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Reshaping the gut microbiota: impact of low calorie sweeteners and the link to insulin resistance?

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Abstract

Disruption in the gut microbiota is now recognized as an active contributor towards the development of obesity and insulin resistance. This review considers one class of dietary additives known to influence the gut microbiota that may predispose susceptible individuals to insulin resistance - the regular, long-term consumption of low-dose, low calorie sweeteners. While the data are controversial, mounting evidence suggests that low calorie sweeteners should not be dismissed as inert in the gut environment. Sucralose, aspartame and saccharin, all widely used to reduce energy content in foods and beverages to promote satiety and encourage weight loss, have been shown to disrupt the balance and diversity of gut microbiota. Fecal transplant experiments, wherein microbiota from low calorie sweetener consuming hosts are transferred into germ-free mice, show that this disruption is transferable and results in impaired glucose tolerance, a well-known risk factor towards the development of a number of metabolic disease states. As our understanding of the importance of the gut microbiota in metabolic health continues to grow, it will be increasingly important to consider the impact of all dietary components, including low calorie sweeteners, on gut microbiota and metabolic health.

~189, (250 word limit)

Keywords: *Insulin Resistance, Low Calorie Sweetener, Microbiota, Glucoregulation, Non-nutritive sweeteners*

1. Introduction

On any given day, it is estimated that 11% of healthy-weight, 19% of overweight, and 22% of obese adults drink diet beverages, prevalent products containing low calorie sweeteners[1]. Women and children are now reported to be the greatest consumers of low calorie sweeteners in the United States. In 2008, it was estimated that nearly 15% of children and 33% of women consume food and beverages containing low calorie sweeteners, a large increase compared to 1999-2000 intake reports[2]. With obesity continuing to rise on a global scale[3], low calorie sweeteners have become a popular sugar substitute, particularly in 'diet' and 'light' foods, allowing a variety of products to retain their palatability without the associated calories, creating a perception of a 'healthier' product. Low calorie sweeteners are ubiquitous within current food products, such as desserts, gum, breakfast foods, and (diet) beverages, and therefore may be unintentionally consumed. For example, saccharin, sucralose, and acesulfame-potassium have all been found in the breast milk of women who did not explicitly report consuming these low calorie sweeteners[4].

The three most popular low calorie sweeteners are sucralose, followed by acesulfame-potassium and aspartame[5]. Although the common characteristic of low calorie sweeteners is to provide sweetness without associated calories, it is important to note that each one is metabolically and chemically distinct. For example, sucralose is a chlorinated disaccharide of which 65-95% is excreted in feces, whereas aspartame is a dipeptide and is completely hydrolyzed into its three moieties (phenylalanine, methanol, and aspartic acid) and absorbed within the small intestine[6,7]. Acesulfame potassium is an acidic cyclic sulphonamide derivative and is primarily excreted in urine[8].

Low calorie sweeteners have been approved for human consumption by regulatory agencies worldwide[9,10] and found to be 'safe' for human consumption. Although low calorie sweeteners

may have value in reducing the energy density of the diet, their impact on health requires further investigation. There is growing recognition that 'safe' and 'healthy' are different considerations. While safety considers disease (e.g. causative in cancer) and/or injury (e.g. toxicity), healthy implies a continued state of optimal physiological functioning (e.g. lack of insulin resistance). However, there is accumulating evidence that changes in gut microbiota may contribute to the development of certain diseases in susceptible individuals, as discussed below. Therefore, observations that low calorie sweetener consumption may result in alterations in the gut microbiota calls to question whether they may still be classified as 'safe'.

2. Epidemiological Data

Epidemiological, observational and biomedical evidence show regular low calorie sweetener consumption over a prolonged period may promote obesity, glucose intolerance, and its related comorbidities[11–14]. Swithers summarized and evaluated prospective cohort studies that examined low calorie sweetened beverage consumption in relation to health outcomes, including weight change, type 2 diabetes, cardiovascular disease and the metabolic syndrome[15]. In this paper, non-consumers were compared to consumers of low calorie sweetened beverages in more than 450,000 study participants in 14 independent investigations over an average 16-year follow-up period. Although results, presented in terms of hazard ratios for all conditions, were not unanimous, there were clear data showing low calorie sweetened beverage consumption to increase weight gain and disease risk (metabolic syndrome, type 2 diabetes, hypertension and cardiovascular disease) in the majority of the studies considered. Of note, these associations occurred independently of baseline adiposity measures suggesting a fundamental relationship more complex than just an excess of 'calories'[11]. These results are further supported by a study performed by Kuk et al, which examined the relationship between sucrose, fructose, aspartame

and saccharin consumption in 2856 adults in the United States using data from the Third National Health and Nutrition Examination Survey (NHANES III)[16]. Results of this analysis revealed that aspartame intake (a predominant sweetener in diet sodas) significantly influenced the association between body mass index and glucose tolerance, wherein only those reporting aspartame intake had a steeper positive association between body mass index and glucose tolerance, than those reporting no intake[16]

Although these health outcomes were based on peer-reviewed epidemiological studies in a number of highly regarded study cohorts, the conclusions have still been criticized as reverse causality - that individuals who are obese or at risk of developing a chronic disease simply consume more diet products containing low calorie sweeteners, despite data being normalized for body mass index. They have also been blamed for raising "unnecessary alarms" that ultimately create barriers to weight loss management[17]. These views are slowly changing due to data showing evidence that low calorie sweetener consumption perturbs metabolism via the gut microbiota across a number of species including humans. Results are in contrast to previously held beliefs that low calorie sweeteners are 'inert' and pass through the body with no metabolic effect. This review considers the role of gut microbiota in insulin resistance and how this may be affected by chronic low-dose, low calorie sweetener consumption. It further postulates as to why some individuals are affected (responders) while others remain unaffected (non-responders) and potential mechanisms by which the gut microbiota and the presence of low calorie sweeteners are communicated to the host.

3. Gut Microbiota

Collectively, the human gut consists of trillions of microorganisms, a number far exceeding the total number of our somatic cells[18,19]. The dynamic and diverse microorganisms that inhabit

the gut are capable of quick adaptation to varied pathological, dietary and metabolic conditions. This is evident in examination of its biochemical capabilities; the human microbiota has evolved to contain upwards of 60,000 glycoside hydrolases and polysaccharide lyases that are capable of digesting complex carbohydrates and other substrates – in comparison, humans have ~17 such enzymes[20]. Such enzymes serve an important role in both energy extraction from dietary sources and metabolism. Foods with high fibre content, such as plant products, provide large amounts of substrate for the microbiome, which in turn helps to regulate the health of the host[21]. Although the human microbiota is dominated by four main phyla – Bacteriodetes, Firmicutes, Actinobacteria and Proteobacteria – the abundance and diversity (phylogenetic, functional and genomic) of the microbiome may vary widely even in healthy populations[22]. Despite differences in abundance and variation in microbiota, studies point to a common "core microbiome" shared between healthy individuals, with common metabolic capabilities such as carbohydrate and amino acid metabolism[18,22,23].

Disruptions in the microbiome, or dysbiosis, have been linked to numerous metabolic disease states including obesity[24,25], insulin resistance[26] and cardiovascular disease[27] in observational and cross-sectional studies, while direct causation has been proven in animal models employing fecal transplant experiments[28]. Diet is a well-known modulator of the gut microbiota[29 30](reviewed in[31]). 'Western' or cafeteria type diets that are low in plant based products and dietary fibre, high in saturated fat and sugar[32], shift ratios of major microbial phyla and species and reduce its diversity[33]. Although not unanimous across all studies, obese phenotypes have been characterized by a reduction in *Bacteriodetes* and a significant increase in *Firmicutes*[25,34]. Further, this phenotype may be transferred when feces from obese mice are transplanted into germ-free mice[32,33] . Interestingly, a recent study revealed that intermittent consumption of a cafeteria type diet (3 days/week) altered rats gut microbiota composition to

model that of continuous cafeteria diet consumption[37]. These results suggest that short bouts of exposure to western type food are sufficient to negatively change the composition of gut microbiota, illustrating the sensitivity of the microbial community to diet.

4. Involvement of the Gut Microbiota in Obesity and Insulin Resistance

The severity and prevalence of obesity has dramatically increased on a global scale, and nearly 39% and 13% of adults are now considered overweight or obese respectively[38]. These statistics are cause for concern since obesity is a known contributor to many chronic disease states including type 2 diabetes and cardiovascular disease, which is increasing in developed and developing countries alike[38]. There is now abundant evidence that the microbiota is an environmental factor actively contributing to the development of obesity and insulin resistance. Early transplant experiments, wherein fecal matter from lean and obese mice was transferred to germ-free mice, showed that mice receiving the obese animal fecal matter gained more weight despite no increase in energy consumption[39]. These results show the microbiota profile to be intricately involved in energy harvest. Observations such as these have spurred a tremendous amount of research aimed at understanding differences between lean and obese microbiota and how the microbiome communicates with the host to affect body weight and metabolic health[24]. Although controversial, it appears that both obesity and type 2 diabetes are linked to proportional shifts in microbial composition at either taxonomic or gene level as well as reductions in microbial diversity. Comparison of microbiota from lean and obese individuals reveals that low bacterial diversity is associated with increased adiposity, insulin resistance, dyslipidaemia and low-grade inflammation compared to those with high bacterial diversity[40,41].

It is well known that obesity is a risk factor for the development of glucose intolerance, type 2 diabetes, and the metabolic syndrome. Diabetes accounts for 1.5 million deaths globally

and is projected to be the 7th leading cause of death worldwide by 2030[42]. Those with type 2 diabetes are at risk of serious health complications including cardiovascular disease, retinopathy, neuropathy, and a lower quality of life[42]. Several studies link the microbiota to insulin resistance, a condition leading to the development of type 2 diabetes[43]. Observationally, bariatric surgery, which has multifaceted effects on the gut microbiota, improves and even resolves insulin resistance prior to significant weight loss[44,45]. Other studies have shown the microbiota to be perturbed in young adults at high risk of developing type 2 diabetes leading to speculation that such individuals have distinctive microbiome features that make them more susceptible to the disease[46]. A key indicator of type 2 diabetes is impaired glucose disposal, the ability to dispose of a glucose load in the presence of insulin. The specific role of the microbiota in glucose disposal was examined by Vrieze and colleagues, wherein men diagnosed with the metabolic syndrome were given fecal transplants from either healthy donors, or had their own fecal matter returned to them[28]. After 6 weeks, individuals receiving transplants from lean donors showed dramatic improvements in insulin sensitivity with a near doubling of their glucose disposal rate under euglycemic-hyperinsulinemic conditions. The experiment showed clear evidence to suggest that the growth of certain bacteria can predispose an individual to insulin resistance with the gut microbiota modulating the influence of lifestyle factors involved in the disease[47]. One predisposing factor in the diet contributing to the development of insulin resistance may be the regular, long-term consumption of low-dose, low calorie sweeteners. These negative effects may also extend to pregnancy where low calorie sweeteners are considered to be safe, but are recommended to be consumed in moderation[48]. A recent study in mice by Collison and colleagues observed greater insulin resistance in males exposed to aspartame neonatally with continued consumption until 19 weeks of age when the insulin tolerance test was performed[49].

5. Low Calorie Sweeteners and the Gut Microbiota

Our inherent craving for sweet foods starts at birth and is shared with many other animal species[50]. Some of the best work to recognize an impact of low-calorie sweeteners on both feeding behaviour and the gut microbiota is derived from the agricultural sector where low calorie sweeteners are added to starter feeds. Although results are variable, the inclusion of either sugar based sweeteners or low calorie sweeteners in a number of species helps to establish gut microbiota and modulate feed-intake behaviour at specific times, which in some cases lead to increased 'performance'[51,52]. In agriculture, 'performance' often refers to feed efficiency – or the amount of weight gained per amount of feed consumed. While low calorie sweeteners may be beneficial in enhancing animal production and efficiency by establishing early commensal gut microbiota, their regular consumption may also lead to alterations in gut microbiota that perpetuate obesity and insulin resistance in an environment of 'over-nutrition'. Such a mechanism may be responsible for findings of numerous studies showing chronic, low dose, low calorie sweeteners to disrupt the gut microbiota.

Potential relationships between sweetener consumption and gut microbiota profile were examined in 1980, where Anderson and colleagues[53] showed that male rats exposed to 7.5% saccharin for ten-days had an altered aerobic to anaerobic fecal bacteria ratio by a specific deletion of anaerobic bacteria and increase in aerobic bacteria. Obligate anaerobic microbiota are densely colonized in the colon and most commonly targeted by dietary interventions[54]. An altered aerobic to anaerobic ratio has also been observed in mice with induced cirrhosis[55] further illustrating negative health effects coinciding with gut microbiota dysbiosis.

Significant dysbiosis was discovered nearly thirty years later in response to various loads of a sucralose-containing sweetener in rats gavaged for 12 weeks[56]. Abou-Donia et al. found a methodical pattern of continued reduction in bacterial counts over the 12-week period for

approved sucralose dosages[56]. The decrements varied by bacterial type with the greatest suppression (up to 70% or more) for the generally beneficial anaerobes (e.g., bifidobacteria, lactobacilli, and Bacteriodes) and with less inhibition for more detrimental strains (e.g. enterobacteria). Anderson et al also found that rats displayed cecal enlargement and increased stool hydration that were associated with an increase in hygroscopic polysaccharides suggesting differences in carbohydrate absorption with treatment[57].

More recently, a study by Suez and colleagues showed low calorie sweeteners to disrupt the gut microbiota in both mice and humans using a number of models and experimental interventions[58]. The first experiments administered three commercial low dose formulations to mice that each contained one of three sweeteners - sucralose, aspartame or saccharin. Compared to control mice consuming water, sucrose or glucose, the mice consuming low calorie sweetener developed glucose intolerance. All subsequent studies employed saccharin given that administration of this low calorie sweetener resulted in the most pronounced impairment. Transplantation of saccharin treated fecal matter into germ-free mice established that the glucose intolerant phenotype could be transferred to host animals, thereby implicating the microbiota as causative in the observed metabolic impairment in mice. Demonstration of metabolic impairments has not been limited to animal models. When researchers examined long-term sweetener consumption in non-diabetic humans, adverse changes in body composition including the waist-to-hip ratio and metabolic profile (fasting blood glucose, glycosylated haemoglobin, glucose tolerance and serum alanine aminotransferase) were positively correlated with sweetener consumption as assessed by validated food frequency questionnaires. These results alongside earlier observational and epidemiological studies suggest that effects of low calorie sweeteners may be subtle and accumulate over time. In other words, these effects are likely missed in shorter term randomized controlled trials, intervention-type studies, or when directly compared to sugar

sweetened beverage consumption (also a risk factor for the development of insulin resistance). Lastly, when Suez et al administered saccharin (at the acceptable daily intake levels, 5mg/kg body weight) to individuals who did not normally consume low calorie sweeteners (n=7) and then followed them for 7 days, a subset of these individuals (n=3) developed glucose intolerance[58]. Termed 'responders' and 'non-responders', there were clear differences in the microbiota profiles of these individuals following sweetener consumption. Fecal transplant from individuals in each group into germ-free mice also demonstrated that insulin resistance could be transferred via the gut microbiota, but only in responders. This again suggests that not all individuals are equally affected by sweetener consumption and response may depend on an individuals starting gut microbiota profile.

Another study by Palmnäs and colleagues reported similar effects in a rat model after consumption of aspartame-containing product[59]. They showed that in low doses (5-7 mg/day, ~2-3 cans of diet soda per day in humans), aspartame administered in drinking water affected gut microbiota and glucose tolerance in lean and diet-induced obese rats. Compared to water alone, aspartame consumption resulted in a reduction in energy intake in both dietary groups. Despite this, animals consuming aspartame displayed elevated fasting glucose levels and impaired insulin responses. Examination of the gut microbiota showed aspartame to induce distinctive changes in microbial composition that were partly dependent on diet. Fecal analysis of gut bacterial composition showed aspartame to increase abundance of *Enterobacteriaceae* and *Clostridium leptum.* An interaction between diet-induced obesity and aspartame was also observed for *Roseburia* with increased abundance in aspartame treated animals. Differences in microbiota composition with aspartame were also manifested in the serum metabolome, the analysis of circulating metabolites by proton nuclear magnetic resonance spectroscopy. Of interest, aspartame administration increased circulating levels of the short chain fatty acid propionate,

suggesting the microbiome shift favours propionate-producing bacteria. The mechanism by which aspartame altered the microbiota in this work and other aspartame studies is unexpected since the majority of aspartame is quickly metabolized into its component amino acids (aspartic acid, phenylalanine) and methanol, and would not reach the microbiota. However, small fractions of aspartame (~1.5%) can form the cyclization product aspartyl phenylalanine diketopiperazine, a compound with known antimicrobial properties[60]. Similarly, the methanol produced from aspartame metabolism also has the potential to affect the gut microbiota[61].

In contrast, a recent cross-sectional study examined the relationship between sweetener intake and microbiota in American adults. Participants were classified as 'consumers' (n=7) or 'non-consumers' (or 'unintentional' consumer; n=24) based on a four-day food intake journal, and further stratified by the type of sweetener consumed (principally aspartame and acesulfame-K)[62]. No differences in bacterial abundance and gene function were noted, however, there were significant differences in microbial diversity between consumer and non-consumers, which the authors proposed to be driven by bacteria in low abundance. This small change in diversity may be a driving force mediating metabolic changes observed following sweetener consumption.

6. Potential Mechanistic Considerations and Knowledge Gaps

At present, the exact mechanisms by which low calorie sweeteners perturb the gut microbiota are not known. Although we have grouped sweeteners into one class in this review, it is important to recognize that each one has a distinct structure, metabolism and acceptable daily intake level. Going forward, studies assessing each individual sweetener and their impact in health, pregnancy, and metabolic disease states are required. In this section we briefly highlight the potential mechanisms that may be involved in mediating sweetener induced insulin resistance.

6.1 Microbiome Diversity. Gut microbial diversity is the number and abundance distribution of distinct types of microorganisms[22]. In this type of analysis, microbiota are viewed as a community with a complexity inherent to ecologies composed of multitudes of different interacting species in a continually fluctuating environment. Interfering with one member of the community affects the balance of other species, especially if other species 'feed' or are dependent on its metabolites for survival. Reduced microbiome diversity has been implicated in several human diseases: low diversity being linked to obesity[23], inflammatory bowel disease[63] and arthritis[64] among others. In the case of low calorie sweeteners, the recent reports of disturbed diversity with the consumption of aspartame and acesulfame-K[62] could represent a plausible mechanism for observed sweetener induced metabolic disturbances, but this requires further study. In considering diversity, it is also important to realize that there is considerable inter-individual variation. Examination of existing low calorie sweetener data reveals that some individuals appear to be more affected than others in response to consumption. There are indications that there are responders and non-responders, possibly due to inherent differences in an individual's genetics, microbiome composition[65], lifestyle[66,67] and even prescription drug use[68,69]. These differences not only extend to the gut microbiota[58], but may also explain other low calorie sweetener induced sensitivities as well[70,71] .

6.2 Microbiota Generated Metabolites. Compositional and functional changes in the microbiome are manifested and communicated to peripheral sites by the release of metabolites[72]. Microbial metabolites appearing in serum consist of metabolic intermediates, organic acids and bacterial fermentation end products. The best-studied metabolites include the short chain fatty acids that can be used by the host and can also serve as a source of salvageable energy from otherwise

inaccessible dietary substrates. In the intestine, the short chain fatty acids are important signalling molecules and can help regulate intestinal gluconeogenesis[73]. It is now recognized that intestinal gluconeogenesis can contribute to 20-25% of total endogenous glucose production during fasting[74]. Given this, previously reported elevations in fasting glucose and elevated serum levels of the short chain fatty acids with aspartame induced metabolic dysfunction is a finding that requires further investigation[59]. It is also plausible that changes in the gut microbiota or short chain fatty acids induced by low calorie sweetener consumption could be communicated downstream in the gut as well, influencing bile acid metabolism[75], gene expression[76], sweet taste receptors[77], satiety[78] and the secretion of peptide YY (PYY) and the incretin hormones glucagon like peptide-1 (GLP-1)[79] and glucose-dependent insulinotropic polypeptide (GIP)[80], all of which likely influence glucoregulation.

6.3 Chronic Inflammation and the Innate Immune System. It is well documented that gut microbiota shapes intestinal immune responses during both health and disease and that low-grade inflammation, activated by the innate immune system, is a player in contributing to the development of insulin resistance[81]. Through pattern recognition receptors (i.e. Toll-like receptors (TLRs), the innate immune system can recognize microbe-associated molecular patterns (MAMPs) produced by microbes and coordinate an appropriate immune response if bacteria are recognized as pathogenic[82]. Obesity and high fat diets have been associated with intestinal hyperpermeability and greater serum lipopolysaccharide (LPS, outer membrane components of gram-negative bacteria) concentration in the blood, a condition also referred to as endotoxemia[26]. This phenomenon is a recognized link between inflammation and insulin resistance[83]. Everard and colleagues revealed that increases in *Akkermansia muciniphila*, a mucin degrading colonic bacteria, alleviated endotoxemia induced by high fat feeding and increased

mucous layer thickness in the gut in mice fed a high-fat diet[84]. *Akkermansia muciniphila* was also found to be underrepresented in mice fed saccharin for 11 weeks, and may provide a mechanistic link to negative responses elicited from sweetener consumption[58]. However, to date, little work exploring the interaction of low calorie sweeteners and obesity on gut barrier function, intestinal permeability and inflammation has been conducted.

7. Conclusions

Accumulating evidence suggests that low calorie sweetener consumption perturbs the gut microbiota and disrupts metabolic health in susceptible individuals. This concern was echoed by the US Scientific Report of the 2015 Dietary Guidelines Advisory Committee that provides the US Federal government with a foundation for developing national nutrition policy. These recommendations acknowledge a potential relationship between low calorie sweetened soft drinks and type 2 diabetes risk. Their consensus was that *"future experimental studies should examine the relationship between artificially sweetened soft drinks and biomarkers of insulin resistance and other diabetes biomarkers"*[85]. Nevertheless, there is no defined gut microbiota signature since the results from each study differ in terms of the microbes that are affected by low calorie sweetener consumption. This lack of consensus may be a result of individual variability, unique chemical composition and metabolism of each sweetener and the dose that is consumed. Therefore, studies investigating the role of each sweetener type and dose and its metabolic impacts are needed. Additionally, mechanisms and interactions potentially driving microbiota dysbiosis are an area that requires greater attention in future studies to address the current knowledge gaps.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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Highlights:

- Diet can influence the gut microbiota profile.
- The gut microbiome is a factor contributing to obesity and insulin resistance.
- Artificial sweeteners may perturb the gut microbiota – contributing to metabolic disease.
- Further studies examining the influence of sweeteners on metabolism are needed.