Tg is produced by the thyroid follicular cells and secreted into the follicular lumen, where it is stored as colloid. Small amounts of Tg are present in the circulation, which is primarily of clinical importance in diagnosing the persistence or recurrence of thyroid cancer after ablative therapy (Spencer *et al.*, 1999). However, Tg in serum is increased in almost all kinds of thyroid disease, including goiter (Knudsen *et al.*, 2001) and subacute thyroiditis (Hidaka *et al.*, 1994), the concentrations overlapping with those in healthy individuals.

Human Tg is one of the main autoantigenes in thyroid disease caused by AITD (Salvi *et al.*, 1988), but antibodies against Tg are also frequently measurable in serum from apparently healthy subjects from the population (Hollowell *et al.*, 1998; Pedersen *et al.*, 2003). Iodination of Tg may induce major changes in its stereochemical configuration (Dunn *et al.*, 1983), which may change its immunoreactivity and be important in the generation of thyroid autoantibodies (Saboori *et al.*, 1998).

Methods for the measurement of Tg-Ab

Tg-Ab was initially measured by passive tanned red cell hemagglutination, and the results were reported simply as positive or negative or as the dilution of serum giving a positive response (titer).

Subsequently, much more sensitive methods including radioimmunoassays (RIA), immunoradiometric assays (IRMA), enzyme-linked immunosorbent assays (ELISA), and chemiluminescence assays have been developed.

The latter methods are now used in clinical practice, and the results are usually given quantitatively, in some cases in international units calibrated against the Medical Research Council (MRC) 65/93 Feldt-Rasmussen (1996). Several studies have found large differences in the performance of different assays for measuring Tg-Ab (Arai *et al.*, 2000; Feldt-Rasmussen, 1996; Lindberg *et al.*, 2001).

Figure 60.1 illustrates the discrepancy between the results obtained by different generations of assays. A new assay based on precipitation of ^{125}I Tg bound to Tg-Ab by the use of polyethylene glycol was compared with a commercially-available passive hemagglutination test (Laurberg and Pedersen, 1988). Twelve of 60 normal subjects and nearly all of 72 patients with various thyroid disorders had Tg-Ab detected by the new assay. On the other hand, none of the normal subjects and very few of the patients had Tg-Ab measured by the hemagglutination assay. The hemagglutination assay had not only a much lower sensitivity, but also a lower specificity, as some of the sera with very high concentrations of Tg-Ab did not give detectable agglutination.

Thyroid peroxidase and thyroid peroxidase antibody

Thyroid peroxidase (TPO) is a 100-kDa poorly glycosylated membrane-bound enzyme containing a heme prosthetic group. The enzyme is expressed on the apical membrane of thyrocytes, facing the colloid, where it is

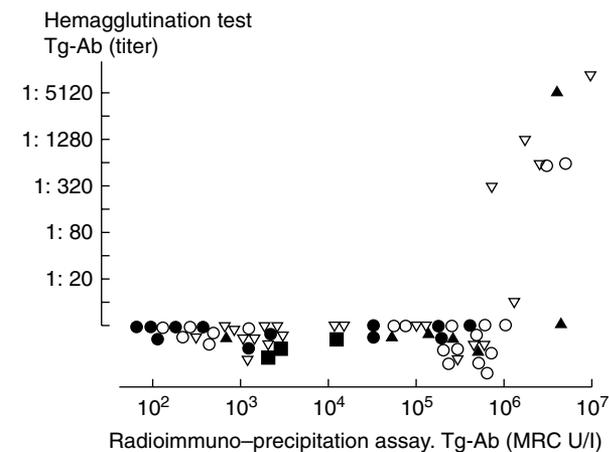


Figure 60.1 Tg-Ab concentrations in sera measured with both a newly-developed radioimmunoprecipitation assay and a commercially available passive hemagglutination assay (Wellcome, UK). (●) Normal subjects (12 out of 60 had Tg-Ab with the new assay); (▽) patients after treatment for Graves' disease (23 out of 25 had Tg-Ab); (○) patients after treatment for multinodular toxic goiter or with atoxic multinodular goiter (20 out of 37 had Tg-Ab); (▲) patients after treatment of spontaneously developed hypothyroidism ($n = 7$, all had Tg-Ab); (■) patients after previously subacute thyroiditis, ($n = 3$, all had Tg-Ab). Data from Laurberg and Pedersen (1988) with permission.

responsible for catalyzing iodine oxidation, iodination of tyrosine residues and coupling of iodotyrosines to generate thyroid hormones (McLachlan and Rapoport, 1992). TPO is identical with the previously defined thyroid microsomal antigen (Marcocci and Chiovato, 2000), and measurement of TPO-Ab has replaced measurements of antibodies against the microsomal antigen (Mic-Ab).

TPO, together with Tg, is the main autoantigen in AITD. Like Tg-Ab, TPO-Ab is found in the majority of patients with autoimmune thyroid diseases, and is also commonly measurable in apparently healthy subjects without symptoms or signs of thyroid disease (Hollowell *et al.*, 1998; Pedersen *et al.*, 2003). However, in the population, there is a considerably stronger association between elevated serum TSH, as a sign of impending thyroid failure, and the presence of TPO-Ab, than between TSH and Tg-Ab (Figure 60.2) (Bülöw Pedersen, *et al.*, 2005).

Methods for measurement of TPO-Ab

Several methods are available for measurement of TPO-Ab in serum. Most early studies were done with assays based on immunofluorescence or passive tanned erythrocyte hemagglutination using crude thyroid microsomes as antigen, and the results were reported as positive or negative or as an antibody titer. After identification of TPO as the microsomal antigen, more sensitive methods for detecting TPO-Ab have been established, including RIA, IRMA, ELISA and chemiluminescence methods (Dherbomez

et al., 2000; Feldt-Rasmussen, 1996). Most TPO-Ab assays provide the antibody results in international units by using the standard MRC 66/387 with a calibration factor (Feldt-Rasmussen, 1996).

Measurement of antimicrosomal antibody has been associated with a low specificity due to interfering factors, such as the presence of Tg in the “purified” microsomes or other antibodies also reacting with the thyroid microsomal fraction (Feldt-Rasmussen *et al.*, 1991). The higher specificity and sensitivity of the newer assays have been shown in a number of studies (Arai *et al.*, 2000; Kasagi *et al.*, 1996; Feldt-Rasmussen *et al.*, 1983; Lindberg *et al.*, 2001; Roti *et al.*, 1992).

Detection of Tg-Ab and TPO-Ab in healthy individuals

Most authors define TPO-Ab and Tg-Ab positivity as the detection of antibodies above a certain assay detection limit (Hollowell *et al.*, 1998; Laurberg *et al.*, 1998; Pedersen *et al.*, 2003). However, it has also been suggested that antibodies are present at low concentrations in nearly all subjects (Jensen *et al.*, 2006; Zophel *et al.*, 2003). Zophel *et al.* (2003) found TPO-Ab in 1277 out of 1295 healthy subjects without signs of thyroid abnormalities. In the low measurable range below 1.1 IU/ml, TPO-Ab values followed a normal distribution that was independent of age and sex, and the authors concluded that a reference range independent of the population investigated

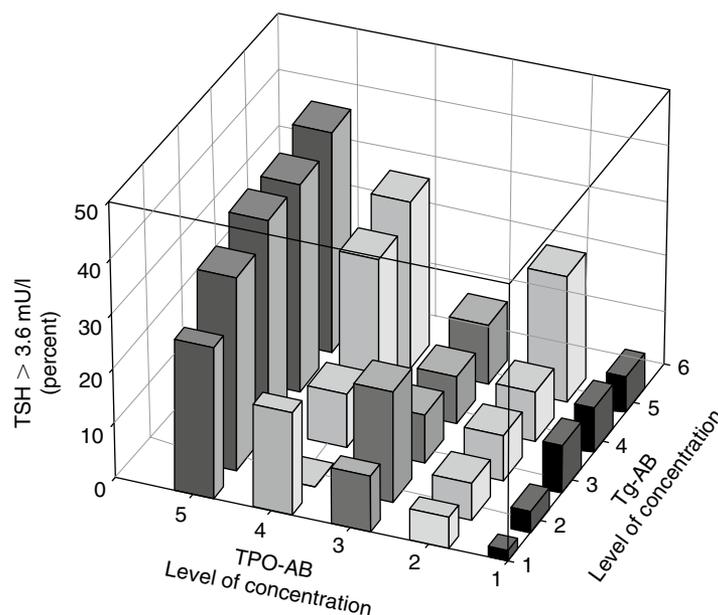


Figure 60.2 Prevalence rates (%) of elevated serum TSH at different concentrations of TPO-Ab and Tg-Ab (level 1: no antibodies, level 2–5: increasing antibody concentrations, i.e., quartiles of antibody positive participants). The prevalence of elevated TSH increased with increasing level of TPO-Ab (p for trend < 0.005). A less prominent but significant TPO-Ab-independent association between TSH and Tg-Ab was observed. The trend was statistically significant both in participants with Tg-Ab measured alone (TPO-Ab level 1, p for trend < 0.001) and in participants with TPO-Ab present (>30 U/l), (p for trend < 0.001). Data from Bülöw Pedersen *et al.* (2005) with permission.

could be defined. It remains, however, to be finally proven that the low signals given by the majority of normal sera really reflect antibodies specifically directed against thyroid antigens, and not just “background” noise.

Thyroid autoantibodies and thyroid autoimmunity

A good correlation has been found between the presence of lymphocytic infiltration of the thyroid and the presence of thyroid antibodies in serum (Kasagi *et al.*, 1996; Roth *et al.*, 1997; Yoshida *et al.*, 1978), and between the severity of histological thyroiditis and the level of antibody in serum (Arai *et al.*, 2000). Currently, it is not known whether TPO-Ab and Tg-Ab are directly involved in the pathogenesis of chronic AITD or if one or both are nonpathogenic antibodies generated secondary to the tissue damage.

Unfortunately, most studies comparing lymphocytic infiltration in the thyroid and circulating autoantibodies in serum have been performed in areas with sufficient or excess iodine intake, and knowledge of the relationship between thyroid antibodies and histological findings in the thyroid in areas with low iodine intake is limited.

Is it possible to compare the prevalence rates of TPO-Ab and Tg-Ab in different studies?

There are many problems associated with the comparison of results obtained from different epidemiological studies on thyroid autoantibodies. As described above, one of the major problems is the differences in the use of biochemical/immunological methods, including absence of standardization of the assays, use of assays with different sensitivity and specificity, and possible differences in other technical details of the assays applied. Cut-off values for TPO-Ab and Tg-Ab were not always well-documented and varied largely (Hollowell *et al.*, 1998; Laurberg *et al.*, 1998; Pedersen *et al.*, 2003). In some studies, the cut-off values corresponded to the functional sensitivity given by the manufacturer, whereas rather high cut-off values were chosen by other investigators (Teng *et al.*, 2006).

The other problem is the differences in the epidemiological methods applied. It is well-established that thyroid autoantibodies are more commonly measurable in females than in males (Hollowell *et al.*, 1998; Laurberg *et al.*, 1998; Prentice *et al.*, 1990; Tunbridge *et al.*, 1977), and that the prevalence of antibodies increases with age, at least in females (Pedersen *et al.*, 2003). It is possible that this increase is present only up to a certain age, after which there may be a plateau (Aghini-Lombardi *et al.*, 1999; Hawkins *et al.*, 1980; Tunbridge *et al.*, 1977). The results obtained from the population studies should therefore be age and sex standardized before comparison. Some studies included highly selective participants, such as subjects above 70 years

(Brochmann *et al.*, 1988), hospitalized geriatric patients (Szabolcs *et al.*, 1995), or elderly ambulatory women (Martinez-Weber *et al.*, 1993), whereas other investigators included randomly selected subjects from the population. A racial difference in susceptibility to thyroid autoimmunity has been shown in autopsy studies (Okayasu *et al.*, 1991, 1994) and in epidemiological studies (Hollowell *et al.*, 1998). Likewise, many other differences in genetic background and environmental factors, such as smoking habits, influence the results (Prummel *et al.*, 2004).

Comparison of epidemiological studies on the prevalence of circulating thyroid autoantibodies in areas with different iodine intakes is therefore difficult, unless the studies are designed as comparatives with exactly the same methods applied in two or more regions.

Intervention Studies on Iodine Intake

In areas where iodine deficiency (ID) was prevalent but disappeared after iodine prophylaxis, histological studies of thyroid glands surgically removed because of cancer or goiter have shown a higher prevalence of lymphocyte infiltration after the increased iodine intake (Harach and Williams, 1995). Clinical studies have confirmed an increase in the production of thyroid antibodies in association with an increase in iodine intake (Kahaly *et al.*, 1998; Koutras *et al.*, 1990; Zimmermann *et al.*, 2003). Epidemiological data to some degree support such an enhancing effect of an increased iodine intake on thyroid autoimmunity. It seems as if a sudden increase in iodine intake may be more important for the development of thyroiditis and generation of thyroid antibodies than exposure to a constant but high iodine level (Kahaly *et al.*, 1998; Laurberg *et al.*, 1998).

Intervention studies in animals

Experimental animal studies have shown that excessive iodine intake can precipitate thyroiditis with lymphocytic infiltration in the thyroid in genetically predisposed strains of rats (Allen and Braverman, 1990; Cohen and Weetman, 1988; Ruwhof and Drexhage, 2001), mice (Rose *et al.*, 1999) and chickens (Safran *et al.*, 1987; Sundick *et al.*, 1992). The opposite phenomenon, with increased thyroid autoimmunity in iodine-deficient animals has also been observed, albeit in genetically different strains of rats (Ruwhof and Drexhage, 2001). In the nonautoimmune prone Wistar rat, a low dietary iodine intake led to not only goiter formation and hypothyroidism, but also an intrathyroidal accumulation of dendritic cells and raised production of Tg-Ab (Mooij *et al.*, 1994; Ruwhof and Drexhage, 2001). It was suggested that this accumulation of dendritic cells could have a physiological function of regulating the growth and function of the thyrocytes

deprived of iodine. Furthermore, the generated Tg-Ab could have the physiological role of clearing the excess Tg, which is released from the thyroid in ID (Ruwhof and Drexhage, 2001). In iodine excess, the autoimmune reactivity of the nonautoimmune prone Wistar rat was depressed and a relatively high dietary iodine intake seemed optimal to keep thyroid autoreactivity at a minimum (Ruwhof and Drexhage, 2001).

The autoimmune prone BB-DP rat, on the other hand, more easily developed AITD under the influence of a diet rich in iodine, depending on the previous state of the thyroid (Ruwhof and Drexhage, 2001). In severe ID, those animals had less severe lymphocytic thyroiditis and the production of Tg-Ab was depressed, probably as part of a general lowering of thyroid autoimmunity induced by severe ID. The results obtained with these different strains of rats may be viewed as a model for the individual heterogeneity in response to changes in iodine intake.

Intervention studies in humans

Most intervention studies performed in humans have been in areas with ID. In a number of studies, administration of iodine in varying doses has been followed by an increase in thyroid autoimmunity (Kahaly *et al.*, 1998; Koutras *et al.*, 1990); however, this has not been a consistent finding (Benmiloud *et al.*, 1994; Liesenkotter *et al.*, 1996; Simescu *et al.*, 2002).

In a double-blind trial, 500 µg/day iodine orally or 0.125 mg/day L-tyroxine was given to 62 patients with ID (Kahaly *et al.*, 1998). After 6 months, high Mic-Ab and Tg-Ab developed in 6 of the 31 patients receiving iodine compared to none in the tyroxine group. Fine needle biopsy revealed marked lymphocyte infiltration in all six antibody-positive subjects. After the withdrawal of iodine intake, antibody titer and lymphocyte infiltration decreased, and after 3 years, four of the six patients were again antibody negative.

In 30 goitrous subjects treated with 300 µg/day iodine as oral potassium iodide, 9 individuals (30%) developed elevated thyroid antibody titers during 6 months of follow-up. The induction of thyroid autoantibodies tended to be dose dependent, as only 12% of goitrous subjects receiving 0.15 mg/day iodine became antibody positive (Koutras *et al.*, 1990).

In a randomized study from Germany, 40 out of 83 euthyroid patients with AITD were treated with 250 µg/day potassium iodide for a mean period of 4 months. The diagnosis was based on positive TPO-Ab combined with moderate-to-severe hypoechogenic pattern of the thyroid by ultrasound. Urinary iodine excretion increased from a baseline mean of 72 ± 38 µg/g to 268 ± 173 µg/g creatinine. Seven patients in the iodine-treated group and one patient in the control group developed some degree of hypothyroidism, but no significant change in TPO-Ab

level was observed. Unfortunately, Tg-Ab was not reported (Reinhardt *et al.*, 1998).

In concordance with that study, no thyroid autoantibodies appeared in 114 schoolchildren from an iodine-deficient area in Romania within 1 year after a single oral administration of iodized oil containing 200 mg iodine (Simescu *et al.*, 2002). Similarly, no changes were seen in the frequency of TPO-Ab in the early postpartum period in a group of 38 women from an iodine-deficient area who had received 300 µg potassium iodide/day during pregnancy from 10 to 12 weeks of gestation (Liesenkotter *et al.*, 1996). Nor did iodine supplementation (150 µg/day) during pregnancy and the postpartum period to TPO-Ab positive women in an area with mild-to-moderate ID induce or worsen postpartum thyroiditis (Nøhr *et al.*, 2000).

Intervention by Iodine Fortification of Salt in the Population

Iodine fortification governed by national health care agencies and guided by international organizations such as the Internal Council for the Control of Iodine Deficiency Disorders (ICCIDD), the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), has successfully eliminated the risk of ID disorders in most countries throughout the world (WHO, 2001). It has been recommended that iodine fortification programs should be followed by monitoring of thyroid diseases to evaluate the effectiveness of the fortification, and to detect and counteract the unintended effects of iodine enrichment such as an increase in hyper- or hypothyroidism (WHO, 2001). In some areas, an increase in thyroid autoimmunity has been observed after iodine fortification (Harach and Williams, 1995), which can be regarded as an iodine intervention study at the population level, albeit with no parallel control.

In the goitrous region of Salta, Argentina, the prevalence of thyroiditis in females was studied before and after iodine prophylaxis was introduced in 1963 by registering lymphocytic infiltration in goiters that had been surgically removed because of thyroid cancer or adenoma (Harach and Williams, 1995). Urinary iodine excretion in schoolchildren increased from 9.3 µg/g creatinine in 1963 to 152 µg/g creatinine in 1975 and 110 µg/g creatinine in 1983. The frequency of lymphocytic infiltrate of the thyroid rose from 8% before iodine supplementation to 28% in those operated on within 10 years after iodine prophylaxis was introduced, and 23% in those operated on more than 10 years later. It can be argued that one of the causes for these findings may be that surgery was primarily performed for ID goiter and not autoimmune goiter before, and that ID goiter became less common after, the increase in iodine intake.

In a severely iodine-deficient area of Morocco, TPO-Ab and Tg-Ab were measured in 323 schoolchildren before

and up to 1 year after the distribution of iodized salt. The median urinary iodine excretion was $17\mu\text{g/l}$ at baseline, which increased to $150\text{--}200\mu\text{g/l}$ after iodine fortification. A transient increase in the prevalence of detectable TPO-Ab and Tg-Ab was seen, with levels returning to baseline within 1 year (Zimmermann *et al.*, 2003).

Although an iodine prophylaxis program has never been officially implemented in Greece, nutritional iodine intake has improved in recent years largely because of the commercial availability of iodized salt. Better socioeconomic conditions, in general, have also contributed to the improved iodine nutrition of Greek schoolchildren (Zois *et al.*, 2003). When a group of Greek schoolchildren was studied in 1994, the median urinary iodine excretion was $84\mu\text{g/l}$, and the prevalence of AITD diagnosed from the presence of thyroid autoantibodies combined with a characteristic pattern of thyroiditis at ultrasonography of the thyroid was 3.3% (Zois *et al.*, 2003). A new status on iodine intake and thyroid autoimmunity in schoolchildren was made in 2001 at a time when the median iodine excretion had increased to $202\mu\text{g/l}$. Sensitive but different antibody assays were used in the two studies. The prevalence of AITD had increased significantly to 9.6% (Zois *et al.*, 2003). Further, the prevalence of autoimmune stigmata in fine needle aspiration smears from thyroid nodules had increased from 5.9% to 13.9% (Doufas *et al.*, 1999).

Five years after the introduction of iodized salt in Sri Lanka, the prevalence of Tg-Ab was high among schoolchildren. The interpretation of the authors was that this was the result of the increased iodine intake (Premawardhana *et al.*, 2000). Three years later, when the status was re-evaluated, the prevalence of Tg-Ab had decreased significantly (Mazziotti *et al.*, 2003). Unfortunately, no pre-iodine data on thyroid autoantibodies were available.

Comparative Epidemiological Studies on Thyroid Autoantibodies

As described previously, it is difficult to compare the results from different studies on prevalence rates of circulation thyroid autoantibodies. Unfortunately, there are relatively few comparative studies of cohorts with different iodine intake in which sensitive assays have been applied in the analysis of thyroid autoantibodies.

In a comparative epidemiological study performed in Iceland, with a stable high iodine intake (median urinary iodine excretion about $300\mu\text{g/l}$), and Jutland, Denmark with a long-standing low iodine intake (median urinary iodine excretion about $40\text{--}60\mu\text{g/l}$), a randomly selected sample of elderly females and males aged 66–70 years were examined (Laurberg *et al.*, 1998). Except for the different level of iodine intake, the two groups were comparable. Clinical goiter was much more prevalent in Jutland than in Iceland (12.2% vs. 3.2%). The dominating type of hyperthyroidism

in Jutland was multinodular toxic goiter, which was infrequent in Iceland. On the contrary, Graves' disease represented the main proportion of the cases of hyperthyroidism in Iceland. Somewhat unexpectedly, the prevalence rates of both TPO-Ab and Tg-Ab were nearly twice as high in Jutland, compared to Iceland with the highest iodine intake (Figure 60.3).

The DanThyr cohort was studied immediately before iodine fortification of salt was implemented in Denmark (Knudsen *et al.*, 2000). It comprised 4649 subjects living in two areas of Denmark with mild and moderate ID, respectively (Copenhagen, mild ID, median urinary iodine $61\mu\text{g/l}$; Aalborg, moderate ID, median iodine intake: $45\mu\text{g/l}$). The participants were randomly selected from the sex and age groups, females aged 18–22, 25–30, 40–45 and 60–65 years, and males aged 60–65 years. The sex and age distribution was equal in the two subcohorts. Assays with high sensitivity were used. The overall prevalence of antibodies (TPO-Ab and/or Tg-Ab) was 18.8% with higher frequencies in women and with age (Pedersen *et al.*, 2003). Within all age and sex groups, the prevalence rates of TPO-Ab and Tg-Ab were similar (overall TPO-Ab: 13.1%, Tg-Ab: 13.0%). The overall prevalence rate of thyroid antibodies (TPO-Ab and/or Tg-Ab) was the same in the two subcohorts. However, in the age group 60–65 years, a consistent pattern of more thyroid antibodies in moderate than in mild ID was found: 22.1 vs. 17.3%, $P = 0.02$. The prevalence of goiter was highest in the area with the lowest iodine intake due to a high presence of multinodular goiter (Knudsen *et al.*, 2000) as was the incidence rate of hyperthyroidism, whereas the incidence rate

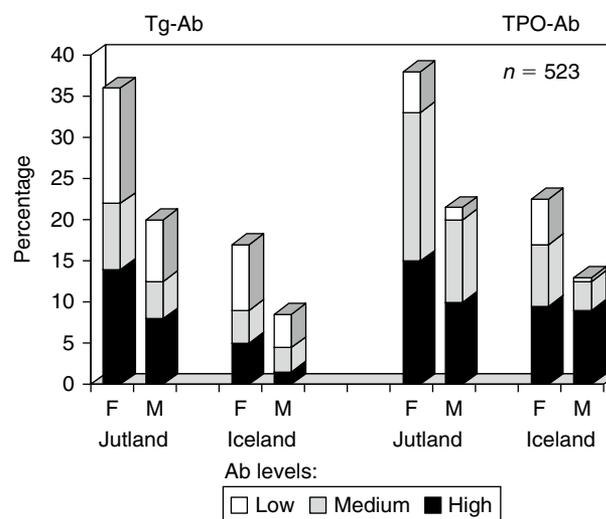


Figure 60.3 Prevalence rates (%) of Tg-Ab and TPO-Ab in elderly subjects from Jutland, Denmark, with mild-to-moderate ID, and from Iceland with a stable high iodine intake. Different levels of antibody concentration are shown in each bar. Samples from the two areas were measured in random order in the same assays. Data from Laurberg *et al.* (1998).

Table 60.1 Thyroid autoantibodies in population studies from areas with different iodine intake

Author [country]	Iodine status	Antibody assays	Cutoff	Age (years)	Prevalence of antibody (%)
Laurberg <i>et al.</i> , (1998) [Denmark]	Moderate ID	Tg-Ab: Radioimmunoprecipitation TPO-Ab: Enzyme-linked immunosorbent assay	Detection limits	68	Tg-Ab: 31 TPO-Ab: 29
Pedersen <i>et al.</i> , (2003) [Denmark]	Moderate and mild ID	Tg-Ab: Radioimmunoassay TPO-Ab: Radioimmunoassay	Detection limits	18–65	Tg-Ab: 13.0 TPO-Ab: 13.1
Hintze <i>et al.</i> , (1991) [Germany]	Mild ID	Tg-Ab: Radioimmunoassay Mic-Ab: Radioimmunoassay	>Detection limits	60+	Tg-Ab: 10.1 Mic-Ab: 23.2
Knudsen <i>et al.</i> , (1999) [Denmark]	Mild ID	TPO-Ab: Enzyme-linked immunosorbent assay	>100 U/ml; Detection limits not given	41 and 71 years	TPO-Ab: 22.8
Aghini-Lombardi <i>et al.</i> , (1999) [Italy]	Mild ID	Tg-Ab: Agglutination TPO-Ab: Agglutination	≥1:100; Detection limits not given	1+	Tg-Ab and/or TPO-Ab: 12.6
Teng <i>et al.</i> , (2006) [Panshan, China]	Mild ID	Tg-Ab: Chemiluminescence TPO-Ab: Chemiluminescence	>Detection limits	36 ± 13 (SD)	Tg-Ab: 9.0 TPO-Ab: 9.2
Fenzi <i>et al.</i> , (1986) [Italy]	Mild ID	Tg-Ab: Agglutination Mic-Ab: Agglutination	≥1:100; Detection limits not given	Young adults	Tg-Ab and/or Mic-Ab: 14.4
Hollowell <i>et al.</i> , (1998) [United States]	Iodine sufficient	Tg-Ab: Radioimmunoassay TPO-Ab: Radioimmunoassay	Detection limits	12+	Tg-Ab: 11.5 TPO-Ab: 13.0
Bjøro <i>et al.</i> , (2000) [Norway]	Iodine sufficient	TPO-Ab: Luminoimmunoassay	>200 U/ml; Detection limits not given	40+	TPO-Ab: 9.7
Tunbridge <i>et al.</i> , (1977) [UK]	Iodine sufficient	Tg-Ab: Tanned red cell technique Mic-Ab: Microhemagglutination	Tg-Ab: ≥1:20 Mic-Ab: ≥1:100	18+	Tg-Ab and/or Mic-Ab: 7.3
Bryhni <i>et al.</i> , (1996) [Norway]	Iodine sufficient	Tg-Ab: Agglutination Mic-Ab: Agglutination	1:10 and 1:100; Detection limits not given	34 ± 8.4 (SD)	Tg-Ab: 2.8 Mic-Ab: 6.1
Laurberg <i>et al.</i> , (1998) [Iceland]	Iodine sufficient	Tg-Ab: Radioimmunoprecipitation TPO-Ab: Enzyme-linked immunosorbent	Detection limits	66–70	Tg-Ab: 13 TPO-Ab: 18
Teng <i>et al.</i> , (2006) [Zhangwu, China]	More than adequate	Tg-Ab: Chemiluminescence TPO-Ab: Chemiluminescence	>Detection limits	36 ± 13 (SD)	Tg-Ab: 9.0 TPO-Ab: 9.8

Note: Prevalence rates of TPO-Ab and/or Tg-Ab in populations with different iodine intake. All studies included both females and males.

of hypothyroidism was highest in the area with only mild ID (Bülow Pedersen *et al.*, 2002).

In a follow-up study, 3761 randomly selected subjects, above 13 years, living in one of three regions in China with mild ID (median urinary iodine excretion: 84 µg/l), more than adequate iodine intake (urinary iodine: 243 µg/l), and excessive iodine intake (urinary iodine: 651 µg/l) were included (Teng *et al.*, 2006). At baseline, the prevalence rates of high level of TPO-Ab (TPO-Ab ≥ 50 IU/ml)

and Tg-Ab (Tg-Ab ≥ 40 IU/ml) were measured using assays with high sensitivity. TPO-Ab was found in 9.2, 9.8 and 10.5%, and Tg-Ab in 9.0, 9.0 and 9.4%, with no statistically significant difference between the subcohorts. After 5 years of follow-up, there were still no differences in the rates of high levels of TPO-Ab and Tg-Ab in the three cohorts.

In Hungary, Szabolcs *et al.* (1997) screened elderly nursing home residents for thyroid disease. The three subcohorts

were comparable with respect to age and sex distribution. They were living in the same geographical and ethnographical region, but in areas with varying levels of iodine intake (median iodine excretion 72, 100 and 513 $\mu\text{g/g}$ creatinine, respectively). The overall prevalence rates of positive thyroid antibodies (TPO-Ab and/or Tg-Ab > 100 U/ml) were similar in the three subcohorts. Further, the prevalence of high TPO-Ab (TPO-Ab > 1000 U/ml) was similar.

Fenzi studied the prevalence of thyroid antibodies in a moderate endemic goiter area of Italy (Fenzi *et al.*, 1986). Schoolchildren ($n = 142$) and their parents ($n = 159$) were included. The overall frequency of Mic-Ab and Tg-Ab in the adult population was 14.4%, which was significantly higher than that in the sex- and age-matched control group living in an iodine-sufficient area. It was observed that antibodies were more common in subjects with goiter compared to subjects without goiter.

In Turkey, mandatory iodization of salt was introduced in 1999. Two years later, a comparative cross-sectional study including 1733 adolescents from two areas with different iodine intakes was performed (Bastemir *et al.*, 2006). ID was still present in one of the two subcohorts (median urinary iodine excretion 61 $\mu\text{g/l}$, $n = 740$), whereas the other subcohort was iodine replete (median urinary excretion 139 $\mu\text{g/l}$, $n = 993$). The prevalence rate of thyroid autoantibodies (TPO-Ab and/or Tg-Ab) was significantly higher in the iodine-replete subcohort (18.5% vs. 6.6%). Considering the low age of the study population (14–18 years) and compared to other studies, the prevalence rates of both TPO-Ab and Tg-Ab were rather high in the iodine-replete subcohort. It is possible that the high values were caused by the sudden increase in iodine intake, and that a decrease in thyroid autoimmunity would occur over the following years (Zimmermann *et al.*, 2003).

Thyroid Autoantibodies in Iodine-Deficient Areas

A number of studies on the epidemiology of thyroid autoantibodies have been performed as descriptive studies in iodine-deficient areas. Results from some population-based studies from both iodine-deficient and iodine-sufficient areas are shown in Table 60.1. The results are ambiguous.

In an Italian survey, 1411 subjects representing the entire population of a small city, Pescopagano, was included (Aghini-Lombardi *et al.*, 1999). The area was an endemic goiter area with long-established, stable, mild ID with a median urinary iodine excretion of 55 $\mu\text{g/l}$. The prevalence of goiter was reported to be 16% in children and 59.8% in adults. Thyroid autoantibodies (TPO-Ab and/or Tg-Ab) were measurable in 12.6% of the entire population (females: 17.3%, males: 7%), increasing from 2.4% in children to 21.7% in the age group 46–55 years, with little change in

older subjects. Antibodies were more frequent in subjects with goiter than without goiter. The antibody assays used for measuring TPO-Ab and Tg-Ab were both based on agglutination. It could therefore be speculated that the given prevalence rates were minimum rates, due to the relatively low sensitivity of such assays.

In a cross-sectional study from a borderline iodine-deficient area in Denmark (Copenhagen), Knudsen *et al.* included 2656 randomly selected subjects aged 41–71 years. The prevalence rate of TPO-Ab, measured by ELISA, was high at 22.8% (Knudsen *et al.*, 1999).

In a moderate ID area of Germany, Mic-Ab and TPO-Ab were measured in 466 randomly selected elderly subjects aged 60–98 years from the population (Hintze *et al.*, 1991). The prevalence rate of elevated Mic-Ab (>500 U/ml) was high at 23.2%, whereas Tg-Ab was significantly elevated (>200 U/ml) at 10%. The values were relatively high considering the low sensitivity of the Mic-Ab assay and the relatively high cut-off values.

In the Sardinian autoimmunity study, 8040 children living in 29 communities with borderline ID or mild-to-moderate ID were included (Loviselli *et al.*, 2001). Thyroid autoantibodies (Mic-Ab by passive hemagglutination technique ($n = 1670$) and TPO-Ab by RIA ($n = 6370$); Tg-Ab by RIA) were measurable in 2.9% of the participants, ranging between 0% and 7.3% without any geographical correlation to goiter prevalence and urinary iodine excretion. Thyroid autoantibodies were more often present in children with goiter, compared to children without goiter. The study may suggest that, at least in the iodine intake level range corresponding to moderate ID to low normal iodine intake, iodine intake does not affect the prevalence of thyroid autoantibodies in children.

Thyroid Autoantibodies in Iodine-Replete Areas

In the NHANES III study, TPO-Ab and Tg-Ab were measured in 15592 randomly selected subjects, representative of the US population (Hollowell *et al.*, 1998). The antibodies were measured with highly sensitive assays based on RIA. Approximately 18% of the disease-free population had Tg-Ab and/or TPO-Ab above the detection limit (TPO-Ab ≥ 0.5 IU/ml, Tg-Ab ≥ 0.1 IU/ml). TPO-Ab was measurable in 13% and Tg-Ab in 11.5% of the population.

In the original Wickham study, thyroid autoantibodies were measured in 2779 subjects from the small city, Wickham, in the UK (Tunbridge *et al.*, 1977). Both Tg-Ab and Mic-Ab were measured with old assays with relatively low sensitivity. The prevalence of one or both antibodies was 11.2% in females and 2.8% in males. At the follow-up 20 years later, 26.4% of the participants were antibody positive (Vanderpump *et al.*, 1996). Possible explanations

for this huge increase in the prevalence of antibody-positive participants could be that the participants were 20 years older at follow-up and that newer and better assays had been used.

In Norway, the iodine intake is generally considered to have been sufficient for many years (Bjoro *et al.*, 2000). In two population-based Norwegian studies, Mic-Ab and Tg-Ab were detected with passive hemagglutination in elderly subjects aged >70 years (Brochmann *et al.*, 1988) and in younger subjects aged around 34 years (Bryhni *et al.*, 1996). Mic-Ab was detected in 15.7% vs. 6.1% of the subjects, respectively, and Tg-Ab in 11.7% vs. 2.8%, respectively. In another population-based Norwegian study, TPO-Ab was measured with a more sensitive luminoimmunoassay in randomly selected subjects above 40 years; 9.7% of the participants had TPO-Ab (females 13.9; males 2.8%).

In South Wales, UK, 414 asymptomatic randomly selected elderly subjects above 70 years were screened for autoimmune thyroid disease (Lazarus *et al.*, 1984): 18.6% of the participants had elevated levels of Mic-Ab and/or Tg-Ab (Mic-Ab 15.4%, Tg-Ab 13.3%, both antibodies present 8.5%).

The Paradox of Iodine Intake and Thyroid Autoimmunity

In spite of the difficulties in interpreting and comparing results from epidemiological studies on thyroid autoimmunity, there are certain tendencies in the relationship between thyroid autoimmunity and iodine intake.

A sudden increase in iodine intake in an iodine-deficient population may induce enhanced thyroid autoimmunity (Harach and Williams, 1995). Both cellular immune response with histological signs of thyroiditis and humoral immune response with circulating thyroid autoantibodies may be increased. It seems as if at least a part of this autoimmunity is reversible, and that the prevalence of antibodies will decrease to a lower level over time if the higher iodine intake is continued (Mazziotti *et al.*, 2003; Zimmermann *et al.*, 2003), or decrease in a relatively short time period if the iodine intake is reduced to the baseline level (Kahaly *et al.*, 1998).

Thyroid autoimmunity with lymphocytic infiltration in the thyroid and circulating thyroid autoantibodies seems to be common in populations with a stable high iodine intake. Subclinical and overt hypothyroidism is prevalent and more common than hyperthyroidism (Hollowell *et al.*, 2002; Vanderpump *et al.*, 1995). It is plausible that the impaired thyroid function associated with high iodine intake is caused by destruction of the thyroid by autoimmune processes in some cases, but not in all. In a population study from the coastal regions of Japan with excessive iodine intake, the authors found a high prevalence of subclinical hypothyroidism in antibody-negative subjects. There was a significant correlation between high iodine intake and

hypothyroidism, which was not found in antibody-positive subjects (Suzuki *et al.*, 1965). It is possible that hypothyroidism in the antibody-negative subjects was secondary to the general inhibitory effect of a high iodine load on many thyroidal processes. Such autoregulation has probably been developed to protect against hyperfunction (Pisarev and Gärtner, 2000).

In mild and moderate ID, the prevalence rate of circulating thyroid autoantibodies in the population is also high (Laurberg *et al.*, 1998; Pedersen *et al.*, 2003). In such areas, nontoxic and toxic multinodular goiters are prevalent and overall hyperthyroidism is more common than hypothyroidism (Laurberg *et al.*, 1999). Results from areas with severe ID are limited and might, in some cases, be influenced by the general immunosuppressive effect of malnutrition, which may occur simultaneously (Salabe *et al.*, 1982).

Speculations on Possible Mechanisms behind Iodine Intake and Development of Antibodies

A number of mechanisms have been suggested to explain the association between thyroid autoimmunity and the level of iodine intake. A sudden shift from very low to high iodine intake may induce damage to the thyroid tissue by free radicals (Li and Boyages, 1994). Also, enhancement of the autoimmunogenic properties of thyroglobulin by increased iodination may play a role (Saboori *et al.*, 1998; Sundick *et al.*, 1987). Apart from these two mechanisms, no other model has been put forward that satisfactorily explains the association between excessive iodine intake and the generation of thyroid autoimmunity.

In iodine-deficient areas, the mechanism behind the development of thyroid autoantibodies may be different. It has been speculated that the development of antibodies in some cases may be secondary to goiter formation with exposure in the thyroid or release of antigens from the thyroid. Tg release from the thyroid is common in iodine-deficient areas with nodular goiter (Knudsen *et al.*, 2001); TPO may also be released from the thyroid. It is possible that mild and moderate ID over the years gradually induces circulating antibodies similar to the gradual induction of formation and growth of nodules. In accordance with such a mechanism, a higher prevalence of antibodies was found in subjects with goiter compared to subjects without goiter in areas with ID (Aghini-Lombardi *et al.*, 1999; Fenzi *et al.*, 1986; Bülow Pedersen *et al.*, 2005).

Autoimmunity, abnormal iodine supply and neoplasia are the three common mechanisms behind thyroid diseases in a population. Further studies are needed to obtain information on the interplay between iodine intake and thyroid autoimmunity. This may allow optimal prevention of thyroid disease by adjusting iodine intake in the individual and in the population.

Summary Points

- AITD is one of the most common autoimmune disorders.
- Nearly all patients with chronic AITD have high concentrations of TPO-Ab and Tg-Ab.
- A sudden increase in iodine intake may enhance thyroid autoimmunity.
- TPO-Ab and Tg-Ab are common in apparently healthy subjects with sufficient or excessive iodine intake.
- TPO-Ab and Tg-Ab are also common in populations with mild and moderate ID.
- The mechanisms behind the development of thyroid autoantibodies in iodine-sufficient and iodine-deficient populations may be different.
- In ID, thyroid autoantibodies may be secondary to goiter formation.
- Further studies are needed to obtain information on the interplay between iodine intake and thyroid autoimmunity.

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Bromide Interference with Iodine Metabolism: Goitrogenic and Whole-body Effects of Excessive Inorganic Bromide in the Rat

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Abstract

Some conclusions drawn from our recent research on the interference of excessive bromide intake with iodine metabolism in the rat are reviewed in this chapter. The biological behavior of bromide ion, especially in the thyroid gland, is compared with the behavior of iodide and chloride. The effects of both organic and inorganic bromides on human health are briefly mentioned. The effects of an enhanced bromide intake in the rat on the thyroid function, and on the whole-body metabolism of iodine are also summarized. In addition, we also proved experimentally our hypothesis that the whole-body biological half life of bromide depends on the magnitude of sodium intake rather than on the intake of chloride. It is suggested that high levels of bromide in the organism of experimental animals can influence their iodine metabolism in two parallel ways: by a decrease in iodide accumulation in the thyroid and skin (and in the mammary glands in lactating dams), and by a rise in iodide excretion by the kidneys. Very high bromide intake in the rat significantly shortens the biological half life of iodine in the thyroid (from about 100 to 30 h). By accelerating the renal excretion of iodide, excessive bromide can also influence the pool of exchangeable iodide in the thyroid. The nature of the goitrogenic effects of bromide and the mechanisms of its interference with the biosynthesis of the thyroid hormones however, remains to be elucidated.

Abbreviations

CNS	Central nervous system
HPGe	High purity germanium (detector)
INAA	Instrumental neutron activation analysis

Introduction

Elements from a given group of the periodic system have repeatedly been shown to exhibit certain similarities of

metabolic behavior. This is particularly pronounced for the elements in group 7 (fluorine, chlorine, bromine and iodine). The physiological significance of the chloride ion has long been recognized. Likewise, the importance of iodine as the main constituent of thyroid hormones is amply documented in a number of chapters in this *Comprehensive Handbook of Iodine*. In contrast, not enough information is available on bromine metabolism. Bromine is very reactive chemically; it has properties that are intermediate between those of chlorine and iodine. Bromine is one of the most abundant and ubiquitous of the recognized trace elements in the biosphere. However, it has not been conclusively shown to perform any essential function in animals, plants or microorganisms (Pavelka, 2004a).

In nature, bromine is found mostly bound to metals in the form of inorganic salts – the bromides. Bromide is also the main degradation product of brominated hydrocarbons (e.g., methyl bromide), excessively used in agriculture for preplanting fumigation of soils and postharvest fumigation of commodities such as grains, spices, nuts, fruits and tobacco; as well as of other bromine compounds (e.g., ethylene dibromide) applied on a large scale in industry. In the course of the twentieth century bromide has been introduced increasingly into the environment as salt-mining waste and a degradation product of fumigants. Therefore at present, the general population will mainly be exposed to bromide via their food. The new role of bromide as a residue in food and water necessitated its broad toxicological evaluation (Van Leeuwen and Sangster, 1987).

Current knowledge about the goitrogenic effects of bromide, including some conclusions following from the results of our recent research on this subject, is summarized in this brief review. Since inorganic bromide is the ionic form of bromine which exerts therapeutic, as well as toxic effects, only studies that deal with exposure of experimental animals to bromide will form the basis of this review.

Metabolism and Function of Bromide

Effects of organic and inorganic bromides on animals and humans

The application of methyl bromide (a gas at temperatures above 4°C) as a soil fumigant results in almost complete eradication of populations of a wide variety of microflora and fauna, as well as other soil organisms. Death from exposure follows a steep dose–response curve (for references, see WHO, 1995). Human exposure to methyl bromide occurs predominantly as an occupational hazard, particularly during fumigation of soil or buildings, but also during chemical manufacture or fire extinguisher accidents. The major health concern is from acute exposure. Since the first case reported by Schuler in 1899, there have been hundreds of cases of methyl bromide poisoning in humans involving systemic poisoning, skin and eye injuries, damage to the central nervous system (CNS) and even fatalities. However, the mode of action of methyl bromide is not yet fully understood. Honma *et al.* (1985) concluded that the CNS toxicity might be due to the methyl bromide molecule itself or the methyl moiety incorporated into tissue constituents. Concentrations of the emerging bromide ion are insufficient to explain methyl bromide toxicity.

Inorganic bromide exerts a very low acute toxicity upon oral administration in rodent species. LD₅₀ values ranging from 3500 mg/kg body weight in rats to 5020 mg/kg body weight in mice have been reported (for references, see Van Leeuwen and Sangster, 1987). In humans, bromide salts have an irritant effect on gastric mucosa, causing nausea and vomiting. Thus, these symptoms can be expected in acute bromide poisoning. Considering the previous use of bromide in clinical medicine for the treatment of neurological and psychic disorders, it is evident that bromide exerts an effect on the CNS. The clinical symptoms of chronic bromide poisoning (so-called bromism) are very well-known and have been described in an almost endless list of publications since the introduction of bromides as medicines in the second half of the nineteenth century. CNS symptoms consist of apathy, disturbed coordination, loss of memory, drowsiness and loss of emotional control. However, the patient may also be agitated and may be overtly hallucinating. Although bromide-containing sedative drugs are now used infrequently, intoxication with this halide and its attendant CNS symptoms still remain a serious problem. The occurrence of bromism is often mentioned in recent medical literature (for references, see Van Leeuwen and Sangster, 1987).

The main origin of observed pharmacological and toxicological effects of the bromide ion appears to be interference with the action of other halides. With regard to the effects on the CNS, it has been proposed that disturbance of the active, as well as passive transport of chloride ions across nerve cell membranes, leading to hyperpolarization of these

cells, might be the mechanism underlying the anticonvulsant activity of bromide (Woodbury and Pippenger, 1982).

Resemblance of bromide to chloride

There is no evidence of bromide accumulation in humans in any particular organ that might indicate a specific physiological function of this ion. After oral ingestion, bromide is rapidly and completely absorbed in the gastrointestinal tract and, analogously to chloride, distributed almost exclusively in the extracellular fluid. The distribution (Pavelka *et al.*, 2000a, b) and action of bromide appears to be directly related to its resemblance to other halides, chloride and, to a lesser extent, iodide (Pavelka, 2004a, b). In the extracellular fluid, bromide ions replace the equivalent amount of chloride ions, the molar sum of total halides remaining constant at approximately 110 mmol/l. Both ions are predominantly excreted by the kidneys, in the case of bromide at a rate of approximately 5% of the administered dose per 24 h (Pavelka *et al.*, 2000b). The greater part of both halogen ions is reabsorbed in the renal tubules after glomerular filtration and, due to the similarity in their physico-chemical properties, bromide and chloride compete for tubular reabsorption (Rauws, 1983).

Resemblance of bromide to iodide: goitrogenic effects of bromide

Considering the chemical similarity of bromine to iodine, on the other hand, goitrogenic effects of bromide may be assumed. Indeed, an enhanced bromide intake in the rat could markedly reduce iodide accumulation in the thyroid (Van Leeuwen *et al.*, 1988; Buchberger *et al.*, 1990; Pavelka *et al.*, 1999), as well as in the skin (Pavelka *et al.*, 2001b). This leads to the general assumption that the biological behavior of bromine is similar to that of chlorine so that administration of bromide results in some displacement of body chloride and *vice versa* (Hellerstein *et al.*, 1960), but this does not appear to be valid in the case of the thyroid gland. In studies on the interaction of bromine with iodine in the rat thyroid under the conditions of enhanced bromide intake (Pavelka *et al.*, 1999; Vobecký *et al.*, 2000) we established that in this tissue, contrary to other tissues, bromide did not replace chloride, but rather iodide. Using the radionuclides ⁸²Br and ¹³¹I and the whole-body measurement of the retained activity, we found that the time course of bromine excretion in adult male rats substantially differed from iodine excretion (Pavelka *et al.*, 1998). The whole-body excretion curve of bromine showed only a single rate constant with a biological half life longer than 10 days. In contrast, iodine was apparently excreted from two different pools: the first with a very short half life (less than 10 h), characterizing the clearance of excess iodine from the organism; the second with a half life of about 108 h, accounting for iodine

release from the thyroid. The rapidity of attainment of a stable $[I]/[Br]$ concentration ratio in the thyroid indicated that the biological half life of bromine in the rat thyroid was substantially shorter than the whole-body half life, and that it was probably close to the half life of iodine. Indeed, we determined the value of the biological half life of bromine in the rat thyroid by measuring the radioactivity of isolated thyroids of animals which received ^{82}Br -labeled bromide in their diet continuously, during a 16-day experimental period (Vobecký *et al.*, 1997). The observed value of this half life (about 110 h), was very close to the measured value of the biological half life of iodine and to the value 106 h, recently published for the biological half life of iodine by Singh *et al.* (1994). The fact that the values of the half lives of bromine and iodine in the rat thyroid are practically identical can be considered as further proof that the biological behavior of bromide in the thyroid, in contrast to other organs and tissues, is more similar to the biological behavior of iodide, rather than of chloride.

Effects of Excessive Bromide on Iodine Metabolism in the Thyroid

Thyrotoxic effects of bromide

Van Leeuwen *et al.* (1983) and Loeber *et al.* (1983) were among the first who proved the toxic effects of high bromide doses on the morphology and function of the thyroid. These authors observed a remarkable complex of presumably related changes in the endocrine system, induced by bromide in male rats fed a very high dose of sodium bromide in the diet (19.2 g NaBr per kg diet) for 4 or 12 weeks. The most striking effects of bromide on the endocrine system were found on the thyroid gland and the gonads. Activation of the thyroid, characterized by an increase in relative weight of the organ and a reduction in follicle size, was observed. These phenomena were accompanied by a decrease in serum thyroxine, indicating typical hypothyroidism induced by excessive bromide (Loeber *et al.*, 1983). Van Leeuwen *et al.* (1988) later stated that bromide affected thyroid peroxidase activity. However, this hypothesis was disproved by Taurog and Dorris (1991), who observed that even a 200-fold excess of bromide, in comparison with iodide, in an *in vitro* incubation system had no effect on the rate of thyroid peroxidase-catalyzed iodination of thyroglobulin. In addition, they concluded that even large doses of bromide did not interfere with iodide transport into the thyroid.

Until recently, studies following the effects of enhanced supply of bromide into the body of experimental animals were carried out mostly on adult individuals and at normal iodine availability. Buchberger *et al.* (1990) were among the first who studied the effects of chronic administration of large bromide doses on the biosynthesis of thyroid hormones in iodine-deficient rats. The results of this study

indicate that bromide toxicity is dependent on the state of iodine stores in the body; the signs of hypothyroidism caused by bromide intake were significantly enhanced under conditions of simultaneous iodine deficiency.

Concentration ratio $[I]/[Br]$ in the thyroid as a sensitive marker of iodine status

In the late 1990s, Pavelka *et al.* took up this subject in a series of studies on the interference of exogenous bromide with iodine metabolism in rat tissues, as well as in studies on bromide and iodine transfer through mothers' milk and its impact on the suckling rat (cf. this book, Chapter 20). We found that, under conditions of increasing bromide intake, the rat thyroid gland responded very sensitively to an increase in a relatively small bromide intake (e.g., approximately 0.4–4 mg bromide/day) by a marked decrease of the $[I]/[Br]$ concentration ratio (approximately from 40 to 6). It is important that the magnitude of the decrease in the $[I]/[Br]$ ratio also depended on the level of iodine supply to the animal. The $[I]/[Br]$ ratio in the thyroid was as much as five times lower in rats with a marginal iodine deficiency than in animals with a sufficient or excessive iodine intake (Figure 61.1).

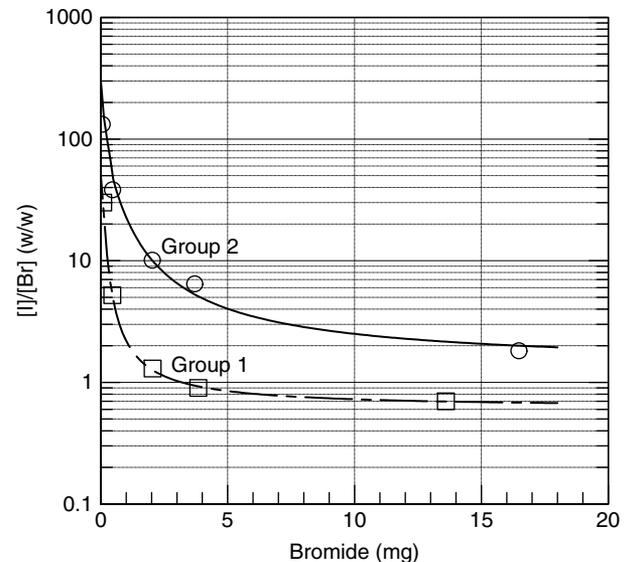


Figure 61.1 Concentration ratios $[I]/[Br]$ (w/w) in the isolated thyroids of rats, depending on the mean daily intake of bromide and iodine. Two groups of adult male Wistar rats, each of 25 members, were maintained for 14 days on a special low-iodine diet (home-made) and drank water with the addition of various amounts of bromide, and no addition of iodide (Group 1) or with the addition of 1 mg iodide/l (Group 2). The concentration ratios $[I]/[Br]$, determined by INAA in the isolated whole thyroids of rats of Group 1 – with a marginal iodine deficiency, are about five times lower than those in the rats of Group 2 – with a sufficient iodine intake.

With the aid of short-term instrumental neutron activation analysis (INAA) of the isolated lyophilized rat thyroids, we found that with enhanced bromide intake, bromide in the thyroid did not replace chloride, as in all other tissues, but iodide (Vobecký *et al.*, 2000). Up to 40% of the amount of iodine in the thyroid was replaced by bromine under our experimental conditions (when bromine was obtained from drinking water supplemented with 0.01–0.5 g bromide/l). Most probably, bromine in the thyroid remains in the form of bromide ion and, in proportion to its increasing concentration, the production of iodinated thyronines decreases. Analyses of fractionated thyroids showed that the most pronounced decrease in the [I]/[Br] ratio occurred in the soluble low-molecular-weight fraction, containing mostly inorganic ions and possibly free halogenated amino acids. In the high-molecular-weight soluble fraction of the thyroid, containing thyroglobulin with covalently bound halogenated residues of tyrosine and thyronines, even a high bromide intake did not displace organically bound iodine, so that the [I]/[Br] ratio changed only slightly (Table 61.1). These results indicate that with sufficient iodine stores in the body, a stable

[I]/[Br] concentration ratio in the thyroid is rapidly established during the exposure of rats to increased concentrations of bromide, while under iodine deficiency iodine atoms in the thyroid are replaced with bromine atoms.

The nature of the toxic effects of bromide on the thyroid gland and mechanisms of its interference with the biosynthesis of thyroid hormones however, has not been explained so far. Most probably, interaction of bromide with iodide uptake by the thyroid gland is the underlying mechanism leading to thyroid dysfunction and, consequently, to the observed alterations in the pituitary–thyroid axis (see below).

Interference of Excessive Bromide with the Whole-Body Metabolism of Iodine

Uptake of iodide by various organs and tissues: an overview

Previously, Van Leeuwen *et al.* (1988) found a decrease in the body weight and marked changes in the morphology of the thyroid, a decrease in serum thyroxine accompanied

Table 61.1 [I]/[Br] concentration ratios (w/w) in whole rat thyroids and in separated fractions of thyroid glands, depending on the mean daily intake of iodine (μg) and bromide (mg)

Group ^a	Subgroup ^b	Mean daily intake		[I]/[Br] (w/w)			
		I (μg)	Br (mg)	Thyroid	Fraction 1 ^c	Fraction 2 ^d	Fraction 3 ^e
0	0	2.5	0.09	30.0	14.9	7.9	254
	10	2.2	0.45	5.2	15.9	0.5	218
	50	2.3	2.0	1.2	7.1	0.2	99
	100	2.2	3.8	1.0	9.5	0.1	111
	500	2.1	13.6	0.8	6.6	0.04	94
1	0	31.6	0.07	131	114	8.9	258
	10	42.0	0.48	37.9	93.4	3.8	505
	50	41.5	2.0	10.0	101	1.0	213
	100	33.6	3.2	6.4	59.9	0.7	200
	500	34.8	16.5	1.1	37.7	0.1	116
10	0	386	0.09	100	168	16.2	605
	10	371	0.45	35.1	132	3.0	368
	50	348	1.8	11.7	172	1.3	415
	100	367	3.7	7.3	60.3	0.6	591
	500	351	17.5	1.5	22.4	0.1	382

Notes: Concentrations of I and Br (as well as those of Cl, Na and K) were determined simultaneously by short-term instrumental neutron activation analysis (INAA) in lyophilized samples of less than 5 mg dry weight of the whole rat thyroids and of the fractions separated from the glands. The magnitude of the decline of the [I]/[Br] ratio with increasing bromide intake in the animals depends markedly on the level of iodine supplied to the organism: cf. rats of Group 0 – with a marginal iodine deficiency, and rats of Group 1 – with sufficient iodine intake and of Group 10 – with excessive iodine intake. The most pronounced decrease in the [I]/[Br] ratio occurred in the soluble low-molecular-weight fraction of the thyroids (Fraction 2), containing mostly inorganic ions (and possibly free halogenated amino acids).

^aGroup 0, no addition of iodide in drinking water; Group 1, addition of 1 mg iodide per liter of drinking water; Group 10, addition of 10 mg iodide per liter of drinking water.

^bSubgroups are characterized by the concentration of bromide (mg/l) in drinking water.

^cInsoluble fraction (remnants of tissue).

^dLow-molecular-weight soluble fraction (inorganic ions and possibly free halogenated amino acids and their derivatives).

^eHigh-molecular-weight soluble fraction (thyroglobulin – covalently bound halogenated residues of tyrosine and thyronines).

by an increase in the concentration of TSH, and a decrease in ^{125}I -iodide uptake by the thyroid in rats fed a semi-synthetic purified diet containing a high concentration of bromide (up to 19.2 g NaBr per kg diet, ensuring an average daily intake of approximately 210–300 mg bromide per animal). Similar signs of hypothyroidism were also described by Buchberger *et al.* (1990) in rats fed an iodine-poor diet with various amounts of added bromide (4–16 g NaBr per kg diet). Under these conditions, besides the above-mentioned findings, death of experimental animals was also encountered. We also followed the effects of enhanced bromide intake on the uptake of ^{131}I -iodide by various organs and tissues in adult male rats, fed either a standard (iodine-sufficient) or an iodine-poor pelleted diet. Moreover, the effects of excessive bromide on the kinetics of iodine elimination from the body, and on the resulting values of the whole-body biological half life of iodine were studied in these animals (Pavelka *et al.*, 1999, 2001a, b; Vobecký *et al.*, 1999). At the same time, we performed detailed studies on the influence of an enhanced bromide intake in the animals on the kinetics of iodide uptake and elimination, especially in the thyroid and skin (Pavelka *et al.*, 2001b). In addition to an extremely high bromide intake (>200 mg bromide per animal per day) we

also used low, moderate and high levels of bromide intake. Because the biological behavior of bromide depends on the state of iodine stores in the body, we performed our studies both under the conditions of sufficient iodine supply and of mild iodine deficiency.

Influence of bromide on the whole-body biological half life of iodine

A significant influence of very high bromide intake in the animals (>160 mg bromide/day) on the values of the whole-body biological half life of iodine was established (Figure 61.2a, b). Very high bromide intake (i) decreased the amount of radioiodide accumulated in the thyroid; (ii) changed the proportion between the amount of iodide retained in the thyroid and the total amount of absorbed iodide; (iii) significantly reduced the biological half life of iodine in the thyroid – from approximately 101 to 33 h in animals maintained on an iodine-sufficient diet (B) and from 92 to about 30 h in rats fed a low-iodine diet (A); and (iv) changed the time course (adding a further phase) of iodine elimination from the body. These changes were caused, with high probability, by an increase of iodine elimination by kidneys due to excess bromide. The overall

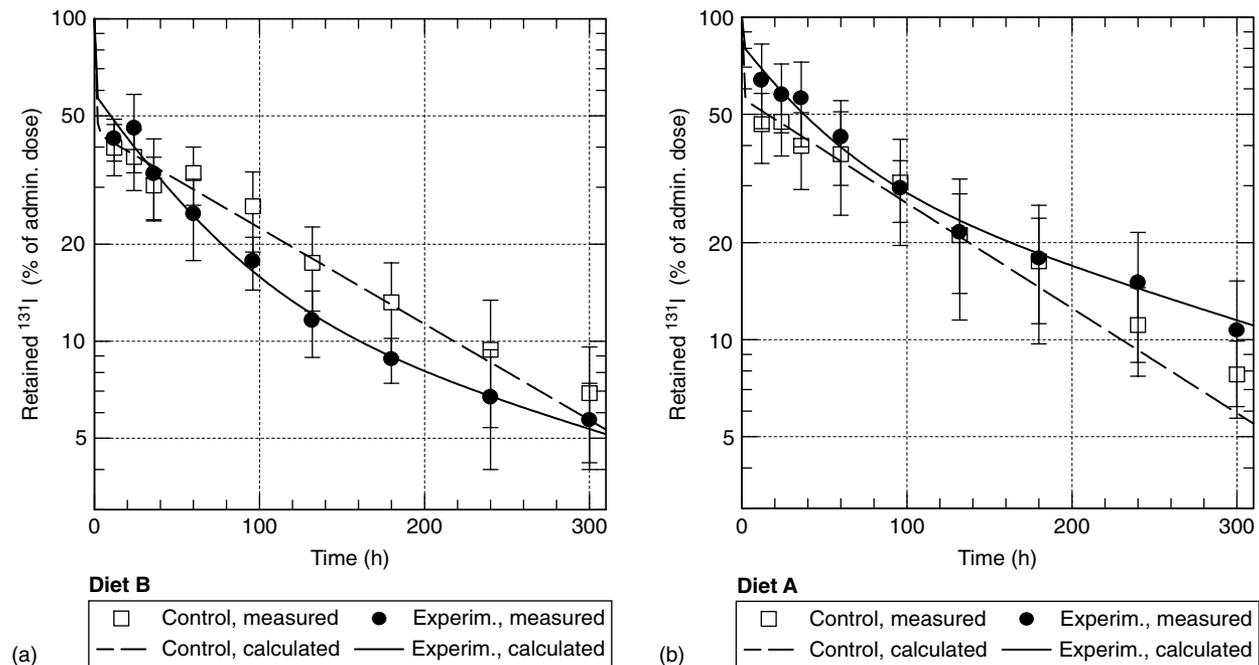


Figure 61.2 Changes in ^{131}I radioactivity retained in the whole body of rats maintained on (a) an iodine-sufficient diet (diet B), and (b) an iodine-deficient diet (diet A), dependent on the time after ^{131}I -iodide administration. (a) Two groups of adult male Wistar rats (each of 8 members) were maintained for 14 days on a standard iodine-sufficient diet (diet B, Bergman). Control rats drank distilled water, while rats of the experimental group drank water with the addition of 5 g bromide/l (concentration of bromide 5000 ppm). (b) The same as in (a) but rats were iodine-deficient, being maintained on a special low-iodine diet (diet A, Altromin C1042) after drinking water containing 0.5% NaClO_4 for 5 days before the beginning of the experiment. On the 14th experimental day, all animals were given approximately 1.2 MBq ^{131}I in the form of carrier-free iodide in saline by subcutaneous injection. The *in vivo* measurement of the retained whole-body ^{131}I radioactivity was performed by using a computerized gamma-spectrometric system equipped with an HPGe detector. Each point represents the mean \pm SD, $n = 8$. Reprinted with permission from Pavelka *et al.*, 2001a.

picture of iodine elimination in animals fed the low-iodine diet was similar to that in animals maintained on an iodine-sufficient diet (Pavelka *et al.*, 2001a).

Influence of bromide on the uptake of iodide by the skin

In rats fed a diet with a sufficient iodine supply ($>25\mu\text{g I/day}$), iodide accumulation in the skin predominated during the first hours after ^{131}I -iodide application (Figure 61.3a, b). Radioiodide was then gradually transferred into the thyroid from the skin. Very high bromide intake ($>150\text{ mg bromide/day}$) in these animals caused a marked decrease in iodide accumulation, especially by the thyroid.

Decreased accumulation was due to an increase in iodide elimination, both from the thyroid and from the skin. In rats kept under conditions of iodine deficiency ($<1\mu\text{g I/day}$) iodide accumulation in the thyroid, but not in the skin, was markedly increased as a result of thyrotrophic stimulation (Figure 61.4a, b). The effect of a high bromide intake ($>100\text{ mg bromide/day}$) in these animals was particularly pronounced, because the rates of iodide elimination were most accelerated both from the thyroid and from the skin (Pavelka *et al.*, 2001b).

Possible mechanisms of excessive bromide actions

The effects of very high bromide intake by animals on the thyroid gland and its function, which we observed in our experiments, notably in animals fed the low-iodine diet (e.g., marked decrease in the 24-h uptake of ^{131}I -iodide; increase in the relative weight of the thyroid; decrease in the serum level of total thyroxine; etc.) were similar to those described by other authors. However, in addition to the thyroid, we also observed the effects of the highest dose of bromide administered to the rats (in drinking water with the addition of 5 g bromide/l, i.e., at a concentration of 5000 ppm), in the stomach and the skin. At the same time, the effects of a lower dose of bromide (500 ppm) were only marginal, and no effects of bromide were observed at the level of 50 ppm (Pavelka *et al.*, 1999). The observed reduction in food intake, and consequently in body weight gain, in rats drinking water with the highest concentration of bromide, could be explained by the assumption that under conditions of high bromide levels in the body a disturbance in the physiological function of the stomach could arise. Because bromide is also concentrated in gastric mucosa and secreted into the stomach lumen (Gross, 1962), possible changes in the composition

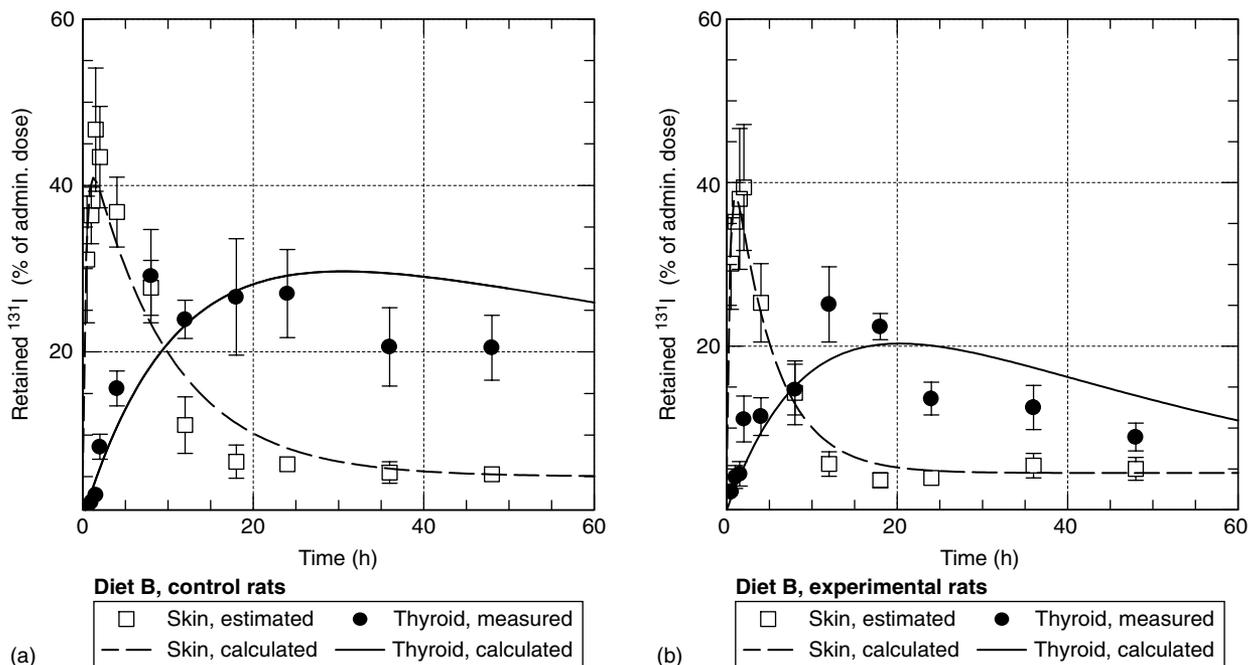


Figure 61.3 Changes in ^{131}I radioactivity retained in the skin (estimated) and in the thyroid of (a) control rats, and (b) experimental rats maintained on an iodine-sufficient diet (diet B), dependent on the time after ^{131}I -iodide administration. Two groups of adult male Wistar rats, each of 66 members, were maintained for 14 days on a standard iodine-sufficient diet (diet B, Bergman). (a) Control rats drank distilled water, and (b) rats of the experimental group drank water with the addition of 5 g bromide/l (concentration of bromide 5000 ppm). On the 14th experimental day, all animals were given approximately 0.3 MBq ^{131}I in the form of carrier-free iodide in saline by subcutaneous injection. At appropriate time intervals the rats were successively killed and samples of whole blood, the thyroid glands and skin without hair were collected. After determination of fresh weight, ^{131}I radioactivity of the samples was measured either by using a computerized gamma-spectrometric system equipped with an HPGe detector or by a gamma-ray counter. Each point represents the mean \pm SD, $n = 6$. Reprinted with permission from Pavelka *et al.*, 2001b.

of the digestive juice (e.g., possible production of hydrobromic acid) could disturb the digestive processes or could produce an organic disorder of the gastrointestinal system. The observed inhibition of the uptake of ^{131}I -iodide by skin under enhanced bromide intake could also be fairly significant for iodine metabolism in the rat, because skin accounts for about 20% of the rat body weight and represents the most important repository of both halogens.

We therefore suggest that high levels of bromide in the body of experimental animals could influence their iodine metabolism in two parallel ways: by a decrease in iodide accumulation in the thyroid; and by a rise in iodide excretion by the kidneys. A high surplus of bromide ions in the blood, which was, under our experimental conditions, several thousand times greater than the concentration of iodide, could competitively inhibit the access of iodide into the thyroid and could replace a part of the iodide in the gland with bromide. By accelerating the renal excretion of iodide, excessive bromide could also influence the pool of exchangeable iodide in the thyroid (Pavelka *et al.*, 1999).

Dependence of the Excretion Rate of Bromide (Iodide) on Sodium Intake

It is well-known that the biological half life of bromide can be markedly shortened by administering surplus sodium chloride (Langley Czerwinski, 1958). (In fact, this is one of the major actions in the treatment of bromide intoxication.) On the other hand, the already long half-life of bromide, which is about 12 days in humans (Söremark, 1960) and approximately 3–8 days in the rat (Rauws and Van Logten, 1975; Pavelka *et al.*, 2000b), may be considerably increased by a salt-deficient diet. In the rat, bromide half-life was prolonged to 25 days on a salt-free diet (Rauws and Van Logten, 1975). This finding was interpreted by the authors as a marked dependence of the biological half life of bromide on chloride concentration in the diet. However, considering the differences between the metabolism of chloride and sodium ions (i.e., sodium is reabsorbed actively in kidney tubules, whereas chloride passively follows the movement of sodium), and the fact that sodium excretion rate depends on the level

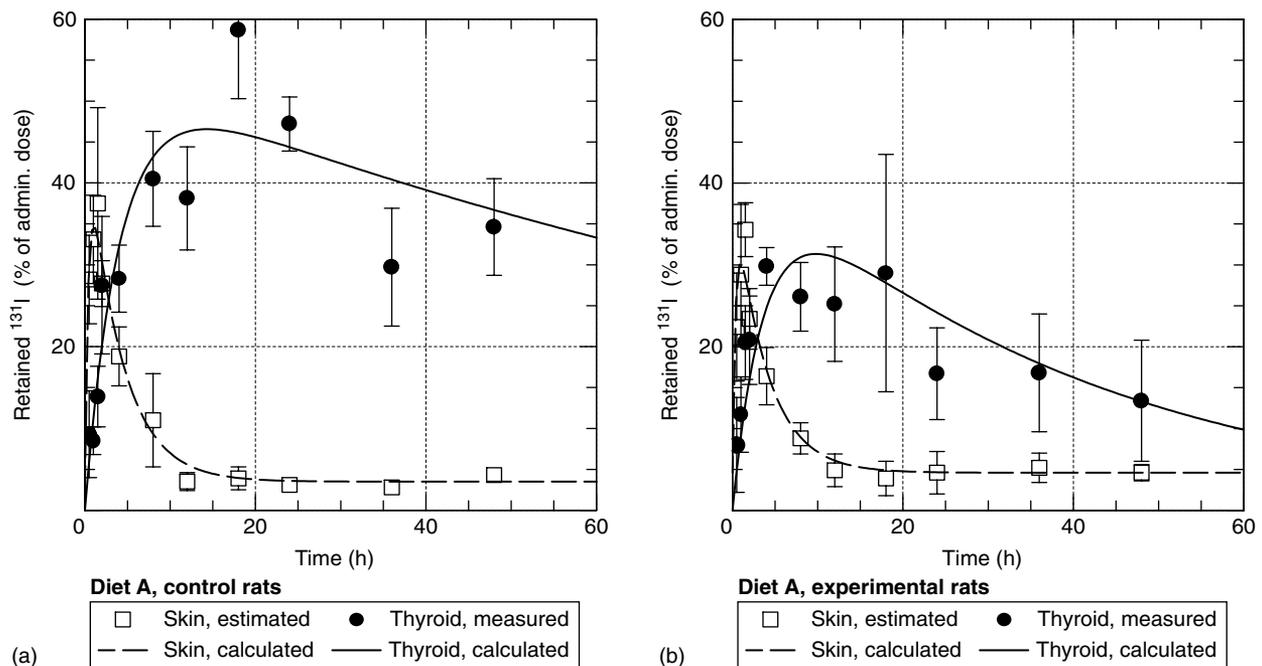


Figure 61.4 Changes in ^{131}I radioactivity retained in the skin (estimated) and in the thyroid of (a) control rats, and (b) experimental rats maintained on an iodine-deficient diet (diet A), dependent on the time after ^{131}I -iodide administration. Two groups of adult male Wistar rats, each of 66 members, were maintained for 14 days on a special low-iodine diet (diet A, Altromin C1042) after drinking water containing 0.5% NaClO_4 for 5 days before the beginning of the experiment. (a) Control rats drank distilled water, and (b) rats of the experimental group drank water with the addition of 5 g bromide/l (concentration of bromide 5000 ppm). On the 14th experimental day, all animals were given approximately 0.3 MBq ^{131}I in the form of carrier-free iodide in saline by subcutaneous injection. At appropriate time intervals the rats were successively killed and samples of whole blood, the thyroid glands and skin without hair were collected. After determination of fresh weight, the ^{131}I radioactivity of the samples was measured either by using a computerized gamma-spectrometric system equipped with an HPGe detector or by a gamma-ray counter. Each point represents the mean \pm SD, $n = 6$. Reprinted with permission from Pavelka *et al.*, 2001b.

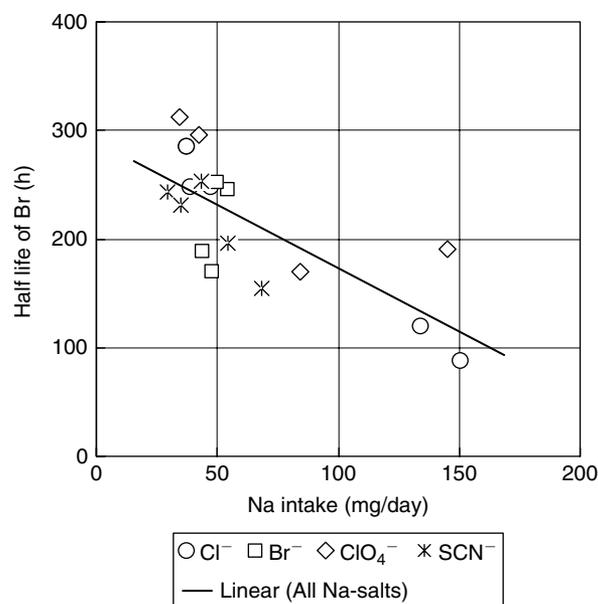


Figure 61.5 Dependence of the biological half life of bromide on the mean daily intake of sodium. Five groups of adult Wistar rats were maintained for 14 days on a special low-sodium diet (home-made; with normal levels of potassium and magnesium and low concentration of chloride). Animals in the individual groups drank distilled water with the addition of NaBr, NaCl, NaHCO₃, NaClO₄, or NaSCN. After establishing constant drinking regimens, on the 14th experimental day, all the animals were simultaneously given approximately 1.7 MBq ⁸²Br and 1.8 MBq ²⁴Na in saline by subcutaneous injection. The retained ⁸²Br and ²⁴Na radioactivity was measured *in vivo* at appropriate time intervals by means of a computerized gamma-spectrometric system equipped with an HPGe detector. The measured values of the biological half life of bromide are inversely proportional to the magnitude of sodium intake in the animals, regardless of the type of anion accompanying sodium ions. The straight line is fitted by linear regression analysis of the values determined in all the individual animals receiving sodium ions accompanied with the specified anions. Derived with permission from Pavelka *et al.*, 2005.

of sodium intake, we hypothesized (Babický *et al.*, 2005) that the biological half life of bromide depends on the level of sodium intake rather than on the intake of chloride. Keeping in mind the above-mentioned relationships between chloride, bromide and iodide ions in the body, we have further tested our hypothesis (Pavelka *et al.*, 2005). We determined, simultaneously, the biological half lives of bromide (with the aid of the radionuclide ⁸²Br) and of the sodium ion (with the aid of the radionuclide ²⁴Na) in five groups of adult female and male rats, which were exposed to various intakes of sodium ions accompanied with five different anions: Br⁻; Cl⁻; HCO₃⁻; ClO₄⁻; and SCN⁻. Regardless of the anion accompanying the sodium ion, the excretion rates of ⁸²Br⁻ and ²⁴Na⁺ ions were proportional to the magnitude of sodium intake in the animals (Figure 61.5). The rates of excretion of bromide and sodium ions run in parallel in rats maintained

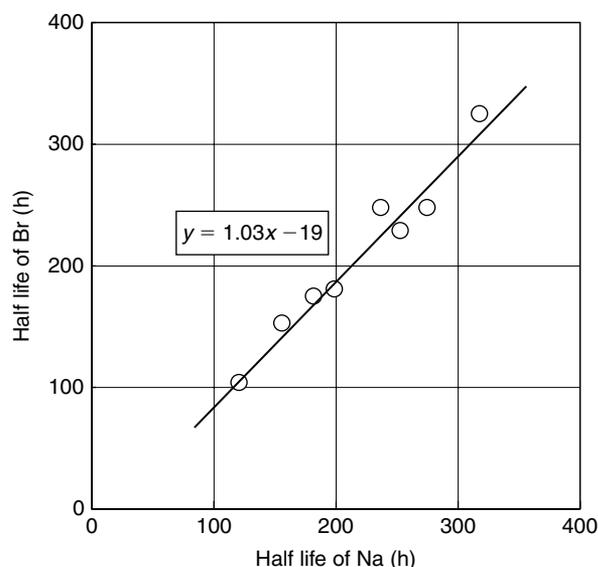


Figure 61.6 Correlation between the rate of excretion of bromide and sodium ions. Each symbol represents the mean of bromide and sodium half life values, measured in two to three animals receiving various amounts of sodium in the form of solutions of sodium bromide, chloride, perchlorate and thiocyanate. Derived with permission from Pavelka *et al.*, 2005.

under the same conditions with regard to the magnitude of sodium intake and metabolic activity (Figure 61.6). Because bromide is excreted slowly and practically entirely in urine, total body chloride may be followed for a considerable time after a single dose of bromide (Hellerstein *et al.*, 1960). As the rat tissues show consistent agreement between the ratios of chloride to bromide in serum, liver, skin and muscle (Hellerstein *et al.*, 1960), we assume that it is also justifiable to use radioactive ⁸²Br-bromide for following the excretion rate of chloride. In addition, due to the close relationship of sodium ions and the attendant anions, the excretion rate of sodium could also be followed by using ⁸²Br-bromide. The results achieved, of simultaneous determination of biological half-lives of sodium and bromide in rats maintained on various sodium intakes (Figures 61.5 and 61.6) corroborate this assumption.

Summary Points

- Inorganic, as well as organic, bromides (methyl bromide) exert toxic effects in animals and humans, especially on the CNS, with a rather steep dose–response curve.
- The biological behavior of bromide in the thyroid gland, in contrast to other organs and tissues, is more similar to the behavior of iodide rather than that of chloride.
- High bromide intake in the rat decreases the amount of iodide accumulated in the thyroid and exerts goitrogenic effects.

- The concentration ratio [I]/[Br] in the thyroid is a very sensitive marker of iodine status in the experimental animal.
- Excessive bromide intake in the rat also inhibits uptake of iodide by the skin.
- Very high bromide intake in the animal (>160 mg bromide/day) significantly shortens the biological half life of iodine in the thyroid (from about 100 to 30 h).
- Our hypothesis that the whole-body biological half life of bromide depends on the magnitude of sodium intake rather than on the intake of chloride was proved experimentally.
- The nature of the toxic effects of bromide on the thyroid gland and the mechanisms of its interference with the biosynthesis of thyroid hormones has not been explained so far.
- We suggest that a high surplus of bromide ions in blood can competitively inhibit the entrance of iodide into the thyroid and bromide can replace a part of iodide in the gland.

Acknowledgments

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Section 3.2

**The Effects on the Brain and Neurological
Aspect**

Iodine Deficiency and the Brain: An Overview

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Abstract

Iodine deficiency is recognized by the WHO as the most common cause of preventable brain damage, with an at-risk population in excess of 2 billion from 130 countries. The most severe effect is endemic cretinism, which is characterized by mental defect, deaf mutism and spastic diplegia. This condition is not reversible, but can be completely prevented by correction of iodine deficiency before pregnancy. The effects of iodine deficiency on the brain have been confirmed by animal studies in sheep, rats and the marmoset monkey. Since 1990 there has been a global program for the elimination of brain damage due to iodine deficiency. By 2000, 68% of the population at risk had been covered by universal salt iodization (USI), which requires all salt for human and animal consumption to be iodized with potassium iodate at a level of 20–40 mg iodine/kg of salt.

Abbreviations

IDD	Iodine deficiency disorders
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IQ	Intelligence quotient
T ₃	Triiodothyronine
T ₄	Thyroxine
TSH	Thyroid-stimulating hormone
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization

Introduction

Iodine deficiency prevails as the most common cause of brain damage in the world today. In 1990, the World Health Organization (WHO) reported that there were at least 20 million people suffering from irreversible brain

damage as a consequence of iodine deficiency during pregnancy. The current estimate declares the at-risk population in excess of 2 billion from 130 countries (WHO, 1994).

The history of the problem of iodine deficiency has been analyzed (Hetzel, 1989), and reports from two comprehensive symposia on various aspects of iodine and brain development have been published (DeLong *et al.*, 1989; Stanbury, 1994).

This chapter reviews the clinical aspects, epidemiology, pathogenesis and the progress made toward global elimination of brain damage caused by iodine deficiency.

Clinical Studies of Endemic Cretinism

The two clinical syndromes of endemic cretinism originally described by McCarrison (1908) have been confirmed by subsequent observations in many parts of the world. These features are summarized in Table 62.1.

Table 62.1 Comparative clinical features in neurological and hypothyroid cretinism

	<i>Neurological cretin</i>	<i>Hypothyroid cretin</i>
Mental retardation	Present, often severe	Present, less severe
Deaf-mutism	Usually present	Absent
Cerebral diplegia	Often present	Absent
Stature	Usually normal	Severe growth retardation usual
General features	No physical signs of hypothyroidism	Coarse dry skin, husky voice
Reflexes	Excessively brisk	Delayed relaxation
ECG	Normal	Small voltage QRS complexes and other abnormalities of hypothyroidism
X-ray limbs	Normal	Epiphyseal dysgenesis
Effect of thyroid hormones	No clinical effect	Improvement

Source: Modified from Hetzel (1989).

More recent studies in Zaire (DeLong, 1987) and China (Boyages *et al.*, 1988) indicate that the two syndromes overlap more often in the same individual than previously thought.

The reasons for the occurrence of the two different syndromes remain unclear. In China, hypothyroid cretinism remains largely confined to the north west (Ma *et al.*, 1989), while in almost every endemic area except in Zaire, neurological cretins are very much more common (Pharoah *et al.*, 1980; Hetzel, 1989). In Zaire, there is regular intake of cassava. This leads to the production of goitrogenic thiocyanates by the liver, through detoxification of the cyanide absorbed during digestion of the linamarin from the cassava, which augments the effect of iodine deficiency on the thyroid (Delange *et al.*, 1982).

Epidemiological Studies of Brain Damage due to Iodine Deficiency

The epidemiological demonstration of the association of cretinism with goiter dates from the Sardinia Commission of 1848. This has been followed by many subsequent studies to date (Hetzel, 1989). In general, cretinism is associated with higher rates of goiter (around 30%) in a population (Pharoah *et al.*, 1980).

A decline in the prevalence of goiter and cretinism was also noted by the Sardinia Commission. A further decline was apparent in the first half of the twentieth century in Cantons, Switzerland, where iodized salt had not been introduced; this also occurred in northern Italy (Costa *et al.*, 1964).

These observations raised doubts regarding whether iodine deficiency was a significant causative factor in cretinism (Lancet, 1972).

The demonstration of the prevention of cretinism with iodized oil in a double-blind controlled trial in Papua New Guinea established the causal role of iodine deficiency in cretinism by an effect on the developing fetal brain (Pharoah *et al.*, 1971). Cretinism could not be prevented unless the iodized oil was given before pregnancy, as injection during pregnancy was ineffective (Table 62.2).

The apparent spontaneous disappearance of cretinism in Europe has been attributed to an increase in iodine intake (in the absence of iodized salt) as a consequence of dietary diversification due to economic and social development,

and with increasing use of iodine supplements, as reported in Switzerland in the 1920s (Burgi *et al.*, 1990).

More recent studies in Papua New Guinea have fully-documented the occurrence of lesser brain defects as a result of iodine deficiency during pregnancy (Pharoah and Connolly, 1987). These include defects in coordination with lesser degrees of loss in intelligence. Studies carried out in Central Java (Dulberg *et al.*, 1983) indicated slowing of the achievement of motor milestones, including walking age in the children of iodine-deficient mothers. Similar data are also available from China (Boyages *et al.*, 1989). A detailed review by Delange (1994) indicates extensive evidence in many endemic diseases of low intelligence quotient (IQ) in populations living in areas with severe iodine deficiency areas (Figures 62.1–62.3).

A series of 18 studies of the effects of iodine deficiency on IQ, reviewed by Bleichrodt and Born (1994), revealed a mean loss of 13.5 IQ points in iodine-deficient populations compared to carefully matched iodine-sufficient populations.

Studies of Pathogenesis

Recent studies in endemic areas have been concerned with three issues.

Maternal hypothyroidism

In Papua New Guinea, Pharoah and Connolly (1987) have described varying scenarios ranging from stillbirths to cretinism, and less severe neurological defects of motor and cognitive performance. These effects could be correlated with the level of maternal thyroxine (T_4), but not with maternal triiodothyronine (T_3), indicating the importance of maternal T_4 to the fetus.

Earlier data indicating the effect of maternal hypothyroidism from other causes on children's subsequent cognitive performance provide further support for this concept (Man *et al.*, 1971).

Fetal and postnatal hypothyroidism

Fetal and postnatal hypothyroidism has been further studied in the Zaire endemic, indicating effects similar to those of other well-known causes of hypothyroidism elsewhere (Thilly *et al.*, 1978; Vanderpas *et al.*, 1986). In China,

Table 62.2 Children born in the western highlands of Papua New Guinea classified according to treatment received by mother^a

<i>Treatment received by mother</i>	<i>Total number of new births</i>	<i>Number of children examined</i>	<i>Number of deaths recorded</i>	<i>Number of endemic cretins</i>
Iodized oil	498	412	66	7 ^b
Untreated	534	406	97	26 ^c

^aSix already pregnant when injected with oil.

^bFive already pregnant when injected with saline solution.

^cSee Pharoah *et al.*, (1971).



Figure 62.1 A mother and child from a New Guinea village who are severely iodine deficient. The mother has a large goiter and the child is also affected. The bigger the goiter, the more likely she will have a cretin child. This can be prevented by eliminating the iodine deficiency before the onset of pregnancy. Reproduced with permission from [Hetzel and Pandav \(1996\)](#).



Figure 62.3 A hypothyroid cretin from Sinjiang, China, who is also deaf mute. This condition is completely preventable. On the right is the barefoot doctor of her village. Both are about 35 years of age. Reproduced from [Hetzel \(1989\)](#).



Figure 62.2 A young cretin with squint, ataxia and mental deficiency from Papua New Guinea. Reproduced from [Hetzel \(1989\)](#).

fetal hypothyroidism has been demonstrated in humans from the 4th month of gestation ([Liu *et al.*, 1982](#)).

Early correction of hypothyroidism due to congenital defects in neonates has now become a standard practice in most developed countries in order to prevent long-term brain damage ([Dussault and Glorieux, 1989](#)). The more severe the fetal hypothyroidism, as indicated by bone retardation, the more likely are residual effects ([Dussault and Glorieux, 1989](#)). Such considerations indicate the urgent need for a preventive approach to the correction of iodine deficiency for developing countries, because treatment of individual cases is not usually possible.

The timing of the lesion of neurological cretinism

[DeLong \(1987\)](#), as a result of careful clinical neurological studies in a number of endemic diseases, has concluded that the major defaults are intellectual deficiency, deafness and motor rigidity, which indicate that the most affected parts of the CNS are the cerebral neocortex, the cochlea and the basal ganglia. They all undergo rapid changes in the second trimester, and would be vulnerable to the effects of iodine deficiency through maternal hypothyroidism at that time.

[DeLong \(1989\)](#) also reports a unique patient with all the features of neurological cretinism occurring in a nonendemic area, who was treated for congenital hypothyroidism

shortly after birth. This indicates that both fetal hypothyroidism and maternal hypothyroidism are necessary to produce neurological cretinism.

Animal Models

Three mechanisms have been suggested for the effect of iodine deficiency on fetal brain development.

- Maternal hypothyroidism.
- Fetal hypothyroidism.
- Elemental iodine deficiency acting directly on the brain (Pharoah *et al.*, 1971).

In 1971, maternal hypothyroidism seemed an unlikely cause, as no such syndrome as cretinism had been noted in children born to hypothyroid mothers; infants suffering from congenital hypothyroidism do not show the features of deaf mutism or neuromuscular defect as seen in the common form of cretinism. This made elemental iodine deficiency an attractive hypothesis (Pharoah *et al.*, 1971).

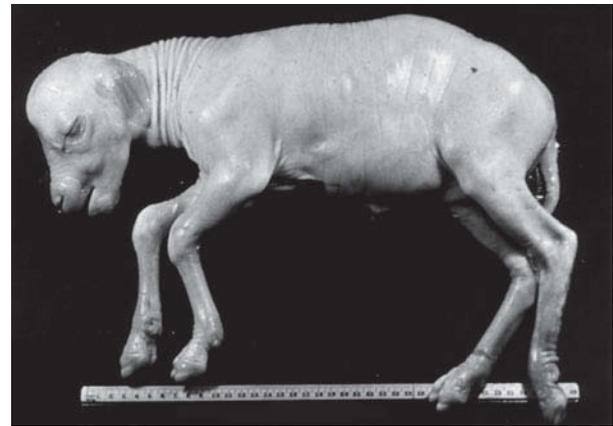
To investigate these various possibilities an animal model was developed in the sheep (Figure 62.4), because of the access provided for both maternal and fetal thyroidectomy and previous experience with trace element deficiencies (Hetzel, 1976). Subsequently, a similar model was developed in the primate marmoset monkey (*Callithrix jacchus jacchus*) (Mano *et al.*, 1987) and in the rat (Li *et al.*, 1985; Morreale de Escobar *et al.*, 1993).

Significant effects of iodine deficiency in slowing fetal brain development have been shown in all three species. In the sheep and marmoset, there was reduction of brain weight and brain DNA, with histological changes characterized by delayed maturation of the cerebellum with greater neuron density in the cerebral hemispheres (motor and visual areas). The effect was significant in the sheep from 70 days gestation, which suggests an effect on neuroblast multiplication that is known to occur at 40–80 days gestation in the sheep (Potter *et al.*, 1982).

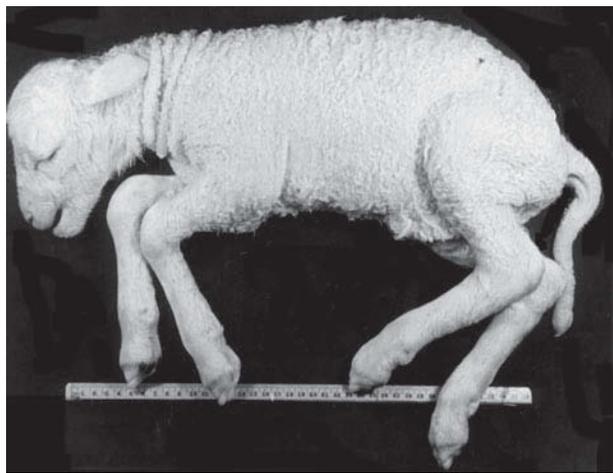
These findings indicated an effect that could be significant in the pathogenesis of cretinism in man.

An analysis of the mechanisms involved in the sheep has shown a significant effect of maternal thyroidectomy (carried out 6 weeks before pregnancy) on the brain at mid-gestation (Potter *et al.*, 1986). There was also an effect at the end of gestation following fetal thyroidectomy at both 60 and 98 days, with a more severe effect following the earlier fetal thyroidectomy (McIntosh *et al.*, 1979). The most striking effect was seen following the double procedure. It was similar, but more severe than that observed in iodine deficiency, associated with a greater reduction in maternal and fetal thyroid hormone levels (McIntosh *et al.*, 1983); indicating more severe maternal and fetal hypothyroidism.

It was concluded that both maternal and fetal thyroid hormones were involved in the effect of iodine deficiency



(a)



(b)

Figure 62.4 The effect of severe iodine deficiency during pregnancy on lamb development in a 140 day old lamb fetus (a) subjected to severe iodine deficiency by feeding the mother an iodine-deficient diet for 6 months prior to and during pregnancy (full-term 150 days), compared to a control lamb, (b) of the same age fed the same diet but with the addition of an iodine supplement. The iodine-deficient lamb showed an absence of a woolly coat, dislocation of the leg joints and a smaller brain. The figure illustrates that iodine is essential for animal development and so is important to the animal industry. Reproduced with permission from Hetzel (1989).

on the fetal brain. This is now supported by evidence of placental transfer of maternal T_4 in the rat (Obregon *et al.*, 1984; Woods *et al.*, 1984) followed by reports in humans (Vulsma *et al.*, 1989).

Recent studies of the regulation of thyroid hormone metabolism in the brain of the rat indicate that the intracellular brain T_3 level is dependent on the serum T_4 and not on serum T_3 , as is the case with the liver, heart and lungs (Larsen, 1989; Oppenheimer and Schwartz, 1997).

Other studies in the iodine-deficient rat confirm low fetal brain T_3 levels despite a five-fold to ten-fold increase

in 5'-deiodinase II activity (Morreale de Escobar *et al.*, 1993). This means that the lowered serum T₄ often evident in iodine-deficient populations leads to a reduced brain T₃, and therefore some impairment of brain function even if there is a normal serum T₃ with maintenance of a euthyroid state in other organs. This situation, now termed as “cerebral hypothyroidism,” may serve as the explanation for the torpor and general inertia characteristic of iodine-deficient populations with no other evidence of clinical hypothyroidism (Hetzel, 2004).

Further studies in sheep of the effect of correction of iodine deficiency by iodized oil injection at 100 days gestation (end of second trimester) revealed only partial correction of the effect on brain structure by the end of gestation (Potter *et al.*, 1984). This requires follow-up with observations of behavior and neurological status into the postnatal period. Such studies could also be carried out in marmosets (primate model) with greater relevance to humans. However, the finding in sheep is consistent with observations in humans that the injection of iodized oil in the latter half of pregnancy may not prevent cretinism in the infant (Pharoah *et al.*, 1971). Similar observations have been made by Cao *et al.* (1994) in China.

There has been no support for the role of elemental iodine deficiency from the animal models. The effect of iodine deficiency in early gestation now appears to be due to maternal hypothyroidism, with reduced T₄ transfer across the placental barrier. However, an additional direct effect of iodine itself cannot be excluded from the existing data.

Quantitative Estimates of Brain Damage in Iodine-Deficient Populations

The term iodine deficiency disorders (IDD) has been generally adopted to denote the spectrum of diverse effects resulting from iodine deficiency in a population, which are all preventable by correction of iodine deficiency (Hetzel, 1983).

The major features of the IDD effects on brain function arise from fetal damage (neurological cretinism) or hypothyroidism at various stages of life – fetus, neonate, juvenile and adult (Table 62.3).

There is much anecdotal evidence coming from observations in Europe over the past 150 years (Hetzel, 1989) supported by reports from China (Ma *et al.*, 1989), India, and Indonesia (Hetzel, 2004), indicating that iodine-deficient village populations suffer from general lethargy, poor work performance and defective school performance in children. These effects are due to hypothyroidism, particularly cerebral hypothyroidism. Beneficial effects of iodization programs on this state have also been described (Li and Wang, 1987; Hetzel, 2004).

Table 62.3 The spectrum of iodine deficiency disorders (IDD)

Fetus
Abortions
Stillbirths
Congenital anomalies
Neurological cretinism:
Mental deficiency, deaf mutism, spastic diplegia, squint
Hypothyroid cretinism:
Mental deficiency, dwarfism, hypothyroidism
Psychomotor defects
Neonate
Increased perinatal mortality
Neonatal hypothyroidism
Retarded mental and physical development
Child and adolescent
Increased infant mortality
Retarded mental and physical development
Adult
Goiter with its complications
Iodine-induced hyperthyroidism (IIH)
All ages
Goiter
Hypothyroidism
Impaired mental function
Increased susceptibility to nuclear radiation

Source: Reproduced with permission from Hetzel (1983), WHO/UNICEF/ICCIDD (2001).

What is required is more quantitative data supporting the impact of iodine deficiency on the brain function of a population. Various approaches have already been made that could be further developed.

In an iodine-deficient population, there is a gradation of effect, an “iceberg,” from the fully-developed picture of cretinism to clinical hypothyroidism.

Less severe cretin populations

It is clear that, apart from fully-developed cretin syndromes, there is a much larger number of individuals affected to a lesser degree, called “subcretins” in China (Ma *et al.*, 1989). Attempts have been made to estimate this larger number by reference to the calculated ratio with the number of fully-developed cretins (Hetzel *et al.*, 1990).

In Ecuador, an estimate of three times is available based on cognitive data (Fierro-Benitez *et al.*, 1986), in Indonesia a similar ratio (three times) exists based on the walking age of infants (Dulberg *et al.*, 1983), and in China the ratio is considered to be up to six times, based on various criteria (Ma *et al.*, 1989).

The WHO (1990) has estimated that 20 million worldwide from an at-risk population of 1 billion are suffering from varying degrees of preventable brain damage, based on a ratio of three to one for the estimates of the numbers of “subcretins and cretins.”

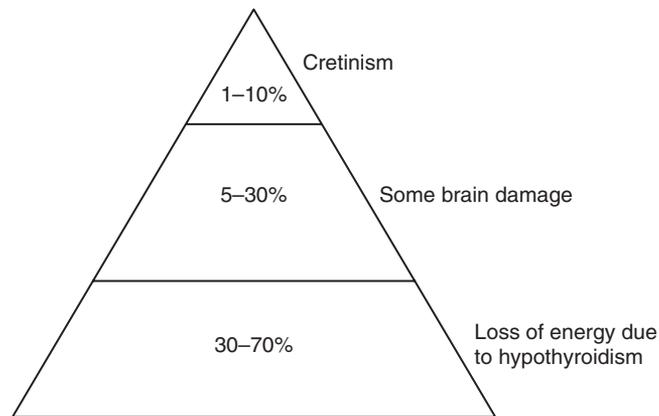


Figure 62.5 The iodine deficiency disorders (IDD) iceberg showing the very large 'hidden' component of IDD associated with lesser degrees of brain effects. Reproduced with permission from Hetzel and Pandav (1996).

Neonatal hypothyroidism

The indicator for this condition is neonatal serum thyroid-stimulating hormone (TSH). A prevalence of 1–10% of elevated TSH has been reported from various endemic diseases (Kochupillai *et al.*, 1986). There is evidence from India of an epidemiological correlation, with subsequent intellectual performance from India (Kochupillai *et al.*, 1986) and elsewhere (Delange, 1994).

Cerebral hypothyroidism

This is quantitatively probably the most frequent component of the IDD spectrum. Cerebral hypothyroidism refers to the effect of hypothyroidism on the brain in childhood and adult life, in contrast to the effect on the fetus and in early infancy. There is a more striking effect of hypothyroidism on the brain than on other organs. This produces the characteristic mental torpor and apathy characteristic of the iodine-deficient subjects – it can be reversed at the population level by correction of iodine deficiency, just as it can be reversed in an individual patient by treatment with thyroid hormones (Hetzel, 2004). In a severe endemic, 30–70% of the population may be suffering from cerebral hypothyroidism, as indicated by a low serum thyroxine level (T_4) (Buttfield and Hetzel, 1967; Kochupillai *et al.*, 1973).

Global Program for Elimination of Iodine Deficiency Disorders

In 1990, the WHO estimated a population of 1 billion at risk of the effect of iodine deficiency. The major populations affected are in Asian countries. More recently, the WHO has estimated the at-risk population to be in excess of 2 billion spread across 130 countries (WHO, 1994).

The great gap between our new knowledge of IDD and the significance of iodine deficiency in brain development,

and the application of this knowledge in national IDD control programs, particularly in developing countries, led to the formation of the International Council for Control of Iodine Deficiency Disorders (ICCIDD) in 1985 (Hetzel, 1989; Hetzel and Pandav, 1996) and the development of a global program for the elimination of brain damage due to iodine deficiency since 1990 (Hetzel, 2004).

This program has relied on the use of iodized salt through a policy known as universal salt iodization (USI). This requires all salt for human and animal consumption to be iodized at a level of 20–40 mg iodine/kg potassium iodate.

By 2000, the distribution of iodized salt had increased iodine intake, covering 68% of the population (2 billion) at risk (WHO/UNICEF/ICCIDD, 1999).

This program is further discussed in Chapter 74.

Summary

- Iodine deficiency is recognized by the WHO as the most common cause of preventable brain damage, with an at-risk population in excess of 2 billion from 130 countries.
- The most severe effect is endemic cretinism, which is characterized by mental defect, deaf mutism and spastic diplegia.
- This condition is not reversible, but can be completely prevented by correction of iodine deficiency before pregnancy.
- There is considerable variation in the effects of iodine deficiency on brain development, with a mean loss of 13.5 IQ points in an iodine-deficient population.
- The effects of iodine deficiency on the brain have been confirmed by animal studies in sheep, rats, and the marmoset monkey.
- Since 1990, there has been a global program for the elimination of brain damage due to iodine deficiency.

- By 2000, 68% of the population at risk had been covered by USI, which requires all salt for human and animal consumption to be iodized with potassium iodate at a level of 20–40 mg iodine/kg of salt.

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Iodine-Deficient Gestation and Neurodevelopment of 3-Year-Old Children

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Abstract

An adequate maternal thyroid function, from the beginning until the end of gestation, is essential for good development of the fetal central nervous system. Iodine deficiency is a critical factor that increases the frequency of hypothyroidism during pregnancy. Children from iodine-deficient areas are at risk of neurological disorders and mental retardation, because of the combined effects of maternal, fetal and postnatal hypothyroxinemia. Our aim was to assess the psychomotor development of 3-year-old children and its relationship with maternal iodine nutrition and thyroid function throughout gestation. A group of 111 women who asked for antenatal assessment in an iodine-deficient area, in the inner west valleys of Asturias (Spain), were initially included in the study. Since only normal pregnancies and normal newborns were selected, only 61 women and their 3-year-old children were kept at the end of the follow-up. Urinary iodine excretion (UIE), serum thyrotropin (TSH), free thyroxine (fT₄) and thyroperoxidase antibodies (TPO-Ab) were determined in pregnant women at the 12th and 32nd week of gestation and at delivery. TSH spotted onto filter paper on the third day of life, was measured in the newborns under study. The McCarthy scales of children's abilities (MSCA) for psychometric assessment was applied to the 3-year-old children. At 12 weeks of gestation, half the women had a UIE below 200 µg/l. Sixty percent had iodized salt in their diet; 81% of these were over the 200 µg/l level, compared with only 33% of those who did not have iodized salt ($p = 0.05$). Fifteen (25%) had serum fT₄ under the tenth percentile and 2 (3%) were under the third Percentile. The mean fT₄ of the women with UIE ≥ 200 µg/l was higher ($p < 0.05$) than the mean of the women under this level. These women had a mean fT₄ very near to the dangerous tenth level. All the women received explicit advice on how to obtain a good iodine supply. At the 32nd week, the UIE of 40% of the women

remained under 200 µg/l. Mean iodine excretion was higher, but the improvement could be seen almost selectively in women usually taking iodized salt from the beginning of gestation. Thyroid function of the pregnant women was significantly lower than at the first trimester ($p < 0.000$). Thirty-eight of them (62%) had fT₄ levels below the tenth percentile and 19 (29%) were below the third. There was no reaction of TSH. Neonatal TSH (on the third day of life) was 3.90 mU/l (± 2.12). Twelve (20%) newborns had TSH higher than 5 mU/l, but none were hypothyroid. The TSH of babies of mothers with UIE below 200 µg/l at the twelfth week of gestation, was 4.62 (± 2.20) mU/l; higher than those with normal UIE (3.31 (± 1.53)mU/l; $p = 0.019$). The MSCA score of the entire group was normal, but there were important differences between them. We observed loss in all the subscales of the test, especially verbal and GCI. The losses (about 10–11%) were related to the iodized salt intake of the pregnant female and her UIE at the twelfth week, and the neonatal TSH.

Abbreviations

fT ₄	Free thyroxine
GCI	General cognitive index
IDD	Iodine of deficiency disorders
MSCA	McCarthy scales of children's abilities
T ₃	Triiodothyronine
T ₄	Thyroxine
TPO-Ab	Thyroperoxidase antibodies
TSH	Thyrotropin
UIE	Urinary iodine excretion

Introduction

The fetal thyroid gland begins its hormonal production after 10 weeks of gestation, when many thyroxin-dependent

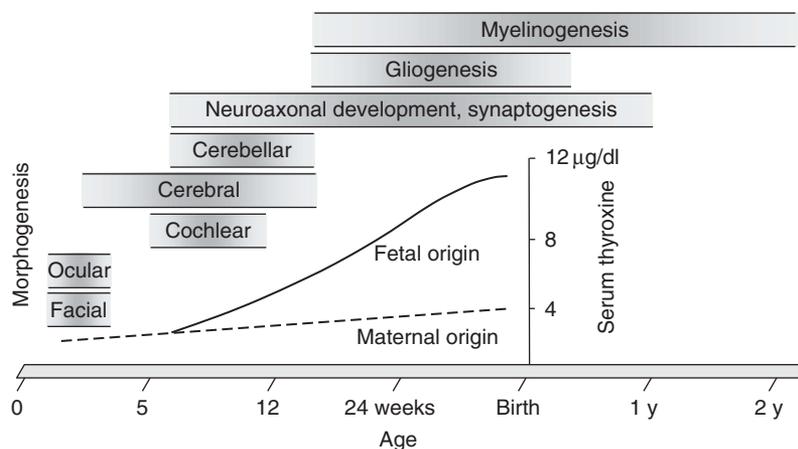


Figure 63.1 The figure shows fetal thyroxinemia and the development of thyroxine-dependent organs. Maternal thyroxine is essential for the neurological development of the fetus.

facial, ocular, and neuronal developmental processes are already in the advanced stage (Morreale de Escobar *et al.*, 2004). The fetus, by itself, is not able to meet its own requirements. Nevertheless, small amounts of thyroxine (T₄) and triiodothyronine (T₃) of maternal origin may be detected in the fetus by the fourth week of gestation and this increases gradually through the gestation. Therefore, an adequate maternal thyroid function, from early gestation, is essential for good development of the fetal central nervous system (WHO, 2001) (Figure 63.1). The intelligence quotient of children of women with elevated thyrotropin (TSH) in the first half of gestation is slightly lower than those of euthyroid ones (Haddow *et al.*, 1999). In addition, a serum-free thyroxine (fT₄) level lower than the tenth percentile at 12 weeks of gestation represents a significant risk for the development of the child (Pop *et al.*, 2003).

Gestational hypothyroidism has been associated with severe complications, such as hypertension, preterm birth, low birth weight, placental abruption and fetal death by Leung *et al.*, (1993) and Allan *et al.* (2000). Many studies in children of hypothyroid pregnant women including Man's group (1976, 1991), and many later ones (Liu *et al.*, 1994; Smit *et al.*, 2000; Klein *et al.*, 2001; Mitchell and Klein, 2004), have demonstrated the importance of maternal thyroid function on the neurodevelopmental evolution of the child.

Higher estrogen serum levels, among several other factors, overload the thyroid function of pregnant women. Therefore, their nutritional iodine needs, increasing from the fifth week of gestation, may exceed 200 µg/day (WHO, 2001) (Table 63.1). For the same reasons, levothyroxine requirements increase by almost 50% during the first half of pregnancy in hypothyroid pregnant women (Mandel *et al.*, 1990).

Insufficient iodine intake is a crucial factor that contributes to the frequency of hypothyroidism during pregnancy

Table 63.1 Recommended dietary intake of iodine

	Iodine (µg/day)
WHO/UNICEF/ICCIDD	
0–59 months of age	90
6–12 years of age	120
Adolescents	150
Adults	150
Pregnancy	200
Lactation	200
US Institute of Medicine	
0–12 months of age	110–130
1–8 years of age	90
9–13 years of age	120
14–18 years of age	150
Adults	150
Pregnancy	220
Lactation	290

Note: Data from WHO/NHD/01.1, 2001; Institute of Medicine of the National Academies (2001).

(Vermiglio *et al.*, 1999). Children born in iodine-deficient areas run the risk of having neurological disorders and mental retardation, due to the combined effects of maternal, fetal and postnatal hypothyroxinemia (Delange, 1994). Iodine deficiency disorders (IDD), as the most widespread cause of maternal hypothyroxinemia, are the most important preventable causes of brain damage worldwide (WHO, 2001). Maternal hypothyroxinemia is a very common condition, perhaps 150–200 times more prevalent than congenital hypothyroidism (Zoeller, 2003). Between 4.5% and 6% of women at gestational age in Colorado (with a sufficient supply of iodine) have elevated serum TSH (Canaris *et al.*, 2000). In Maine, Germany (mildly iodine deficient), serum TSH is elevated in 2.5% of the pregnant women, and 0.3% of these show overt hypothyroidism (serum T₄ concentration below the two standard deviations limit) (Klein *et al.*, 1991). The serum fT₄ of 20% of pregnant women in Madrid (mild iodine

deficiency) is below the tenth percentile of references from women with normal iodine nutrition (Morreale de Escobar and Escobar del Rey, 1999).

The McCarthy scales of children's abilities (MSCA) for psychometric assessment of children between 2.5 and 7 years comprises 18 independent tests, producing six score subscales: verbal (verbal expression and conceptualization); perceptual-performance (conceptualization and reasoning without words); quantitative (numeric aptitude); memory (recall of words, numbers, pictures, and tonal sequences); and motor scale (fine and gross motor tasks). Items from the first three of these subscales are included in the GCI, with a mean value of 100 and a standard deviation of 16. This scale reflects the child's global cognitive function, as the Intelligence Quotient does. All of them have high indexes of internal consistency and stability. A child's profile of MSCA index scores reflects his real and meaningful performance in the domains of cognitive and motor ability.

Objectives

Our aim was to examine the neurodevelopment of a group of 3-year-old children of mothers living in an iodine-deficient area, and to establish the possible existence of a relationship between their performance and maternal iodine nutrition and thyroid function through gestation.

Subjects

The study was carried out in the inner valleys of western Asturias (a northern Spanish region). This is an isolated mountainous area, with an autarkic farmers' population and long-standing IDD. Ten years after the introduction of an iodized-salt diet, infantile endemic goiter has been reduced from 63% to a mild iodine deficiency of 16.4% (Enguix *et al.*, 1995). One hundred and eleven consecutive women who asked for antenatal assessment at the local medical center of the Spanish National System gave their consent to Paloma Sánchez Martínez in order to participate in the study. The Ethics Committee gave its approval to the study.

The educational level of the women ranged from basic schooling to a low college degree. In no case was there information about deficient or abnormal conduct. All of them were viable simple pregnancies, and free of treatment and known thyroid disease. They were examined at the 12th week, 32nd week and at labor. In order to correct pernicious habits and iodine deficiency, all of them received explicit advice on suitable habits and diet to maintain a good iodine supply.

Thirteen women were excluded due to psychosocial or obstetric reasons (miscarriage, premature delivery, etc.) or withdrawal. During the 3 years of postnatal follow-up, another fraction of the women moved outside the region

or refused to continue participating. In order to avoid any confounding variables related to child development, those cases with a low Apgar score, small for gestational age, or any other added pathological condition were excluded. Finally, 61 mothers and their 3-year-old children were included in our study.

Analytical Procedures

Urinary iodine excretion (UIE), serum TSH, fT₄ and TPOAb were determined in pregnant women at the 12th and 32nd week of gestation and at delivery; TSH was spotted onto filter paper on the third day of life in their newborns. UIE ($\mu\text{g/l}$) was measured by the colorimetric ceric ion arsenious acid wet-ash method, based on the Sandell-Kolthoff reaction. Serum TSH was measured by an ultra-sensitive ROCHE electrochemiluminescence double antibody immunoassay (normal range: 0.270–4.2 mU/l; detection limit: 0.005 mU/l); fT₄ was measured by Roche electrochemiluminescence immunoassay competitive (normal range: 0.9–1.90 ng/dl) and thyroperoxidase antibodies (TPO-Ab) by radioimmunoassay (DYNObest). TPO-Ab status was considered positive with levels greater than 100 U/ml.

The Spanish validated version of the MSCA (McCarthy, 1999) was used for the child neurodevelopment assessment. Each child was evaluated by María Pilar Mosteiro Díaz, a development psychologist who was unaware of the mother's data when pregnant and the TSH value of the newborn.

Statistical Analyses

In order to maintain a double-blind design, Isolina Riaño Galán and M. Francisco Rivas Crespo performed the statistical analyses and summarized the results in such a way that it was impossible for them to identify individual cases. Data are expressed as mean values (\pm standard deviation), unless specified. The Pearson chi-square was employed for comparing data among the categories. Differences in continuous variables were tested by the *t* test. Logistic regression was applied to examine the significance for relationships between numeric variables. A $p < 0.05$ was considered statistically significant. All calculations were performed using SPSS 11.0 (SPSS, Inc., Chicago, IL).

Results

The average age of the 61 women who were included was 28.9 (± 4.29) years (range: 20–38). Six of them (10%) were 35–38-year-old. Thirty-four (56%) were primiparae and 27 (45%) were multiparae. Twenty-six women (44%) had a job out of home, and four (6.7%) smoked 10 or more cigarettes a day. Ages, parity, UIE, ingestion of iodized salt and thyroid function at early gestation of the studied women were similar to the 50 women who were

Table 63.2 Maternal urine iodine excretion, thyroid function and TPO-Ab determination at the first and third trimester of gestation

N = 61	12 weeks	32 weeks
UIE ($\mu\text{g/l}$)	195.0 (± 81.0)	246.9 (± 04.6) (a)
≥ 200	51%	60%
< 200	49%	40%
< 100	15%	3%
TSH (mU/l)	1.94 (± 1.32)	2.02 (± 0.96) (b)
fT4 (ng/dl)	1.13 (± 0.13)	0.97 (± 0.12) (a)
$\leq \text{P10}$ (1.03 ng/dl)	25%	62% (a)
$\leq \text{P3}$ (0.9 ng/dl)	3%	29% (a)
TPO-Ab (+) (frequency)	6 (10%)	4 (6.66%) (b)

Note: UIE, urinary iodine excretion. TSH, thyrotropin; fT4, free thyroxine. TPO-Ab, Anti-thyroid peroxidase antibodies. Values are expressed as mean (\pm SD) except for TPO-Ab (number of cases and percentages). (a) $p < 0.05$ for the differences. (b) No significant differences.

Table 63.3 Urine iodine excretion and free thyroxine at the 12th week related to the parity, work outside home and iodized salt intake of the pregnant women

N = 61	UIE ($\mu\text{g/l}$)	fT4 (ng/dl)
Parity		
Primiparae	201.1 (± 87.6)	1.14 (± 0.13)
Multiparae	188.1 (± 70.1)	1.12 (± 0.11)
Iodized salt		
Yes	210.8 (± 80.6)	1.14 (± 0.13)
No	180.7 (± 91.0)	1.10 (± 0.12)
Work outside home		
Yes	209.7 (± 87.1)	1.13 (± 0.12)
No	184.9 (± 74.4)	1.13 (± 0.13)

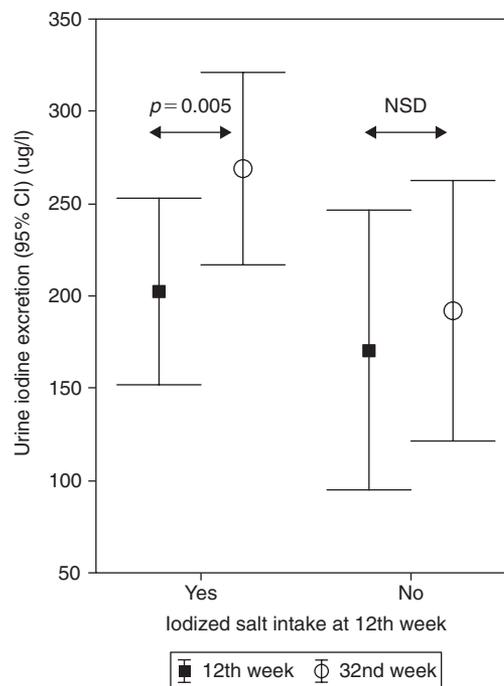
Note: No significant differences between groups. UIE, urine iodine excretion; fT4, serum free thyroxine.

excluded. However, only 25% of those were working out of home, and only 40% used iodized salt.

At 12 weeks of gestation, the UIE of 30 women (49%) was below $200 \mu\text{g/l}$ and 9 (15%) were below $100 \mu\text{g/l}$. Thirty-seven (60%) had iodized salt in their diet. Eighty one percent of them had a UIE of $\geq 200 \mu\text{g/l}$, versus 33% of the women who did not have iodized salt ($p = 0.05$). **Table 63.2** shows maternal UIE, thyroid function and TPO-Ab at the first and third gestational trimester.

Table 63.3 shows UIE and fT4 at this time in relation to parity, work outside home and iodine salt intake. Unexpectedly, we did not find significant differences in the women's UIE, related to their incorporation of iodized salt in their diet. Nevertheless, 14 of them (23%) did not have a clear idea on this question and their answer was not included.

Three women (4.7%) had slightly elevated serum TSH with fT4 below the normal range. Serum fT4 was lower than the third percentile level (0.9 ng/ml) in two (3.2%) and was lower than the tenth (1.03 ng/ml) in 15 (25%)

**Figure 63.2** The figure shows that only the pregnant women previously taking iodized salt improved their urinary iodide excretion through gestation. CI, confidence interval. NSD, no significant differences.

of them. At this time, serum fT4 is positively correlated with UIE, which it significantly depends on ($r = 0.417$; $p = 0.001$). The mean fT4 of pregnant women with UIE of $200 \mu\text{g/l}$ or more ($1.18 \pm 0.11 \text{ ng/dl}$), was significantly higher ($p = 0.001$) than those of the pregnant women with UIE lower than this level, who were almost in the tenth percentile ($1.07 \pm 0.12 \text{ ng/dl}$). UIE of women with fT4 higher than the tenth percentile was $209.76 (\pm 77.3) \mu\text{g/l}$, greater ($p = 0.022$) than those below this level ($150.9 \pm 78.2 \mu\text{g/l}$).

Six women (10%) were TPO-Ab (+) with values ranging from 1890 to 607 U/ml. No repercussion on their own or the fetal thyroid function was detected. There were no significant differences between UIE or serum fT4 between smokers and nonsmokers.

At the 32nd week of gestation, UIE was higher than that at early gestation, but 40% of the women remained under the $200 \mu\text{g/l}$ level. A positive correlation in UIE between the 12th and 32nd weeks of gestation ($r = 0.437$; $p = 0.018$) was found, but dimorphic behavior could be distinguished. The women receiving iodized salt at early gestation were more receptive to our advice about suitable diet, reacting with a clear increase in their UIE ($202.4 \pm 83.4 \mu\text{g/l}$ at the 12th week vs. $269.2 \pm 86.3 \mu\text{g/l}$ at the 32nd week; $p = 0.005$). The UIE of those not receiving iodized salt in their diet in the first weeks of gestation remained at the same level at the 32nd week ($180.7 \pm 91.1 \mu\text{g/l}$ at the 12th week vs. $192.1 \pm 76.3 \mu\text{g/l}$ at the 32nd week; $p \geq 0.05$; **Figure 63.2**).

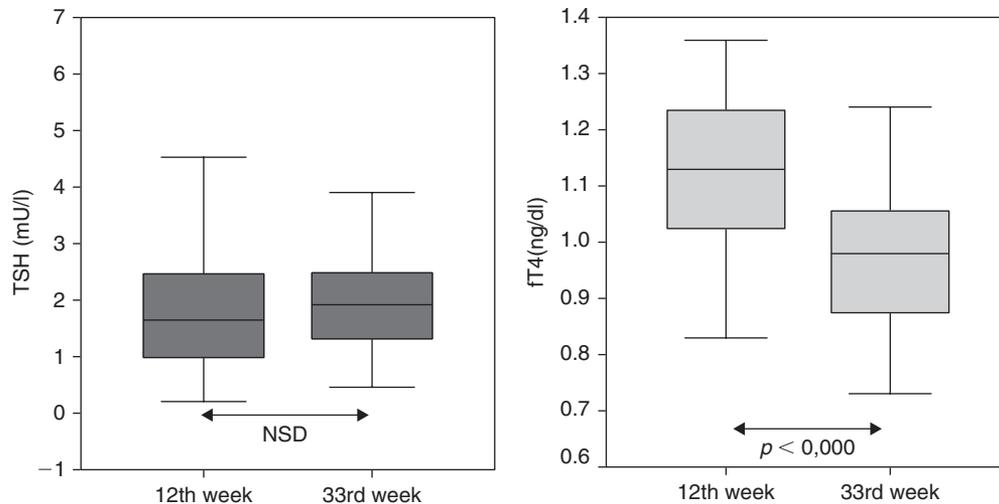


Figure 63.3 The figure shows the evolution of maternal thyroidal function. TSH, serum thyrotropin. fT4, serum free thyroxine. NSD, no significant differences.

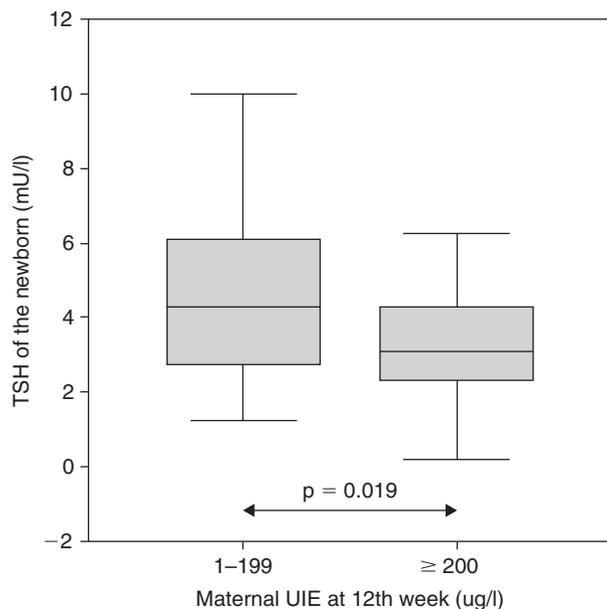


Figure 63.4 The figure shows that the neonatal TSH is related to maternal iodine status at the first trimester of gestation. UIE, urine iodine excretion.

At this point, the thyroid functional status of the pregnant women was significantly poorer than at early gestation ($p < 0.000$). The fT4 level of 38 of them (62%) was below the tenth percentile, and 19 (29%) were hypothyroid (below the third). There was no reaction of TSH (Figure 63.3). Thyroxinemia and UIE followed diverging evolutions: at this time the rate of fT4 is not UIE-related. Only four women were TPO-Ab (+), with a maximum value of 562 U/ml, and two were just over the upper level of normality. They had no thyroid function repercussions.

The weight of the 61 full-term newborns (46% males) after 39.6 ± 1.25 weeks of gestation was $3279 (\pm 487)$ g.

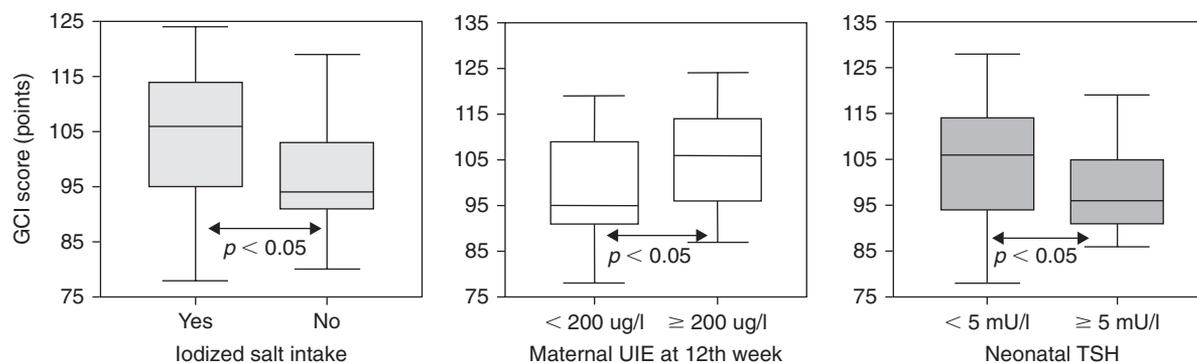
Thirty four (56%) were the first offspring. Their neonatal TSH (third day of life) was $3.90 \text{ mU/l} (\pm 2.12)$ with a maximum value of 10 mU/l . In twelve (20%) babies, TSH was higher than 5 mU/l , but no cases of neonatal hypothyroidism were registered. The TSH of newborns whose mother had UIE below $200 \mu\text{g/l}$ at the 12th week of gestation was $4.62 (\pm 2.20) \text{ mU/l}$, which was higher than those with normal UIE ($3.31 (\pm 1.53) \text{ mU/l}$; $p = 0.019$) (Figure 63.4). Fifteen percent of the newborn babies whose mother had UIE beyond $200 \mu\text{g/l}$ had TSH greater than 5 mU/l , versus 33 of those of mothers with UIE lower than this level. Nevertheless, 87% of the newborns whose mother had UIE below $100 \mu\text{g/l}$ had TSH under 5 mU/l .

The MSCA test was applied to the 61 children at a mean age of 40 months (range: 37–47). The score of the six subscales of the test are shown in Table 63.4. We found some important differences between the abilities of the children which were related to intake of iodized salt and maternal UIE in the first weeks of pregnancy, and neonatal TSH. Children whose mothers received iodized salt had scored higher in all items than the others, reaching significant differences ($p < 0.005$) in verbal, perceptual, motor scales and GCI (7.77 points higher). In the same way, children of mothers with UIE higher than $200 \mu\text{g/l}$ at the 12th week scored better in all items of the test, reaching significant differences in verbal development (5.8 points) and GCI (7.8 points) compared with the other children. Those children with a neonatal TSH lower than 5 mU/l , also reached a better level in all the MSCA scales, with significant differences in verbal, perceptual, memory, motor scales and GCI (9.8 points higher) (Figure 63.5). We did not find significant differences between babies of mothers with fT4 lower than the tenth percentile and those above this cut-off point. The MSCA test did not show any difference related to maternal TPO-Ab or Apgar test score,

Table 63.4 Children's psychometric evaluation results and its correlation with iodized salt intake and UIE at 12th week of gestation and neonatal TSH

		Verbal	Perceptual	Quantitative	GCI	Memory	Motor
Total score		53.8 (±7.4)	52.9 (±8.7)	46.9 (±7.2)	103.1 (±11.6)	48.7 (±8.1)	54.6 (±9.9)
MSCA scale scores							
Iodized salt intake	No	49.76	48.88	45.94	97.59	46.18	49.06
	Yes:	54.96	53.84	47.04	105.36	49.80	56.72
UIE (a)	<200	49.4	50.1	45.7	97.7	46.0	52.8
	≥200	55.2	54.1	45.9	105.5	49.4	55.6
Neonatal TSH (b)	≥5	49.2	48.6	43.7	95.1	43.3	50.9
	<5	56.9	54.2	47.3	104.9	50.1	55.1

Note: MSCA: McCarthy scales of children's abilities. GCI, general cognitive index. UIE: urinary iodine excretion at 12 weeks of gestation. TSH, thyrotropin. Bold typed numbers paired: $p < 0.05$ for the difference between subgroups. a: $\mu\text{g/l}$; b: mU/l .

**Figure 63.5** The figure shows that a normal General Cognitive Index requires adequate maternal iodine nutrition conditions and a good neonatal thyroid function. CGI, General Cognitive Index. UIE, urine iodine excretion. TSH, serum thyrotropin.

neither in the size of the newborn nor the position of the child among their siblings. **Figure 63.6** shows the cognitive skills classification of the children of women with a good iodine supply at early gestation, versus children of iodine-deficient mothers (UIE < 200 $\mu\text{g/l}$).

Discussion

In order to study the effect of pregnant women's iodine nutritional situation on their child's development, we have isolated, as much as possible, other major determining factors, such as health assistance, maternal cultural and intellectual status, family structure, and number and order among siblings.

The mean UIE of the pregnant women, in the first trimester of gestation, was under the critical level of 200 $\mu\text{g/l}$ (15% of them were in severe deficiency), while 21.4% of their newborns had TSH over 5 mU/l . These data, which are worse than that of the last decade (**Enguix et al., 1995**), describe the researched zone as in area of a "moderate deficiency," based on the criteria of the **WHO (2001)**. One quarter of the pregnant women had their fT_4 lower than the tenth percentile. Seven of them (11.2%) were hypothyroid, but none showed clinical evidence about their thyroid insufficiency. A similar observation was referred to by

Fukushi et al. (1999) in 15.2% of the screened women in Sapporo. Once again, the need for checking the thyroid function of women in early pregnancy is affirmed (**Haddow et al., 1999**).

Hypothyroidism of pregnant women is frequently related to antithyroid autoimmunity: 77% of the hypothyroid mothers in **Haddow's group (1999)** were TPO-Ab (+). Autoimmune thyroid disease could be responsible for congenital hypothyroidism, which even though transitory, disrupts the psychomotor development of the child (**Dussault and Fisher, 1999; Matsuura and Konishi, 1990**). Though the TPO-Ab serum level is simply a marker, it is evident that starting from as low as 50 mU/l , the incidence of maternal hypothyroxinemia is on the rise (**Pop et al., 2003**). Autoimmunity was positive in over 10% of the women at 12th week. Nevertheless, at the 32nd week, the autoimmune reaction was milder and less frequent (6%). Neither the women nor the newborn showed thyroid alteration.

The etiology of hypothyroidism in our group of women, as expected in a deficient area, is iodine deficiency. At the 12th week, only half of the mothers were over the level of 200 $\mu\text{g/l}$ of UIE, and only these women reached a serum fT_4 over the tenth percentile. The mean fT_4 of those women whose UIE was below 200 $\mu\text{g/l}$ was very

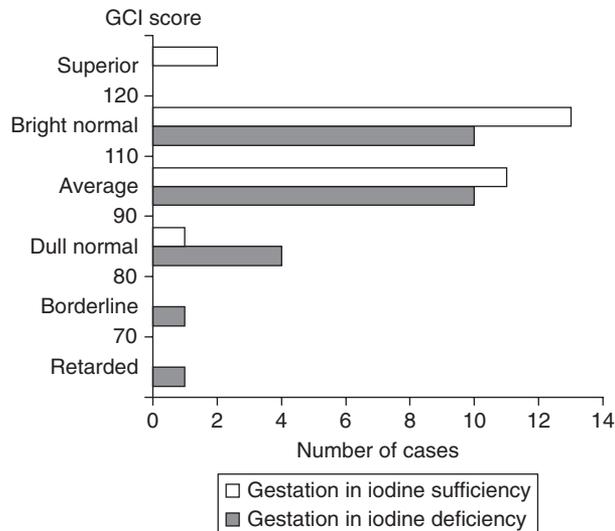


Figure 63.6 The figure shows cognitive skills in children of women with normal iodine nutrition status at early gestation, versus children of iodine-deficient mothers. Children of iodine-deficient mothers have poor cognitive performance compared to normal ones. CGI, General Cognitive Index.

close to the tenth percentile. This is a dangerous limit, below which the psychomotor development of the child is reduced (Pop *et al.*, 2003).

In this situation of deficiency, iodized salt plays a very important role, the average UIE of the women who included it in their diet was higher than 200 $\mu\text{g}/\text{l}$; whereas in the rest it was below this critical level. Almost two thirds of the women not using iodized salt are iodine deficient as opposed to the 20% who do use it. In other words, iodized salt intake has a positive effect on the nutritional status of iodine in the pregnant women, but it is insufficient as the only source of iodine. Nevertheless, this observation has a limited reliability since, frequently, people lacking knowledge confuse iodized salt with noniodized salt, labeled as “marine salt,” and almost 23% of the women do not know what kind of salt has been bought for home consumption.

At the 32nd week, UIE increased (47.5% on average) to an apparently good level (Table 63.2). But this interpretation is partially an illusion: there was an important reduction in the number of severely iodine-deficient women; the number of deficiency cases diminished to 9%. It seems that our “advice for a healthy pregnancy” were followed better by women adequate previous iodine nutrition (Figure 63.2). The advice given in the only medical consultation, after a request by the woman herself for information about healthy habits, has not been sufficient. An improvement in nutritional habits of pregnant women can be achieved through more prolonged education.

MSCA is a widely practiced test, whose results have a high correspondence with estimations of educators (Simons and Goh, 1982) and other methods of assessment

(Arinaldo, 1982; Karr *et al.*, 1992), showing some predictive capacity about the subject’s later intellectual performance (Hansen *et al.*, 2002). However MSCA, as with other scales used, does not predict the child’s future intellectual capacity with absolute certainty. In addition, specific circumstances under which the child is going to be assessed (the time of day, tiredness, health status, emotional color, motivation, empathy with the evaluator, etc.) may influence the results significantly. Therefore MSCA, as with other similar tools, must be used and interpreted cautiously, in order to avoid hasty or groundless predictions. Despite its restrictions, MSCA clearly detected lower performance in children of iodine-deficient women compared to those of well-nourished ones. As the thyroxine dependency of fetal cerebral development determines the performance loss detected in children of iodine-deficient women, a preventive policy is urgently required in areas of deficiency such as ours. It must be implemented immediately in the whole population and reinforced especially as pre-gestational advice. It should also be a requirement to monitor the thyroid function of all pregnant women at their first obstetric visit.

The thyroxine effects on fetal neurological development (where the thyroid maternal contribution plays its own role until the end of gestation) are not limited to early gestation. Several phenomena of neuronal migration and organization of the central nervous system, which will determine intellectual performance, take place later (Morreale de Escobar, 2001). Furthermore, fT_4 values decrease steadily and significantly during gestation, as a direct consequence of the elevation of thyroxine-binding globulin. In well-nourished women, serum fT_4 at delivery is lower than in nonpregnant women by an average of 10–15%. The increased iodine renal clearance and diversion of a part of the available iodine to the feto-placental unit, worsen the situation in iodine-deficient women (Glinoe, 1997). The mean fT_4 of our mothers lowered by 0.16 (± 0.12) ng/ml (about 13.3%) between the first and third trimester of gestation, causing a number of pregnant women to suffer from hypothyroidism. Despite the maternal fT_4 drop at the 32nd week, neither its tenth nor third percentile separated groups of children with different MSCA scores.

Summary Points

- The hypothyroid status of the pregnant woman is not usually clinically evident. The thyroid function of all pregnant women should be checked at the early phases of pregnancy.
- The serum tenth percentile of a pregnant women’s free thyroxine is a dangerous level below which psychomotor development of the child is at risk. Only pregnant women with a urine iodide excretion over 200 $\mu\text{g}/\text{l}$ reach a serum-free thyroxine (fT_4) level over the tenth percentile.

- Thyroid autoimmunity is not a relevant cause of hypothyroidism in pregnant women from iodine-deficient areas, which is in contrast to the experience in nondeficient areas.
- A significant percentage of pregnant women do not have sufficient knowledge about the concept of iodized salt and its importance for both the child's and their own health.
- A simple medical act is not enough to make pregnant women aware of the necessity of including iodine in their diet. A suitable policy is required in order to inform women in gestational age.
- Intake of iodized salt has a positive effect on the nutritional status of pregnant women, but it is insufficient if it is their only source of iodine.
- Intake of iodized salt and maternal excretion of urinary iodide in the first weeks of pregnancy determine the differences in neurodevelopment of 3-year-old children.
- Neonatal TSH levels of 5 mU/l or higher, especially in an iodine-deficient area, such as ours, are associated with a lower neurodevelopment score in 3-year-old children.
- The greatest deficits registered in the children of iodine-deficient pregnant women, in our "moderately deficient" area, are in the verbal subscale, and in the General Cognitive Index. The magnitude of this loss represents 10–11% of the adequately nourished children.

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Table 64.1 Clinical features in neurological cretinism and in untreated congenital hypothyroidism

Clinical features	Neurological cretinism	Congenital hypothyroidism
Mental retardation	Present, often severe	Present, less severe
Deaf mutism	Usually present	Absent
Cerebral diplegia	Often present	Absent
Stature	Normal	Severe growth retardation usual
General features	No physical signs of hypothyroidism	Coarse dry skin, husky voice
Reflexes	Excessively brisk	Delayed relaxation
ECG	Normal	Small voltage QRS complexes; other anomalies of hypothyroidism
X-ray of limbs	Normal	Epiphyseal dysgenesis
Prevention	Preferably before midgestation	As soon as possible after birth
Effect of thyroid hormones	No effect	Clinical effect

Source: Adapted from Hetzel (1994).

Studies done in Papua New Guinea (Choufoer *et al.*, 1965) suggested that the birth of cretins was causally related to incapacity to increase plasma T₄ (as measured by PBI) during pregnancy, even if the women were not hypothyroid. Thereafter, several groups in New Guinea, Peru and China presented evidence that hypothyroxinemia of pregnant women was the cause of the birth of neurological cretins, as well as of the reproductive failure of women (Fierro-Benitez *et al.*, 1970; Pharoah *et al.*, 1971; Pretell *et al.*, 1974; Zhu *et al.*, 1981; Hou *et al.*, 1982). The birth of cretins could be avoided by the administration of iodine to pregnant women before or early in pregnancy (Pharoah *et al.*, 1976; Wang and Yang, 1985; Cao *et al.*, 1994).

The clinical features of people affected by neurological cretinism are completely different from those affected by congenital hypothyroidism (CH) (Boyages *et al.*, 1988; Boyages and Halpern, 1993). A summary is presented in Table 64.1. Neurological cretins show no physical signs of hypothyroidism, but severe and irreversible mental retardation, deficits in hearing and speech, and motor abnormalities (spasticity, muscle wasting, flexion dystonia, diplegia, tetraplegia and so on). These defects point to early damage of specific brain structures (Cao *et al.*, 1994) and, e.g., in the case of deafness, to damage in the cochlear development that occurs in the first trimester of pregnancy. On the contrary, in CH several signs of hypothyroidism are present (epiphyseal dysgenesis, growth retardation, coarse and dry skin, mental retardation), which can be corrected by administration of thyroxine soon after birth, but the defects are not related to early damage during brain development, suggesting that during this early period the development of the fetal brain is protected by placental transfer of maternal thyroid hormones.

Table 64.2 Conditions in which CNS damage has been associated with thyroid hormone deficiencies early in development and their prevention

Condition and consequences	Effective preventive measures
Severe iodine deficiency (ID): neurological cretinism (deafness, mental retardation, cerebral palsy, etc.)	Correction of iodine deficiency before or very early in pregnancy (before midgestation)
Congenital hypothyroidism: mental retardation, dwarfism, poor maturation	Prompt postnatal treatment with T ₄
Maternal hypothyroxinemia: decreased I.Q. of progeny	Early correction of maternal hypothyroxinemia
Combined maternal and fetal hypothyroidism: severe neurological damage, reminiscent of ID cretinism	Maintenance of normal maternal T ₄ throughout pregnancy, and prompt postnatal treatment of the newborn with T ₄
Thyroid hormone resistance syndrome: in many cases, mental retardation, deafness	Not yet known
Prematurity: increased risk of neurodevelopmental impairment and cerebral palsy	Postnatal correction of transient neonatal hypothyroxinemia

Source: Morreale de Escobar (2001); Morreale de Escobar *et al.* (2002).

During the last decades there has been increasing evidence of the protective role of maternal thyroid hormones. This is evident not only in situations of severe iodine deficiency, but also in cases of milder iodine deficiency or in situations of hypothyroxinemia during pregnancy, where a decrease in the IQ of the progeny or attention deficit hyperactivity disorders (ADHD) have been described (Man and Serunian, 1976; Pharoah *et al.*, 1984; Pop *et al.*, 1999, 2003; Vermiglio *et al.*, 2004). In Table 64.2 we summarize the situations in which brain damage has been associated with thyroid hormone deficiency during development: iodine deficiency, CH, maternal hypothyroxinemia, combined maternal and fetal hypothyroidism, prematurity and thyroid hormone resistance syndromes. This list has increased during the last years considering situations, such as prematurity, where deprivation of maternal thyroid hormones during the last part of gestation has negative effects on the neurological development of the baby. Most of the effects of iodine deficiency during brain development are related to hypothyroxinemia of the mother, as will be explained below. For more information, we refer to several reviews on the effects of iodine deficiency on brain development (DeLong, 1989; Morreale de Escobar *et al.*, 1989, 1997, 2004; Morreale de Escobar and Escobar del Rey, 2003; Obregon *et al.*, 2005).

The Maternal Transfer of T₄ during Gestation: Experimental Studies

To understand the brain damage observed in neurological cretinism it is important to keep in mind the main findings related to the presence and protective role of maternal

Experimental Models of Iodine Deficiency and Cretinism during Development: The Role of the Mother

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Abstract

Neurological cretinism is caused by severe iodine deficiency during pregnancy, and is characterized by severe mental retardation, neurological and motor abnormalities. It can be corrected by administration of iodine before conception. The clinical features of cretinism are different from the clinical signs of hypothyroidism found in congenital hypothyroidism. In iodine deficiency the maternal plasma T₄ is usually low, even with normal plasma T₃ and slightly elevated or normal TSH. The cause of the neurological abnormalities is thought to be the lack of thyroid hormones during the first period of brain development, and specifically maternal hypothyroxinemia early in pregnancy. Maternal transfer of T₄ during gestation has a protective role during the development of the fetal brain. Most of the T₃ in the fetal brain is produced from T₄ via the D2 deiodinase present in the fetal brain early in gestation (12–20 weeks of gestation). The development of the neocortex can be affected by short periods of maternal hypothyroxinemia before the onset of fetal thyroid function. Therefore, normal maternal thyroxinemia is of maximal importance to maintain T₃ levels in the fetal brain.

List of Abbreviations

PBI	protein bound iodine
CH	congenital hypothyroidism
ADHD	attention deficit hyperactivity disorders
LID	low iodine diets
MMI	methylmercaptoimidazole
BW	body weight
GH	growth hormone
D2	deiodinase type 2
D3	deiodinase type 3
CNS	central nervous system

IQ	intellectual quotient
RIA	radioimmunoassay
rT ₃	reverse T ₃
rT ₃	triiodothyronine
rT ₄	thyroxine

Cretinism: Historical and Epidemiological Studies

Cretinism is a condition of severe physical and mental retardation due to iodine deficiency, and specifically due to deficiency of thyroid hormones during early pregnancy. This condition is irreversible, even after treatment with thyroid hormones or iodine soon after birth, but can be corrected if treatment with iodine starts prior to or early in gestation.

Iodine deficiency has been recognized as the most frequent cause, after starvation, of preventable mental defects, affecting hundreds of million people to different degrees. The eradication of iodine deficiency has been approved in resolutions or declarations from most of the international conferences and organisms (United Nations, UNICEF, WHO, FAO, ICCIDD). Adequate iodine nutrition is important for the prevention of brain damage that could be irreversible by birth, and is only preventable when administered very early in gestation.

Endemic cretinism has been a common finding in the mountains or isolated regions, such as the Alps or the Himalayas and has been described or depicted in drawings over the centuries. McCarrison (1917) studied this condition in the Himalayas, describing two types of cretinism: the nervous and myxedematous types. He already suspected the influence of the maternal thyroid function on the fetus. Early observations in the Alps (Hunziker-Shild, 1915) already reported that the fetus could receive the hormone from the mother, and proposed the prophylactic administration of iodine from conception.