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<td>5:00-6:30</td>
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<td>6:30-6:45</td>
<td>V. Yaylayan – President IMARS Department of Food Science and Agricultural Chemistry, McGill University, Quebec, Canada (<a href="mailto:varoujan.yaylayan@mcgill.ca">varoujan.yaylayan@mcgill.ca</a>)</td>
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<td>6:45-7:15</td>
<td>Paul J. Thornalley - President Elect IMARS Clinical Sciences Research Laboratories, University of Warwick, Coventry, UK (<a href="mailto:P.J.Thornalley@warwick.ac.uk">P.J.Thornalley@warwick.ac.uk</a>)</td>
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<td>7:15-7:45</td>
<td>Monika Pischetsrieder, Department of Chemistry and Pharmacy, Friedrich Alexander University Erlangen-Nuremberg, Erlangen, Germany (<a href="mailto:monika.pischetsrieder@fau.de">monika.pischetsrieder@fau.de</a>)</td>
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<td>7:45-8:15</td>
<td>Thomas Henle, Department of Food Chemistry, Technische Universität Dresden, Germany (<a href="mailto:thomas.henle@tu-dresden.de">thomas.henle@tu-dresden.de</a>)</td>
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“Metabolic origins of dicarbonyl stress in hyperglycemia linked to the development of vascular complications of diabetes”

“Beyond glucose and lysine: the relevance of less common Maillard reactions”

“Glycation in the light of evolution: A Darwin-inspired view on Maillard reactions in food”
Methylglyoxal and insulin resistance: novel mechanism and unique target. Casper Schalkwijk. Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands. (c.schalkwijk@maastrichtuniversity.nl)

Insulin resistance is characterized by an impaired responsiveness to the action of insulin at its multiple target organs. The accumulation of methylglyoxal has been demonstrated in clinical settings of insulin resistance such as in diabetes, hypertension, and obesity. We have focused on methylglyoxal as a modulator of insulin resistance. Structural and functional abnormalities of the insulin molecule by methylglyoxal may contribute to the pathogenesis of insulin resistance. In addition, it is likely that AGEs interfere in the complex molecular pathways of insulin signaling and as such in insulin resistance.

Ascorbic acid oxidation as a source of methylglyoxal in the aging human lens and brain. Vincent M. Monnier (Xingjun Fan, Benlian Wang, David R. Sell, Daniel Wesson) Case Western Reserve University, Cleveland, Ohio, USA (vmm3@case.edu)

PURPOSE: Human lens crystallins become progressively pigmented and crosslinked with age, in part due to reactive carbonyl compounds producing advanced glycation end products (AGEs). The most prevalent AGE modification found in aged human lens crystallins, i.e. the arginine modification by methylglyoxal (MG)-derived hydroimidazolones (MG-H1) is usually attributed to glucose-derived MG formation during glycolysis. Below, we hypothesized that ascorbic acid oxidation could be an important source for MG-H1 formation in the lens. To demonstrate this we determined the in vitro and in vivo mechanism of MG-H1 formation from ascorbic acid.

METHODS: In vitro: lens protein extract was incubated with glucose, ASA and ASA catabolic compounds at various concentration at 37°C for 7 days. The MG-H1 formation was determined by both LC/MS and western blot with an MG-H1 antibody. The lens extract was also incubated with C3-13-ASA, C4-13-ASA and C6-13-ASA to determine the mechanisms of MG-H1 formation via ASA oxidation. In vivo: MG-H1 formation was determined in somatic and lens specific Vitamin C transporter 2 (SVCT2) transgenic mouse tissues compared to WT mouse. Universal C13 labeled ASA (U6-C13-ASA) was also delivered into glutathione biosynthesis KO (Gclm KO) and WT mouse brain via intra-ventricular injection, and the brain C13-MG-H1 was analyzed two weeks after injection.

RESULTS: In both anaerobic and aerobic conditions, ASA and most of its catabolic compounds can form MG-H1 after incubation with lens protein extract. The isotopic labeling experiments indicated that ASA was undergoing C3-C6 break during oxidative catabolism, and subsequent C4-6 backbone was involved in MG-H1 formation. There was five-fold elevation the level of lens protein bound MG-H1 (p<0.0001) and positive association (r²=0.732) with aging in the lens specific SVCT2 transgenic mouse compared to WT. Similar
results were found in the brain cerebral cortex of somatic SVCT2 transgenic mouse at 12 mos of age compared to age-matched WT. Direct evidence of MG-H1 formation from ASA oxidation was achieved by intra-ventricular injection of 10mM U6-C^{13}-ASA in both wild type and Gclm knockout mouse. From this experiment, we were able to detect C^{13}-labeled MG-H1 two weeks after injection with a three-fold increase in C^{13}-MG-H1 in Gclm KO brain compared to WT.

**CONCLUSIONS:** While much of MG-H1 formation is attributed to glucose degradation, the finding of elevated MG-H1 levels in tissues rich in ASA, as in the lens and brain, suggests that MG-H1 accumulation in these tissues reflects oxidative stress likely linked with impaired GSH homeostasis.

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### 8:50 - 9:05

**Dicarbonyl stress and glyoxalase enzyme system regulation in human skeletal muscle**

**Jacob M. Haus** (Jacob T Mey, Brian K Blackburn, Edwin R Miranda, Alec B Chaves, Joan Briller, Marcelo G. Bonini). University of Illinois at Chicago, IL, USA; University of Michigan, Ann Arbor MI, USA  

Skeletal muscle insulin resistance is a hallmark of type 2 Diabetes (T2DM) and may be exacerbated by protein modifications by methylglyoxal (MG), known as dicarbonyl stress. The glyoxalase enzyme system composed of glyoxalase 1/2 (GLO1/GLO2) is the natural defense against dicarbonyl stress, yet its protein expression, activity and regulation remain largely unexplored in skeletal muscle. Therefore, this study investigated dicarbonyl stress and the glyoxalase enzyme system in the skeletal muscle of subjects with T2DM (age: 56 ± 5 yrs.; BMI: 32 ± 2 kg/m²) compared to lean healthy control subjects (LHC; age: 27 ± 1 yrs.; BMI: 22 ± 1 kg/m²). Skeletal muscle biopsies obtained from the *vastus lateralis* at basal and insulin-stimulated states of the hyperinsulinemic (40 mU/m²/min) –euglycemic (5 mM) clamp were analyzed for proteins related to dicarbonyl stress and glyoxalase biology. At baseline, T2DM had increased carbonyl stress and lower GLO1 protein expression (-78.8%), which inversely correlated with BMI, percent body fat and HOMA-IR while positively correlating with clamp derived glucose disposal rates. T2DM also had lower NRF2 protein expression (-31.6%), which is a positive regulator of GLO1, while Keap1 protein expression, a negative regulator of GLO1, was elevated (207%). Additionally, insulin stimulation during the clamp had a differential effect on NRF2, Keap1 and MG-modified protein expression. These data suggest that dicarbonyl stress and the glyoxalase enzyme system are dysregulated in T2DM skeletal muscle and may underlie skeletal muscle insulin resistance. Whether these phenotypic differences contribute to the development of T2DM warrants further investigation.

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### 9:05 - 9:10

**The impact of angiotensin receptor type 1 glycation on its signaling pathways.**

**Sophie Touchette** (Ulrike Froehlich, David St-Pierre, Brian Holleran, Jean-Luc Parent, Richard Leduc and Michel Grandbois). Université de Sherbrooke, Quebec, Canada  

Glyoxal (GO) is formed under hyperglycemic conditions and acts as advanced glycation end product (AGE) precursor, forming adducts on proteins containing lysine and arginine residues. It has been previously shown that GO exposure can alter tyrosine kinase receptors signaling activity, in particular PDFRβ, EGFR and the insulin receptor signaling which is an important component of hyperglycemia in diabetes. It is now assumed that GO could alter the signaling activities associated to other receptor such as the large family of G-protein coupled receptors. Indeed, GO was previously shown to increase the cell contractile response following agonist stimulation of the angiotensin II type 1 receptor (AT1R)⁴. In this context, elevated GO levels could be involved in the development of hypertension related to chronic hyperglycemia through AT1R homeostasis. GO could react with the lysine and arginine residues present on the receptor (AT1R) and affect the binding of AngII, its activation or the coupling of G proteins and β-arrestins to the receptor. In order to test this hypothesis, we conducted immunoprecipitation and immunoblotting experiments on HEK293-AT1R cells in order to observe potential glycation of the AT1R.

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### 9:10 - 9:25

**Dicarbonyl stress and glyoxalase enzyme system regulation in human skeletal muscle**

**Jacob M. Haus** (Jacob T Mey, Brian K Blackburn, Edwin R Miranda, Alec B Chaves, Joan Briller, Marcelo G. Bonini). University of Illinois at Chicago, IL, USA; University of Michigan, Ann Arbor MI, USA  

Skeletal muscle insulin resistance is a hallmark of type 2 Diabetes (T2DM) and may be exacerbated by protein modifications by methylglyoxal (MG), known as dicarbonyl stress. The glyoxalase enzyme system composed of glyoxalase 1/2 (GLO1/GLO2) is the natural defense against dicarbonyl stress, yet its protein expression, activity and regulation remain largely unexplored in skeletal muscle. Therefore, this study investigated dicarbonyl stress and the glyoxalase enzyme system in the skeletal muscle of subjects with T2DM (age: 56 ± 5 yrs.; BMI: 32 ± 2 kg/m²) compared to lean healthy control subjects (LHC; age: 27 ± 1 yrs.; BMI: 22 ± 1 kg/m²). Skeletal muscle biopsies obtained from the *vastus lateralis* at basal and insulin-stimulated states of the hyperinsulinemic (40 mU/m²/min) –euglycemic (5 mM) clamp were analyzed for proteins related to dicarbonyl stress and glyoxalase biology. At baseline, T2DM had increased carbonyl stress and lower GLO1 protein expression (-78.8%), which inversely correlated with BMI, percent body fat and HOMA-IR while positively correlating with clamp derived glucose disposal rates. T2DM also had lower NRF2 protein expression (-31.6%), which is a positive regulator of GLO1, while Keap1 protein expression, a negative regulator of GLO1, was elevated (207%). Additionally, insulin stimulation during the clamp had a differential effect on NRF2, Keap1 and MG-modified protein expression. These data suggest that dicarbonyl stress and the glyoxalase enzyme system are dysregulated in T2DM skeletal muscle and may underlie skeletal muscle insulin resistance. Whether these phenotypic differences contribute to the development of T2DM warrants further investigation.
following exposure to GO. We then quantified the whole-cell integrated AT1R mediated-response using a cell impedance assay in order to evaluate the impact of the exposure to GO. These experiments point toward a decrease in the G₁₂/₁₃/Rho/ROCK and ERK1/2-dependant pathways activation following GO exposure. We also investigated whether AT1R potential glycation had an impact on the activation profile of the AT1R signaling pathways. In a targeted manner, BRET-based biosensor assays revealed that β-arrestin 2 recruitment and Gq activation undergo a decrease following GO treatment. We also evaluated inositol 1-phosphate (IP₁) production, using the IP-One Assay. AT1R treated with GO induced a decrease in inositol phosphate formation. MAP kinases phosphorylation was also evaluated, in particular ERK1/2, using the AlphaScreen SureFire assays, which showed a reduction of ERK1/2 phosphorylation following GO exposure. This is consistent with the results of the β-arrestin 2 recruitment which also undergoes a decrease following GO treatment. Taken as a whole, our results suggest that exposure of the HEK293-AT₁R cells to high concentration of GO affect the activation profile of the AT₁R dependent signaling pathways.

9:30-9:45

Glyoxalase I expression, copy number and survival analysis in clinical treatment of breast cancer. Muhanad Alhujaily (Naila Rabbani and Paul J Thornalley)
University of Warwick, University Hospital, Coventry, UK. (M.Alhujaily@warwick.ac.uk)

Background and aims: Glyoxalase 1 (Glo1) catalysed the metabolism of methylglyoxal and thereby prevents the formation of quantitatively major protein and nucleotide advanced glycation endproduct formation, hydroimidazolone MG-H₁ and imidazopurinone MGdG, respectively. In cancer, increased Glo1 expression is permissive for high glycolytic rate and tumour growth and also mediates multidrug resistance (MDR) in cancer chemotherapy. Increased Glo1 expression is linked to increased GLO1 copy number and expression of transcription factor Nrf2. In this study we examined factors linked to Glo1 expression in human tumour cell lines and association of Glo1 expression to the effectiveness of clinical breast cancer treatment.

Materials and methods: Expression data in human tumour cells lines was accessed from the Cancer Cell Line Encyclopaedia (CCLE; https://portals.broadinstitute.org/ccle) – a transcriptomic database of 1043 human tumour cell lines. Glo1 expression and breast cancer survival was analyzed by the Kaplan Meier plotter analysis tool (http://kmplot.com/analysis/) where data on endocrine and/or chemotherapy treatment and relapse-free survival of 3951 breast cancer patients is available.

Results: In the CCLE database, Glo1 expression correlated positively with GLO1 copy number (r = 0.43, P=3.9x10⁻⁴⁴ P<0.01 n=1043) but not with expression of glyoxalase 2 (HAGH), Nrf2 not the Nrf2 antagonist, KEAP1. Increased GLO1 copy number was of highest prevalence in breast cancer cell lines (35%) where Glo1 expression also correlated positively with GLO1 copy number (r = 0.57, P=0.0001 P<0.01; n = 59), We therefore explored the association of Glo1 expression to survival in clinical breast cancer. For breast cancer patients of all genotypes receiving endocrine and/or chemotherapy, increased Glo1 expression was a negative survival factor (hazard ratio 1.38, logrank P = 3 x 10⁻⁸); median relapse-free survival was 185 and 217 months for high and low Glo1 expression, respectively. The effect was larger for estrogen-receptor-positive (ER⁺) cancer: hazard ratio 1.62, logrank P = 2 x 10⁻⁸; median relapse-free survival was 163 and 217 months for high and low Glo1 expression, respectively (n = 2061). The highest effect was for ER⁺ cancer with tamoxifen treatment: hazard ratio 1.8, logrank P = 2 x 10⁻⁴; median relapse-free survival was 85 and 148 months for high and low Glo1 expression (n = 740).

Conclusion: Increased GLO1 copy number is a determinant of increased Glo1 expression in human tumour cell lines with high prevalence in breast cancer lines. Increased Glo1 expression is a negative survival factor in endocrine/chemotherapy treatment of clinical breast cancer, consistent with a role for Glo1 in MDR.
Skin autofluorescence improves the Finnish Diabetes Risk Score in detection of present diabetes, and predicts diabetes in a large population based cohort — the LifeLines cohort study. Andries J. Smit (Van Waateringe RP, Fokkens BT, Wolffenbuttel BHR). University of Groningen, University Medical Center Groningen, The Netherlands. (a.j.smit@umcg.nl)

In type 2 diabetes mellitus (T2D), a long, clinically latent period often exists in which diabetes is not detected, but silent development of complications frequently occurs. Earlier studies showed that skin autofluorescence (SAF), measured with the AGE-reader, increases with ageing, and is independently associated with type 2 diabetes, and its complications. We aimed to investigate whether SAF, as estimate of advanced glycation endproducts accumulation, improves the currently used Finnish diabetes risk score (FINDRISC) in detecting undiagnosed diabetes, and also predicts 4-year risk of incident T2D, in the general population.

Methods: Subjects included were participants of the Lifelines cohort study, a large population-based cohort. SAF was assessed in an unselected subset of participants. After exclusion of participants with previously diagnosed diabetes (n=1635), pregnant women (n=58), and participants using corticosteroids (n=345), 79,248 subjects were available for analysis at baseline, and 74172 participants with at least one year, and median 4 years follow-up. Diabetes was defined at baseline by fasting blood glucose ≥7.0 mmol/l, non-fasting blood glucose ≥11.1 mmol/l, or HbA1c ≥6.5%. Incident type 2 diabetes was scored by self-report, or fasting blood glucose ≥7.0 mmol/l, or HbA1c ≥6.5% (mmol/mol) at follow-up.

Results: Diabetes was detected at baseline in 1042 participants (aged 55 ± 12 yr; 54% male). SAF improved the area under the receiver operating curve (AUROC) of the FINDRISC model from 0.802 to 0.811 (p<0.001). Furthermore, addition of SAF to the FINDRISC reclassified 8-15% of all participants into more accurate risk categories (NRI = 0.080, 95% CI 0.052 – 0.110). The proportion of participants reclassified was especially high (>30%) among the intermediate risk categories. Furthermore, when SAF was added to a simplified model (using age and BMI categories only), its performance was similar to the full model+SAF (AUROC =0.806, p=0.062). After median 4 years follow-up (range 1-9 years): 994 subjects (1.2%) had developed T2D; Baseline SAF was elevated in subjects with incident T2D (p<0.01), and predicted development of T2D, independent of several traditional risk factors.

Conclusion: SAF is a non-invasive tool that may be used to further improve the FINDRISC in diabetes detection. The proposed new model is especially useful in reclassifying participants in intermediate risk categories in which additional blood glucose testing is needed to confirm diabetes presence. Importantly, the simplified model (age, BMI and SAF) performed as well as the full model. Furthermore, SAF is also clinically valuable in screening for future T2D risk, particularly in a priori low-risk individuals.

Break

Glycation and oxidation in Diabetes and Other Diseases
Chairs: Naila Rabbani and Ryoji Nagai

Higher dietary advanced glycation endproduct intake increases circulating and hepatic free advanced glycation endproducts and consequently leads to hepatic inflammation. Jean L. Scheijen1,2 (Mitchell Bijnen1,2, Suzan Wetzels1,3, Coen D.A. Stehouwer3,2, Kristiaan Wouters2,3 and Casper G. Schalkwijk1,3)
1 Dept. of Internal Medicine, MUMC, Maastricht, The Netherlands, 2 CARIM, MUMC, Maastricht, The Netherlands, 3 Dept. of Immunology and Biochemistry, Biomedical Research Institute, Hasselt University, Hasselt, Belgium.
j.scheijen@maastrichtuniversity.nl
Background: Advanced glycation endproducts (AGEs) in the body are formed by the reaction of reducing sugars with amino acids in proteins, and have been associated with several age-related diseases such as diabetes mellitus and cardiovascular disease. However, AGEs can also be derived from exogenous sources such as the diet; in particular high-heat treated and processed foods. In humans, we have recently demonstrated that higher intake of dietary AGEs is associated with higher levels of free AGEs in plasma and urine. However, it is unknown whether dietary AGEs accumulate in organs such as the liver. Therefore, the aim of our study was to investigate the association of dietary AGEs with circulating AGEs and AGE levels in the liver and whether dietary AGEs cause hepatic inflammation in mice.

Methods: We first quantified the protein-bound form of the major AGEs N\(^\epsilon\)-(carboxymethyl)lysine (CML), N\(^\epsilon\)-(carboxyethyl)lysine (CEL), and N\(^\delta\)-(5-hydroxy-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H\(_1\)) with UPLC-MS/MS in different type of diets. In mice, fed with a standard chow diet (standard CD), a low fat diet (LFD) or a baked chow diet (baked CD) for 5 weeks, the free form of CML, CEL, and MG-H\(_1\) were determined in plasma and liver homogenates. Hepatic inflammatory gene expression was measured by real-time PCR.

Results: Baking the CD strongly increased protein-bound AGE content (3 to 10-fold higher). Feeding mice with baked CD increased circulating free CEL (42\(\pm\)2 vs 54\(\pm\)4 nmol/g) and MG-H\(_1\) (3.7\(\pm\)0.1 vs 9.9\(\pm\)2.0 nmol/g) in comparison to standard CD-fed mice. Compared to standard CD, baked CD increased the hepatic expression of TNF-\(\alpha\) (1\(\pm\)0.2 vs 1.6\(\pm\)0.25; \(p=0.057\)) and CRP (1.3\(\pm\)0.13 vs 1.37\(\pm\)0.06; \(p=0.04\)). A LFD contains up to 50-fold less protein-bound AGEs as compared to standard CD. Switching the diet from standard CD to LFD, resulted in lower circulating concentrations of free CML (534\(\pm\)27 vs 87\(\pm\)6 nmol/g), free CEL (100\(\pm\)7 vs 21\(\pm\)1 nmol/g) and free MG-H\(_1\) (120\(\pm\)3 vs 8.4\(\pm\)1.7 nmol/g) and hepatic free CML (54\(\pm\)1 vs 72\(\pm\)0.6 nmol/g), CEL (6.5\(\pm\)0.4 vs 5.3\(\pm\)0.4 nmol/g) and MG-H\(_1\) (1.16\(\pm\)0.1 vs 0.72\(\pm\)0.06 nmol/g) within 3 weeks. The reduction of hepatic AGEs was accompanied by a reduction of hepatic inflammatory gene expression.

Conclusions: Dietary AGEs are a contributor to circulating and hepatic AGEs and higher dietary AGE intake results in increased hepatic inflammation. This effect is reversible by a switch to low dietary AGE intake.
**Results:** Subjects in the non-CVD/high MetSO group had significantly higher levels of Met relative to those in the CVD/low MetSO group (35±10 vs 24±5 μmol/l, p=0.0003), and by design also higher levels of MetSO (3434±676 vs 895±94 nmol/l, p<0.0001). The ratio of MetSO to Met was also significantly higher in the non-CVD relative to the CVD group (0.11±0.03 vs 0.04±0.005, p<0.0001). The percentage of the S isomer was significantly higher in the CVD (64±5%) compared to the non-CVD group (59.7±5%, p=0.02) indicating greater enzymatic control of MetSO levels, since direct chemical production results in a close to 50/50 ratio of the S and R isomers.

**Conclusions:** Patients with diabetes but no CVD have significantly higher levels of Met, which is known to protect against oxidative stress by formation of less chemically reactive methionine sulfoxide. This protective effect is also supported by the significantly higher levels of MetSO in the non-CVD group, relative to lower levels in the CVD group. Higher levels of a marker of enzymatic control reflected by the S isomer of MetSO in the CVD group, suggests control by regulated pathways that increase propensity to vascular damage. Different levels of MetSO for given levels of methionine in the non-CVD relative to the CVD groups also suggest a regulated process. Further research is needed to identify the specific mechanisms responsible for these unique observations and their clinical implications.

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**Advanced glycation end-products, dityrosine and arginine transporter dysfunction in autism – a source of biomarkers for clinical diagnosis. Naila Rabbani (Attia Anwar, Provvidenza Maria Abruzzo, Sabah Pasha, Kashif Rajpoot, Alessandra Bolotta, Alessandro Ghezzo, Marina Marini, Annio Posar, Paola Visconti, Paul J Thornalley)  
Clinical Sciences Research Laboratories, University of Warwick, University Hospital, Coventry UK, Department of Experimental, Diagnostic and Specialty Medicine, School of Medicine, University of Bologna, 40126 Bologna, Italy, Department of Computer Science, University of Birmingham, Birmingham, UK, Don Carlo Gnocchi Foundation ONLUS, IRCCS “S. Maria Nascente”, 20148 Milan, Italy, Child Neurology and Psychiatry Unit, IRCCS Institute of Neurological Sciences, 40139 Bologna, Italy, Department of Biomedical and Neuromotor Sciences, University of Bologna, 40139 Bologna, Italy and Research Technology Platform – Proteomics, University of Warwick, Coventry, UK (N.Rabbani@warwick.ac.uk)**

**11:00-11:15**

**Background and aims:** Autism Spectrum Disorder (ASD) is a group of developmental disorders mainly affecting social interactions and range of interests and causing a wide spectrum of other disabilities, such as speech disturbances, repetitive and/or compulsive behaviors, hyperactivity, anxiety and difficulty to adapt to new environments, with or without cognitive impairment. Clinical chemistry tests for ASD are currently unavailable. Behavioural and psychological tests have 60 – 70% accuracy with sometimes long delay for referral to expert assessors. The aim of this study was to explore the diagnostic utility of plasma protein glycation, oxidation and nitration adducts and related glycated, oxidised and nitrated amino acids (free adducts) in plasms and urine for the clinical diagnosis of ASD.

**Materials and methods:** 38 children with ASD (age 7.6 ± 2.0 years) and 31 age-matched healthy controls (age 8.6 ± 2.0 years) were recruited for this study. Plasma protein glycation, oxidation and nitration adducts and amino acid metabolome in plasma and urine were determined by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry. Machine learning methods were then employed to explore and optimise combinations of analyte data for ASD diagnosis.

**Results:** Children with ASD had increased advanced glycation endproducts (AGEs), Nε-carboxymethyl-lysine (CML) and Nω-carboxymethylarginine (CMA), and increased oxidation damage marker, dityrosine (DT), in plasma protein, with respect to healthy controls. Children with ASD also had increased CMA free adduct in plasma ultrafiltrate and increased urinary excretion of oxidation free adducts, alpha-aminoacidic semialdehyde and glutamic semialdehyde. From study of renal handling of amino acids, children with ASD had decreased renal clearance of arginine and CMA, with respect to healthy controls.
Algorithms to discriminate between ASD and healthy controls gave strong diagnostic performance with features: plasma protein AGEs - CML, CMA, and 3-deoxyglucosone-derived hydroimidazolone, and oxidative damage marker, DT; sensitivity, specificity and accuracy were 92%, 84% and 88%, respectively.

**Conclusions:** Data-driven combination of plasma protein AGEs and DT gave diagnostic algorithms of high sensitivity and specificity for ASD. Changes in plasma AGEs were likely indicative of dysfunctional dicarbonyl metabolism in ASD. Increased DT implicates dual oxidase activity in ASD, possibly linked to impaired gut mucosal immunity. Decreased renal clearance of arginine and CMA in ASD is indicative of increased arginine transporter activity which may be a surrogate marker of disturbed neuronal availability of amino acids.

Both simple analysis and precise analysis of AGEs are important to evaluate the metabolic disorders. Ryoji Nagai Jun-ichi Shirakawa¹, Rei-ichi Ohno¹, Hikari Sugawa¹, Hiroyo Yamaguchi², Shoutaro Arakawa², Ryusuke Suzuki², Hikari Satou², Shiori Sakake³, Nana Katsuta³, Mime Nagai³, ¹Laboratory of Food and Regulation Biology, Graduate School of Agriculture, Tokai University, Japan; ²Orthopaedic Surgery, Jikei University school of Medicine, Japan (nagai@agri.u-tokai.ac.jp)

AGE content in biological samples is estimated by measuring fluorescence intensity, brown color, or reactivity with anti-AGE antibodies, or by detecting the AGE structure using instrumental analysis. These methods have been used to clarify the biological significance of glycation with age-related diseases. However, because many pathways have been reported to be involved in the generation of AGEs in vivo, it is difficult to clarify the relationship between pathology and glycation by measuring only a single AGE structure. Therefore, monitoring for multiple AGEs in biological samples by instrumental analyses, such as liquid chromatography tandem mass spectrometry (LC-MS/MS), is widely done to assess the biological significance of AGEs. However, LC-MS/MS analysis requires multiple preparation steps before the analysis can be performed and it is difficult to run a large number of clinical samples. For this reason, we developed a device, called the AGE sensor, that detects skin fluorescence intensity. The results using this sensor indicated that the fluorescence intensity at the fingertip was increased in the presence of diabetic microvascular complications (1). This device allows us to estimate the subject's AGE levels in one minute and the involvement of AGEs in various pathological conditions is currently being investigated. Therefore, I would like to explain the advantages and disadvantages of this simple analysis and the precise analytical methods used for AGEs.

Localization of Advanced Glycation Endproducts and fluorescence in dark skin. Isabella Azteni (Jeltsje Boersema¹, Hendri H. Pas¹, Gilles F.H. Diercks¹, Jean L. Scheijen², Casper G. Schalkwijk², Piet van der Zee³, Andries J. Smit⁴). ¹Department of Internal Medicine, Division of Vascular Medicine, Department of Dermatology and Department of Pathology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. ²Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands. Diagnoptics Technologies (P.v.d.Z.), Groningen, The Netherlands. (i.m.azteni@umcg.nl)

**Background and aims:** Skin autofluorescence (SAF) is a non-invasive method to estimate dermal accumulation of advanced glycation endproducts (AGEs). SAF correlates with dermal AGEs in Caucasians and Asians, but in dark-skinned subjects AGE localization, its dependence on age and diabetes mellitus (DM), and effects of absorption of excitation/emission light by melanin during fluorescence measurements, are not validated. We aimed to qualitatively assess localization of AGEs and autofluorescence/SAF in the (epi)dermis in dark-skinned subjects. Notably, in this pilot study our goal was not to quantify the usability of the AGE Reader, which measures SAF, in the dermis of dark-skinned subjects nor to interpret SAF as a function of age or DM. Our primary goal was to check whether there will be a contrast between young and healthy versus old and diabetic in AGE accumulation, and in SAF signal, as previously seen in Caucasians.
Patients and methods: In 6 healthy young subjects (median age 22 years) and in 6 older DM patients (median age 65 years) with Fitzpatrick skin type IV-VI, volar forearm SAF, skin pigmentation level measurements, and skin biopsies were obtained. SAF was measured with the AGE Reader, skin distribution of autofluorescence using confocal microscopy, and distribution of AGEs with anti-AGE-antibodies for N^ε-(carboxymethyl)lysine (CML) and N^δ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) in the skin biopsies. Biochemically, dermal AGEs (CML and MG-H1) were measured using UPLC-MS/MS+HPLC analysis.

Results: Antibody-assessed CML and autofluorescence intensity were higher in the dermis of older DM dark-skinned patients, as was biochemically assessed CML. Anti-AGE antibody assessed CML was preferentially visible around blood vessels and fibroblasts in the dermis. Anti-AGE antibody assessed MG-H1 was preferentially visible around blood vessels, colocalized with collagen and fibroblasts in the dermis, and was also present in the epidermis, on a sweat gland and hair follicle. Autofluorescence was most prominent in the dermis, but was also present in the epidermis.

Conclusion: Dermal AGE (CML) accumulation during aging and diabetes occurs qualitatively similar in subjects with dark skin. This supports further attempts to develop methods for estimating skin AGE levels using SAF in dark-skinned subjects.

12:00-2:00 Lunch/IMARS members meeting

Food A Controlling the Maillard Reaction Chairs: I. Blank & M. Pischetsrieder

Opportunities and challenges to guide the Maillard reaction cascade. Imre Blank^1 (Tomas Davidek^2) ^1Nestec Ltd., Nestlé Research Center, 1000 Lausanne 26, Switzerland, ^2Nestec Ltd., Nestlé Research Orbe, Switzerland. (imre.blank@rdls.nestle.com)

Food chemistry is known as an interdisciplinary research area addressing quality, safety, and nutrition of foods and beverages. The major constituents, i.e. carbohydrates, amino acids and their derivatives, lipids as well as polyphenols, react with each other resulting in desirable effects, such as aroma, taste, color, and health-promoting compounds. However, at the same time, using very similar reaction pathways, certain undesirable compounds can also be generated leading to off-flavors, loss of nutritional value or the formation of toxic compounds. This is in particular valid for the Maillard reaction cascade, requiring a better understanding of the parameters that influence the molecular composition and the overall product quality. The identification of acrylamide as a food-borne toxicant formed from asparagine through the Maillard reaction cascade has triggered research looking at mitigation strategies while keeping the product quality. The large number of publications has shown the complexity in finding the best compromise between quality and safety. It turned out that the early phase of the Maillard reaction cascade is key in favoring the reaction pathways towards vinylogous compounds as compared to Strecker aldehydes or the corresponding thermogenic amines. Similarly, the formation of furan under roasting conditions^3 is associated with flavor generation that is a major quality attribute of many foods and beverages. The strong interplay of the Maillard reaction cascade with lipid oxidation^4 has become evident, which calls for a more holistic approach in food research. The lecture will give an overview elaborating on various strategies that favor the formation of desirable compounds using coffee roasting and extrusion cooking as examples. The lecture will also address the need to perform studies in both model systems and in real food including labelling experiments.

Diversity of the Maillard reaction-Searching for regular patterns in a complex reaction cascade. Daniel Hemmler (Chloé Roullier-Gall^1,^2, James W. Marshall^3, Michael Rychlik^1, Andrew J. Taylor^3, Philippe Schmitt-Kopplin^1,^2)
The Maillard reaction produces a multitude of compounds through interconnected chemical pathways. Many of the produced reaction products significantly contribute to the taste, aroma and color of foods. In order to control the Maillard reaction towards desired reaction products, it is of great importance to understand the entire chemical “collective”. However, the complexity and diversity in chemical properties often complicates simultaneous analysis of the whole range of reaction products. Here we demonstrate that non-targeted (ultra-)high resolution mass spectrometry tools, such as high-field (12 Tesla) Fourier transform cyclotron resonance mass spectrometry (FT-ICR-MS) and tandem liquid chromatography, can comprehensively fingerprint initial and intermediate Maillard reaction products (MRPs). Even in simple sugar – amino acid model systems hundreds of different reaction products, originating from only two initial precursors, could be resolved in the same time. Moreover, relatively slow reaction kinetics allowed time-resolved monitoring of the reaction intermediates produced. While the sugar precursor predominantly defines reaction rates, the amino acid is responsible for the molecular characteristics of the reaction products. Despite a large chemical diversity, many reaction products, independently of the amino acid precursor, follow simple and regular reaction patterns. Conceptual reaction pathways are presented which provide novel and holistic insights into the scope of the complex reaction cascades in Maillard chemistry.
Maillard Reaction in black garlic. Hao Jing. College of Food Science and Nutritional Engineering, China Agricultural University, China (haojing@cau.edu.cn)

Garlic (Allium sativum L.) is a widely distributed plant, and has been used as food, spice, and traditional Chinese medicine. Garlic is reported to have antimicrobial, anti-atherosclerotic, immune-modulatory bioactivities. Its characteristic pungent flavor limited its application in food product development. Japanese scientist first, then followed by Korean scientist reported transformation production from garlic to black garlic under certain temperature and relative humidity. Black garlic tastes slightly sweet without pungent flavor, and have strong antioxidant activity and extended shelf-life. Black garlic is produced from garlic through thermal process, without adding enzymes and microorganisms. It is mis-leading to call the process a fermentation process. It is a Maillard reaction process. Here presents some recent development in studying Maillard reaction in black garlic. Maillard reaction products have been identified in black garlic. During the thermal process of black garlic production, garlic fructan degraded into oligosaccharide, fructose and glucose, which react with amino acids in garlic through Maillard reaction. 2-furoylmethyl-amino acids (2-FM-AA), including 2-furoylmethyl-lysine, 2-furoylmethyl-arginine, and 2-furoylmethyl-γ-amino butyric acid, were identified in black garlic, which are formed after the acid hydrolysis of Amadori and Heyns products. Intermediate products of 5-(hydroxymethyl)furfural (HMF) and 2-acetylypyrrole, and late stage products melanoids (brown nitrogenous polymers) were also identified. Further research would be carried out in identification of Maillard reaction products, exploration of Maillard reaction pathways, and modulation of Maillard reaction development in black garlic.

Amines and metal cations – how do they influence α-dicarbonyl formation? Sabrina Gensberger-Reigl (Andrea Auditore, Ingrid Weigel, Monika Pischetsrieder. Department of Chemistry and Pharmacy, Emil Fischer Center, Food Chemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany (sabrina.gensberger@fau.de)

The formation of reactive α-dicarbonyls during sugar degradation is well established. Heating processes or storage lead to glucose degradation products (GDPs) which can negatively influence product properties e.g. the biocompatibility of drugs. The α-dicarbonyl content of various foods as well as drugs were intensively investigated during the last decade indicating the presence of glucosone, 3-deoxyglucosone (3-DG), 3-deoxygalactosone (3-DGal), glyoxal, 3,4-dideoxyglucosone-3-ene (3,4-DGE), and methylglyoxal (MGO). The reaction mechanisms leading to these GDPs are based on dehydration or oxidation of (mono)saccharides and are highly influenced by ambient conditions such as pH value or heating temperature. It is further supposed that amines catalyse the formation of α-dicarbonyls in glucose containing matrices. Studies which verify this assumption, however, are rare. In the present study, the influence of primary amines and inorganic cations were investigated. Two different amines, glucosamine and γ-amino butyric acid (GABA), were used at different concentration levels to analyse a possible catalytic effect. Therefore glucose was heated with glucosamine or GABA respectively and the α-dicarbonyl profile was analysed. Additionally, the influence of inorganic cations on the α-dicarbonyl content is of interest, since this knowledge can be used to improve biocompatibility of drugs. Hence, the influence of eleven different cations during heat treatment of glucose was investigated. Experiments containing complexing agent diethylenetriaminepentaacetic acid (DTPA) were also conducted. Most remarkable, the presence of amines did not enhance the α-dicarbonyl formation from glucose. Using GABA no differences between control and samples could be detected. Glucosamine, however, can be degraded itself to GDPs and thus, contributed only additively and not catalytically to the total α-dicarbonyl load. These findings are in contrast to the widespread assumption that amines catalyse glucose degradation. The experiments with cations revealed three important results: (i) the addition of DTPA reduced α-dicarbonyl formation even without
added metals indicating that metal traces which are present in the raw materials enhance glucose degradation; (ii) the formation of α-dicarbonyls which are originated by dehydration processes like 3-DG, 3-DGal, 3,4-DGE are not enhanced by metal ions; (iii) the formation of glucosone, glyoxal, and MGO is highly increased in the presence of pro-oxidative cations. This was expected for glucosone and glyoxal, but not for MGO, since the formation of MGO is mainly described by a retro-aldol cleavage of glucose. Our findings, however, point out that additionally oxidation mechanism may lead to the formation of MGO.

### 3:45-4:00

**The Strecker reaction: a pathway leading to desired aroma-active compounds but also to undesired toxicologically relevant products. Michael Granvogl.** Technical University of Munich, Chair of Analytical Food Chemistry, Freising, Germany. (michael.granvogl@tum.de)

The Strecker reaction, based on the reaction of an amino acid and an α-dicarbonyl compound is well known for > 100 years leading to different reaction products. Probably the most desired ones are the so-called Strecker aldehydes eliciting pleasant aroma impressions, e.g., malty or honey-like attributes. In contrast, to the classic pathway mentioned above, also other possible formations of these aldehydes were proven, based on Amadori rearrangement products or on 3-oxazolines, a group of newly found odorless precursors releasing the aldehydes by the influence of water or human saliva during food consumption. All these pathways will be presented in the lecture based on results obtained both in model systems and in real foods. On the other side, also toxicologically relevant compounds are formed within the Strecker reaction. Thereby, acrylamide was probably the most “famous” compound during the past years. The lecture will summarize the results in regard to its formation pathway. However, a complete formation pathway starting without any carbohydrate at the beginning will be presented. The last part of the lecture will deal with recently found “Strecker amines”, which are the corresponding amines to the well-known Strecker aldehydes. These amines can act in both ways, as food odorants, for example, in chocolate, but also as compounds influencing human physiology, e.g., causing migraine attacks.

### 4:05-4:20

**Break**

### 4:20-4:35

**Novel Maillard Reaction Chemistry Chairs: M. Granvogl & D. Peterson**

**Reactivity of uronic acids in comparison to reducing sugars. Alexandra Urbisch (Lothar W. Kroh) Berlin Institute of Technology, Chair of Food Chemistry and Food Analysis, Berlin, Germany** (a.urbisch@tu-berlin.de)

Investigations in the past show the high browning potential during caramelization of sugar acids especially in comparison to reducing sugars. The heat treatment of aqueous model-systems of d-GalA for example show a ten times higher browning compared to that of d-Gal. The ring opening velocity may play an important role for understanding the drastic differences in the reaction speeds. Wegener et al. (2017) postulated that carboxyl groups influence the mutarotation velocity of carbohydrates and thus lead to an enhanced degradation. The carboxylic function present in amino acids could thus have an impact on the speed of the Maillard reaction. To investigate whether the carboxylic group of uronic acids also influences the ring opening speed polarimetric experiments where performed. These measurements show that the speed of mutarotation of d-GalA exceeds that of d-Gal by nearly 4.5 times. To examine whether the high reactivity of d-GalA goes back to its carboxylic functionality, formic acid was mixed in equal concentrations with d-Gal. The mutarotation rate constant was measured again and an enhancement of a factor of 1.7 was registered. But not only the ring opening velocity differs between the two carbohydrate structures. One other factor influencing the degradation reactions is the release of CO₂.
Experiments measuring the concentration of CO₂ during a time series of heated d-GalA at 60 °C show a steady increase. Already after 2 hours the amount starts to rise, correlating with a decrease of the d-GalA concentration. After 48 hours approximately 6% of degraded d-GalA has set free CO₂. One of the degradation reactions postulated for the release of CO₂ leads to α-ketoglutaraldehyde which is responsible for the formation of several chromophoric substances. Apart from an enhanced ring opening velocity and the release of CO₂, GC-MS investigations of aquatic model-systems of d-GalA at 130 °C show next to the typical degradation products such as norfuraneol and furfural, that are also generated during the degradation of d-Gal, the formation of 2,3-dihydroxybenzaldehyde, catechol and 3,8-dihydroxy-2-methylchromone. Model-systems of 2,3-dihydroxybenzaldehyde already show an intense colour formation after 120 minutes. These results indicate that the formation of chromophoric substances within uronic acid model-systems derive from two different pathways. One leading from the formation of caramelization reactions typical for sugar degradation and the other one resulting from oxidative polyphenolic coupling reactions that do not take place within model systems of reducing sugars.

### Extraordinary potential of 'iso-oligosaccharides' in Maillard reaction

Ondrej Novotny (Thierry DUFOSSE, Tomas DAVÍDEK). Nestlé Research Orbe, Nestec Ltd., Orbe, Switzerland. (ondrej.novotny@rdor.nestle.com)

'iso-oligosaccharides' encompassing sugar oligomers with β-→6 glycosidic bonds are found naturally in some foods, as well as being manufactured commercially. Multiple health benefits of iso-oligosaccharides have been widely reported, such as prebiotic and anticariogenic properties, impact on hormone production, lipid and carbohydrate metabolism and immune response; yet very little is known about their reactivity in Maillard reaction. Kato et al. reported that iso-oligosaccharides isomaltose and melibiose mixed with ovalbumin strongly induced brown colorization, production of fluorescent compounds, and protein polymerization, whereas maltose, cellobiose, and lactose, disaccharides with β-→4 linkage, did so very weakly. To extend our knowledge, generation of selected Maillard-derived odorants from several iso-oligosaccharides (isomaltose, isomaltotriose, isomaltulose, melibiose) was followed and compared with disaccharides having β-→4 linkage (maltose, lactose) and monosaccharides, from which they are composed of (glucose, fructose, galactose). Generation of odorants was studied in binary mixtures of sugar and amino acid (glucose or proline or cysteine) either in simple model system (heating in buffer) or in food system (wafer baking). Selected odorants were followed by HS-SPME-GC/MS/MS; quantification was accomplished by Stable Isotope Dilution Assay (SIDA). The objective of the study was to get more information about the relationship between the sugar structure and generation of selected Maillard-derived odorants, in particular the yields of odorants generated from oligosaccharides with β-→6 linkage were compared to oligosaccharides with β-→4 linkage and monosaccharides. The formation mechanisms explaining the different odorant yields will be discussed.

### Formation and Structure of Colorants formed from heterocyclic intermediates of the MAILLARD reaction.

Clemens Kanzler (Lothar W. Kroh). Technische Universität Berlin, Chair of Food Chemistry and Analytics, Berlin, Germany. (clemens.kanzler@tu-berlin.de)

Thermally induced degradation of carbohydrates in presence of amino acids or proteins during food processing contributes substantially to intended – but also to undesired – changes in functional and organoleptic properties of food items. Aroma, taste, texture, reducing potential, or complexing abilities are well studied in this context, but the formation mechanisms and structures of colorants are still widely unresolved. Considering, that the estimates intake of the colored end products of the Maillard reaction is about 10 g per day every insight into their chemistry is of importance. Early studies were conducted by Heyns and Hauber who postulated the polymerization of heterocyclic intermediates. Tressl et al. (J. Agric. Food Chem. 1998, 46, 1765–1776.995) took up this idea and could verify
a mechanism based on electrophilic aromatic substitution. Aldol condensation could be identified as important reaction step for the formation of colorants on basis of 3-deoxyglucosone by Kroh et al. (Ann. N.Y. Acad. Sci. 2008, 1126, 210–215) and heterocycles by Ledl and Severin (Lebensm. Unters. Forsch. 1978, 167, 410–413). In recent investigations our working group used heterocyclic intermediates as precursors in browning reactions under aqueous and dry conditions. The color formation in systems consisting of 4-hydroxy-2-methylfuran-3(2H)-one and furan-2-carbaldehyde was by a multiple more intense than the color formed by each compound alone. In aqueous solution the formed colorants precipitated partially indicating a low solubility in water, most probably in consequence of the elimination of water and increasing sp²-hydridization. The use of HRMS and MS-MS experiments allowed for structural elucidation of the resulting polymers. Through comparison of products obtained from furan-2-carbaldehyde and its corresponding nitrogen derivative pyrrol-2-carbaldehyde the exact linking patterns and steps in which water was eliminated were revealed. Under dry conditions the reaction is more directed and produces homogeneous polymers formed by aldol condensation and Michael addition. On the other hand, in aqueous solution the product range is more complex.

### 3 short oral poster presentations: Food chemistry (15 min)

1) 3-DG and methylglyoxal derived hydroimidazolones of creatine in Meat. **Stephanie Treibmann**

2) A novel compound isolated from the reduced ribose-tryptophan Maillard reaction products on the expression difference of mainly inflammatory factors. **Lin Li**

3) Inhibition of acrylamide by glutathione in model system and cookies. **Yuchen Zhu**

**Banquet**
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<td>8:00 - 8:20</td>
<td>Fructose-amino acid Maillard Reaction Products elicit chemoprotective activity towards ethanol-induced oxidation of Caco-2 relative resistance and transmembrane protein components. David D. Kitts (Chloe Chen). Food, Nutrition and Health. Faculty of Land and Food Systems. The University of B.C. Vancouver. (<a href="mailto:david.kitts@ubc.ca">david.kitts@ubc.ca</a>)</td>
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| 8:30 - 8:45 | Islet AGER expression associates with genes and pathways that regulate T cells in individuals diagnosed with type 1 diabetes. Sherman Leung¹, Nataliya Lenchik², Clayton Mathews³, Alberto Pugliese¹, Adam Ewing⁴, Mitchell Sullivan⁵, Mark Harris⁶, John Miles⁶, Kristen Radford⁷, Danielle Borg⁸, Ivan Gerling⁹, Josephine M Forbes¹⁰. ¹Glycation and Diabetes, Mater Research Institute - The University of Queensland (MRI-UQ), Translational Research Institute (TRI), Brisbane, Australia; ²Department of Medicine, Division of Endocrinology, University of Tennessee Health Science Center, Memphis, TN, USA; ³Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL,
The receptor for advanced glycation end products (RAGE), encoded by the AGER gene, is expressed in pancreatic islets and polymorphisms in the AGER gene confer risk for type 1 diabetes (T1D). Here, we analysed gene expression in laser captured islets procured from control (n=18; 36.0±16.16 yrs; M:F -9:9), non-diabetic autoimmune-positive (n=12; 37.21±18.24 yrs; M:F-7:5) and T1D donors (n=20; 19.97±9.67 yrs, P<0.05 vs. remaining groups; M:F-10:10), obtained from the Network for Pancreatic Organ Donors with Diabetes (nPOD) tissue bank. Analyses were targeted on a prospective selection of 118 genes, which had known association with AGER or adaptive immunity. In islets from T1D donors that were bisected based on AGER gene expression, the balance of gene expression for the remaining islet transcripts was noticeably different, where a positive relationship was observed between principal component 1 (PC1) and PC2 in AGERhi T1D donors, but the inverse was seen in their AGERlo counterparts. Using Gene Ontology of PC1, 7 significant biological processes separated AGERhi and AGERlo T1D donors (P<0.05 for all). The pathways included "positive regulation of lymphocyte proliferation", "positive regulation of T cell activation" and "interleukin-17 production". There was enrichment for processes that regulate immune function, but the ten highest ranked PC1 genes included both immunological (IL2RA, IL13, TNF, CTLA4, IL21, MMP9) and non-immunological genes (FASLG, BBC3, GCGR, IDO1). Histological assessment of pancreata from T1D donors revealed no change in the proportion of donors with insulitis, when stratified into AGERhi (80%, 8 of 10) and AGERlo (60%, 6 of 10). Changes in islet AGER gene expression did not associate with any differences in the control and autoimmune-positive donors when interrogated by PCA or Gene Ontology. Altogether, these findings suggest that islet AGER gene expression may influence genes and pathways that regulate T cells, as well as non-immunological factors implicated in the pathogenesis of T1D.

High dietary glycemic load is associated with increased levels of plasma and urinary methylglyoxal hydroimidazolones (MG-H1) in individuals with Type 2 Diabetes: The CODAM Study. Kim Maasen (Maasen, K.1,2, van Greevenbroek, M.M.1,2, van der Kallen, C.J.H.1,2, Stehouwer, C.D.A.1,2 and Schalkwijk, C.G.1,2. 1CARIM School for Cardiovascular Diseases, Maastricht University Medical Centre, Maastricht, the Netherlands. 2Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, the Netherlands. Kim.maasen@maastrichtuniversity.nl

Background: Accumulation of advanced glycation end-products (AGEs) and AGE-precursors (dicarbonyls) contributes to development of diabetic complications. We previously found increased circulating levels of the dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) after an oral glucose tolerance test and mixed meal test. Glycemic Index (GI) is a value assigned to foods based on how quickly they affect blood glucose and Glycemic Load (GL) represents carbohydrate quality and quantity in a serving of that food. In this study, we examined associations of dietary GI and GL with dicarbonyls and AGEs.

Methods: Cross-sectional analyses were performed in the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM [n=574, 25% Type 2 Diabetes (T2DM), 59±7 years]). GI and GL were derived from a Food Frequency Questionnaire. Dicarbonyls and AGEs were
measured in fasting state by UPLC-MS/MS. MGO, GO and 3-DG were measured in plasma and free forms of hydroimidazolone (MG-H1), Nε-(carboxymethyl)lysine (CML), Nε-(carboxyethyl)lysine (CEL) were measured in both plasma and urine. Protein-bound CML, CEL and pentosidine were measured in plasma. Linear regression was performed with log-transformed and standardized dicarbonyls and AGEs (dependent variables), and standardized dietary GI or GL content (main independent variables). Models were adjusted for age, sex, glucose metabolism status, BMI, smoking, physical activity, medication, kidney function, alcohol intake, and dietary fat and fiber.

**Results:** GI was not significantly associated with dicarbonyl or AGE level (-0.070<β<0.051, 0.1<p-value<0.8). GL was associated with plasma MG-H1 after age and sex adjustment (β=0.137, 95%CI [0.048;0.227], p=0.003). Statistical significance was just lost in the full model (β=0.133, 95%CI [-0.018;0.284], p=0.084). A similar positive association was observed between GL and urinary MG-H1 (β=0.168, 95%CI [-0.001;0.338], p=0.052). After stratification for glucose metabolism status, GL was most strongly associated with circulating and urinary MG-H1 in T2DM individuals (β=0.369, 95%CI [0.038;0.700], p=0.029 and β=0.367, 95%CI [0.014;0.721], p=0.042 respectively). In addition, GL was in the total cohort inversely associated with GO (β=-0.174, 95%CI [-0.327;0.021], p=0.026) and protein-bound pentosidine (β=-0.184, 95%CI [-0.335;0.033], p=0.017) in the full model.

**Conclusion:** Higher GL in the habitual diet is associated with increased levels of plasma and urinary MG-H1. These associations maintained statistical significance after adjustments for potential confounders in T2DM but not in the total cohort. The lack of association with GI suggests that dietary carbohydrate quantity rather than quality is important for the effect of diet on circulating AGEs. The fact that the most consistent and positive association was observed for MG-H1 suggests that dietary GL induces MG-H1 via transient increases in MGO.

**9:10-9:25**

Surveying the salivary AGEom - quantitation of glycation compounds in saliva and the dietary impact of an AGE-rich diet. **Friederike** (Michael Hellwig, Franziska Pietz, Thomas Henle). Food Chemistry, Technische Universität Dresden, Germany

Maillard reaction products formed of lysine and arginine play a key role as both amino acids provide nucleophilic side chains that could be easily glycated. During the last years it was shown that certain dietary glycation compounds can pass into blood and urine in a component-dependent manner and degree. Up to now, there are no investigations concerning AGEs in saliva. Saliva is a body fluid that mirrors the blood level of many compounds, among them amino acids and carbohydrates. Saliva has been gaining more attention as a diagnostic tool due to a non-invasive sampling procedure and an easy sample handling. The aim in the current study was to investigate the presence of Nε-carboxymethyllysine (CML), Nε-carboxyethyllysine (CEL), pyrraline and Nε-fructosyllysine (FrLys) as well as methylglyoxal-derived hydroimidazolinone 1 (MG-H1) in saliva and to study the influence of a dietary intake of glycation compounds on salivary concentrations. Analytical characterization was performed following an optimized sampling protocol with an LC-MS/MS system by using a multimethod to quantitate the analytes of interest. For this aim, specific target AGEs and the respective isotopologues were synthesized. We show for the first time that i) different AGEs can be detected and quantitated in saliva, ii) that a raw food diet with low amount of glycation compounds leads to a lowering of salivary AGEs level and iii) the level of some specific AGEs in saliva can be modified by the diet. This study is embedded in the EU-wide SALIVAGES project as part of the JPI ERA-HDHL project „Biomarkers for Nutrition and Health“.

**9:30-9:45**

Highly processed foods contain Advanced glycation end-products (AGEs), which have been linked to the development of diabetes complications. Diet-derived AGEs largely escape digestion and are fermented by gut bacteria, however, it is not known whether AGEs can alter gut microbial ecology leading to the progression of chronic kidney disease (CKD). This study aimed to investigate if diet-derived AGEs could worsen CKD progression and whether CKD progression could be arrested via remodelling the gut microbiome using resistant starch supplementation. These studies utilized a mouse model of type 2 diabetes, the db/db mouse, in which a low- or high-AGE (baked AIN93G) diet, plus or minus 25% resistant starch was supplied for 10 weeks. Gut microbiota composition was assessed using 16S-rRNA sequencing of cecal digesta. Transcriptomic profiling of renal cortex was determined by RNA-Sequencing. In db/db mice, consumption of a high AGE diet led to worsening of renal injury (albuminuria), enhanced intestinal permeability (determined by in vivo FITC-labelled dextran clearance) and a shift in the Firmicutes to Bacteriodetes ratio compared to low AGE-fed db/db mice. Resistant starch restored the Firmicutes to Bacteriodetes ratio and significantly reduced albuminuria. Gene set enrichment analysis showed an upregulation in the complement cascade in high AGE-fed db/db mice, which was normalized by resistant starch. These studies indicate that: (i) dietary AGEs (heat treated foods) can worsen CKD via gut microbiome remodelling and complement activation, (ii) diet therapy with resistant starch can improve renal disease progression and dampen innate immune system activation. Resistant starch may be a readily applicable therapy for protecting the kidney in individuals at risk of CKD.

Quercetin but not epicatechin decreases plasma levels of methylglyoxal in (pre)hypertensive adults; a randomized double-blind, placebo-controlled, crossover trial with pure flavonoids. Mathias D.G. Van den Eynde1,2 (Johanna M. Geleijnse3, Jean L.J.M. Scheijen1,2, Nordin M.J. Hanssen1,2, James I. Dower3, Lydia A. Afman3, Coen D.A. Stehouwer1,2, Peter C.H. Hollman3, Casper G. Schalkwijk1,2). 1 Department of Internal Medicine, Maastricht University Medical Center, Maastricht, The Netherlands; 2 Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands; 3 Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands. m.vandeneynde@maastrichtuniversity.nl

Background and aim: Methylglyoxal (MGO) is the most potent precursor in the formation of advanced glycation end-products (AGEs). MGO and AGEs have been associated with diabetes, its complications, and other age-related diseases. Experimental studies have shown that the flavonoids quercetin and epicatechin are able to scavenge MGO and lower the formation of AGEs. Data about the effects of these flavonoids on MGO and AGE levels in humans are not yet available. We therefore investigated the effect of quercetin and epicatechin on the levels of MGO and AGEs in a randomized controlled trial in (pre)hypertensive individuals.

Design: Thirty-seven apparently healthy, (pre)hypertensive, non-smoking, men and women (40-80y) were included in a randomized double-blind, placebo-controlled, crossover trial. Participants ingested (-)-epicatechin (100 mg/day), quercetin 3-glucoside (160 mg/day) or placebo capsules for periods of 4 weeks, separated by 4-week washout periods. Fasted blood samples were collected at the start and end of each intervention-period and the treatment effect was calculated. Liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was used to determine plasma levels of the dicarbonyl compounds MGO, glyoxal (GO) and 3-deoxyglucosone (3DG) and free and protein-bound AGEs. Microarray analysis and qRT-PCR were used to determine the expression of glyoxalase-1, the enzyme involved in the degradation of MGO.
Results: The treatment effect of quercetin on MGO was -40.2 nmol/L (95% CI: -73.6, -6.8; P = 0.019), a decrease of 11% from baseline values, while GO, 3DG and free- and protein-bound AGEs did not change significantly. Epicatechin did not significantly change the concentrations of dicarbonyls and free- and protein-bound AGEs. We did not find a significant change in expression of glyoxalase-1 by these flavonoids.

Conclusion: In (pre)hypertensive men and women, quercetin but not epicatechin decreased plasma MGO levels. Quercetin may potentially form a new treatment strategy for diseases in which MGO plays a pivotal role.

10:10-10:25 Break

Sources and Impact of Dietary AGEs Chairs: M. Glomb and G. Teodorowicz

10:25-10:40 Association of methylglyoxal urinary levels with visceral adipose tissue area. Claudia Luevano-Contreras. (Evelin Moreno-Vargas, Armando Gómez-Ojeda, Ma. Eugenia Garay-Sevilla, Maciste Macías-Cervantes). Department of Medical Sciences, University of Guanajuato, León, México.(c.luevanocontreras@ugto.mx)

Introduction: Obesity has been associated with dicarbonyl stress (DS) which is defined as an increased concentration of a highly reactive metabolite, methylglyoxal (MG). Urinary MG levels have been proposed as a DS marker. Studies in subjects with obesity have found a higher MG plasma concentration, and a lower amount of glyoxalase-1 in visceral adipose tissue (VAT). Therefore, VAT could have a role in dicarbonyl stress.

Objective: To compare urinary dicarbonyl metabolites, Glyoxal (GO), MGO, and metabolic risk factors in overweight adults with a VAT area ≥100 cm² vs. adults with a VAT area <100 cm².

Materials and methods: Adults 20-40 years old (n=86) with overweight (25-29.9 Kg/m²) were recruited in Central Mexico for a cross-sectional study. After consent, anthropometric measures and body composition were evaluated by bioelectrical impedance analysis (Inbody S10). Subjects were divided into two groups according to their VAT area, (group 1, <100cm² and group 2, ≥100cm²). A blood sample was taken for analysis of glucose, lipid profile, and insulin, and the homeostatic model assessment (HOMA) index was calculated to evaluate insulin resistance. GO, and MGO urine levels were quantified by high-performance liquid chromatography with a fluorescence detector, as markers of dicarbonyl stress. The t-student test was used to compare means between groups, and Pearson or Spearman tests were used for correlations.

Results: Each group included 43 subjects (40% women). Age and metabolic variables were similar for both groups, except for triglycerides. Age, glucose, cholesterol, c-HDL and HOMA-IR values were as follows for group 1: 29.7±0.7 years, 92±13 mg/dL, 181±37 mg/dL, 38±9 mg/dL, 2.7[1.8], and for group 2: 30.9±0.7 years, 96±14 mg/dL, 190±41 mg/dL, 38.9±7.4 mg/dL, 3.0[2.4] respectively. Triglycerides levels were significantly lower in group 1, 128±65 mg/dL vs. group 2, 179±98 mg/dL (p=0.006). After normalizing for creatinine, GO levels were similar in both groups. However, MGO levels were significantly lower in group 1 65.1±48.5 compared to group 2 159.5±104.4 µMGO/g Creatinine (p<0.001). Positive significant correlations for MGO were found with VAT (r=0.52), and triglycerides (r=0.26) (p<0.05).

Conclusions: Overweight adults with a VAT area ≥100 cm² had higher triglycerides and urinary MGO levels in comparison to those with lower VAT area. Furthermore, MGO levels had a positive correlation with VAT and triglycerides.

10:45-11:00 Interaction of Maillard reaction products from bovine milk with human macrophages. Gosia Teodorowicz1, (Porbahaie M.1, Hettinga K.A.1, Wichers H.J.1, van Neerven R.J.J.1,2, Savelkoul, H.F.J.1,3). 1Wageningen University, The Netherlands 2FrieslandCampina,
Background: Maillard reaction products (MRPs) present in food are linked to the increasing prevalence of diet- and inflammation-related non-communicable diseases including food allergy. Although during the last years a better understanding of immunogenicity of MRPs has been achieved, still only little is known about the structural/chemical characteristics predisposing MRPs to interact with macrophages directing the ensuing immune response. Objectives: The objective of this study was to characterize the immunogenicity of glycated bovine milk proteins and milk protein hydrolysates by determining (1) binding to selective receptors present on macrophages and (2) stimulation of secretion of pro-inflammatory cytokines by human macrophages.

Methods: Whey proteins (WP) and β-lactoglobulin (BLG), both isolated from raw bovine milk, were heated in the presence and absence of lactose (100°C, 90 minutes). Whey protein hydrolysates (WPHs) were dissolved and fractionated according to the molecular weight (MW) into the following fractions: ≥100 kDa, 10-100 kDa, 3-10 kDa and <3 kDa. The presence of carboxymethyllysine (CML) in the studied material was confirmed by dot-blot. The biological activity of MRPs was determined by its binding ability to the following receptors: soluble receptor for advanced glycation end products (sRAGE), scavenger receptor class B (CD36), scavenger receptor class A (SR-Al), and galectin-3 (Gal-3) using ELISA-based inhibition assays. Immunogenicity of the MRPs was further determined by induction of pro-inflammatory cytokines by macrophage-differentiated THP-1 cells.

Results: The WP and BLG samples heated with lactose showed binding ability to sRAGE, CD36, SR-Al, and Gal-3. The sRAGE receptor was chosen to study the effect of agglomeration on the ability to bind to receptors present on macrophages. The high MW fractions (aggregates above ≥100 kDa) of milk protein hydrolysates demonstrated the highest binding potential to sRAGE. The sRAGE binding ability was positively correlated with CML content in the fractions. The agglomerates were shown to be responsible for induction of IL-6, IL-1β and IL-8 by macrophage-differentiated THP-1 cells while this effect was not seen for the hydrolysate fractions smaller than 100kDa.

Conclusions: The results of this study indicate that the MRPs formed during processing of bovine milk may interact with macrophages via specific receptors. The MR-induced agglomerates have a high ability to bind to sRAGE accompanied by stimulation of a pro-inflammatory response in macrophages. These findings underscore the need to better understand the influence of MR on the immunogenicity of milk proteins and its role in the development of cow’s milk allergy.
ribose-5-phosphate as the donor and acceptor substrates, respectively. No glycolaldehyde of the intermediate dihydroxyethyl thiamine diphosphate was released to the reaction mixture. In contrast, when glycolaldehyde was added to the incubation the carbonyl served as an alternative acceptor molecule to give erythrulose. In parallel the formation of glycolaldehyde mediated AGEs was significantly suppressed. Second, to gain insights to the physiological situation, we expanded the investigations to human transketolase. In experiments with fructose-6-phosphate and ribose-5-phosphate in presence of glycolaldehyde and human serum albumin the formation of related advanced glycation end-products was diminished by up to 70%. Again, glycolaldehyde was enzymatically converted to erythrulose. However, this setup gave a diverse picture, as it also entailed the formation of reactive carbonyl species as erythrose-4-phosphate, which triggered significantly the formation of CML. The complex reaction pathways in human transketolase reaction were unequivocally assigned by the use of isotopically labeled sugars and mass spectrometric fragmentation experiments. Third, the situation in vivo was assessed by a LC/MS/MS method for the comprehensive detection of all major sugars involved in the transketolase reaction based on reductive amination. Quantitative analyses were validated and conducted for whole blood, plasma and red blood cells and revealed glycolaldehyde amounts up to 2 µM in the comparison of healthy versus uremic human subjects. In conclusion, we were able to identify significant changes in the sugar and sugar phosphate profile of uremic patients and to emphasize the role of the pentose phosphate pathway as a shunt for reactive intermediates. Glycolaldehyde was unequivocally established as crucial intermediate.

| 11:25-11:40 | CML in infant formula: an increased risk of food allergy. C. Delayre-Orthez²,³ (Baskara I, Joly Condette C, Anton P.M.¹,², Gay-Queheillard J.³, Niquet-Leridon- Crist, Barbezier N.¹). ¹Unité Transformations & Agro-ressources UP 2018.C103, Institut Polytechnique UNILASALLE, Beauvais, France; ²Francophone Maillard Reaction Society (FMaRS), Lille, France; ³Unité PériTox UMR I 01, Université de Picardie Jules Verne, Amiens, France. carine.delayre@unilasalle.fr |

Food allergy is an aberrant Th2 immune response to food, whose incidence has increased during the last decades. Environmental factors might be incriminated. Infants are more susceptible to these factors because of the immaturity of their immune system. Dietary Advanced glycation end-products (AGE), generated from the Maillard reaction taking place during food processing, are found in infants’ diets. The role of these contaminants on the development of allergies remains poorly studied. We decided to focus on a specific neoformed compound, the Nε-carboxymethyllysine (CML), since infant milk formulas contain up to 83 times more CML than breast milk. The purpose of the study was to determine if CML can alter the T helper (Th) cells differentiation in vitro and to study the impact of CML in a mouse model of food allergy. For the in vitro studies, human naive Th cells were cultured in the presence of different doses of CML (0 to 550 µM). Phenotypic analysis of Th2 cells was assessed by flow cytometry using specific extracellular (CD4⁺CD25⁺) and intracellular markers (IL4⁺) and by qPCR through the analysis of GATA3, the specific Th2 cell subset transcription factor. For the in vivo studies, four-weeks-old male BalbC mice were sensitized to ovalbumin (OVA) by two i.p. injections. In parallel to this sensitization, mice were orally exposed to increasing doses of CML (0 to 12.8 mg/kg/d) during 6 weeks. One week later, mice were challenged by oral administration of OVA. Allergic symptoms were evaluated by anaphylactic scores. OVA-specific IgE levels were quantified in serum. GATA3 expression in the spleen was determined by qPCR. In vitro, the highest dose of CML induced a greater proliferation of Th2 cells associated with an increase in the production of IL-4, the Th2-specific cytokine, and an overexpression of GATA3, compared to the control conditions. In vivo, CML dose-dependently increased the allergic response. Mice sensitized to OVA in the presence of 12.8 mg/kg/d of CML had higher anaphylactic scores associated with higher OVA-specific IgE levels and splenic Gata3
expression compared to sensitized mice unexposed to CML. These results show that CML, a food contaminant present in infant formulas, may increase the risk of allergy and exacerbate allergic reactions, through a proliferative effect and a possible alteration of the Th cells differentiation.

11:45-12:00
3 short oral poster presentations: Nutrition and Medicine (15 min)

1) Mingzhan Xue: Delay of cell senescence and decreased protein glycation by caloric restriction mimetic effect of sulforaphane

2) Drug library screening to identify new compounds for inducing ectodomain shedding of RAGE and formation of soluble RAGE. Shuhei Kawano

3) Jun-ichi Shirikawa: 2SC level in the peripheral blood of diabetic mice and patients with diabetic complications is significantly increased as compared with other AGEs

12:00-2:00 Lunch Discussion - Vincent Monnier

Medicine B
The AGE-RAGE axis in the kidney and nervous system Chairs: R. Inagi & Y. Yamamoto

2:00-2:15 Pattern recognition receptor RAGE: foe or friend for life? Yasuhiko Yamamoto
Department of Biochemistry and Molecular Vascular Biology Kanazawa University Graduate School of Medical Sciences, Kanazawa, Japan
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The accumulation of advanced glycation end-products (AGE) has been linked to age-related chronic diseases such as type 2 diabetes, diabetic vascular complications, neurodegenerative diseases, and cancer. Among the AGE binding receptors, the receptor for advanced glycation end-products (RAGE) is particularly important and well-characterized. RAGE belongs to the immunoglobulin superfamily and is a member of pattern-recognition receptors (PRRs) like toll-like receptors. It actively participates in the innate and adaptive immunity and inflammation. Our group reported that RAGE could directly interact with lipopolysaccharide (LPS), which resulted in the induction of intracellular NF-κB activation and TNF-α secretion, and leaded to the septic shock to an excessive degree. The elevation of serum TNF-α, IL-6, and endothelin (ET)-1 and the tissue damages induce by LPS were attenuated in RAGE-null Ager−/− mice. We also demonstrated that RAGE could bind to phosphatidylinerine and modulate apoptotic cell phagocytosis. Specifically, RAGE-deficient alveolar macrophages showed impaired phagocytosis of apoptotic thymocytes and defective clearance of apoptotic neutrophils in Ager−/− mice. Recently, we reported evidence of RAGE-mediated transport of oxytocin from the intestinal tract into the blood circulation across the intestinal barrier in mice. Oxytocin is a neuropeptide secreted from the pituitary gland into the circulation and is known to mediate both physiological and psychosocial events surrounding mammalian birth, including uterine contractions, initiation of lactation, and maternal bonding. The physiological roles of RAGE in the oxytocin transport are still under examination. These above-mentioned pathophysiological roles of RAGE will be presented and discussed.

2:20-2:35 Circulating Soluble RAGE Isoforms are Attenuated in Obese, Impaired Glucose Tolerant Individuals and are Associated with the Development of Type 2 Diabetes. Edwin R. Miranda1,2 (Vikram S. Somal1, Jacob T. Mey1, Brian K. Blackburn1, Edward Wang1, Sarah Farabi1, Kristian Karstoft1, Ciaran E. Fealy3, Sangeeta Kashyap3, John P. Kirwan3, Laurie Quinn3, Thomas P.J. Solomon8, Jacob M. Haus6,7) edwinray@umich.edu

1 Department of Kinesiology and Nutrition, University of Illinois at Chicago, Chicago, IL USA. 2 School of Kinesiology, University of Michigan, Ann Arbor, MI USA. 3 College of Applied Health Sciences, University of Illinois at Chicago, Chicago, IL USA. 4 Department of Biobehavioral Health Science, University of Illinois at Chicago, Chicago, IL USA. 5 The Centre of Inflammation and Metabolism and the Centre for Physical Activity Research,
The soluble receptor for advanced glycation end products (sRAGE) may be protective against inflammation associated with obesity and type 2 diabetes (T2DM). The aim of this study was to determine the distribution of sRAGE isoforms, and whether sRAGE isoforms are associated with risk of T2DM development in subjects spanning the glucose tolerance continuum. In this retrospective analysis, circulating total sRAGE and endogenous secretory RAGE (esRAGE) were quantified via ELISA and cleaved RAGE (cRAGE) was calculated in 274 individuals stratified by glucose tolerance status (GTS) and obesity. Group differences were probed by ANOVA and multivariate ordinal logistic regression was used to test the association between sRAGE isoform concentrations and the proportional odds of developing diabetes, versus normal glucose tolerance (NGT) or impaired glucose tolerance (IGT). When stratified by GTS, total sRAGE, cRAGE, and esRAGE were all lower with IGT and T2DM, while the ratio of cRAGE to esRAGE (cRAGE:esRAGE) was only lower (p<0.01) with T2DM compared to NGT. When stratified by GTS and obesity, cRAGE:esRAGE was higher with obesity and lower with IGT (p<0.0001) compared to lean, NGT. In ordinal logistic regression models, greater total sRAGE (odds ratio: 0.91; p<0.01) and cRAGE (odds ratio: 0.84; p<0.01) were associated with lower proportional odds of developing T2DM. Reduced values of sRAGE isoforms observed with both obesity and IGT are independently associated with greater proportional odds of developing T2DM. The mechanisms by which each respective isoform contributes to obesity and insulin resistance may reveal novel treatment strategies for diabetes.

2:40-2:55 Advanced glycation end products contribute to synaptic failure. Shirley ShiDu Yan

Abstract pending end of June

3:00-3:15 Oxidative/glycative stress, a culprit of organelle stress. Reiko Inagi. Division of CKD Pathophysiology, The University of Tokyo, Graduate School of Medicine. (inagi-npr@umin.ac.jp)

Oxidative/glycative stress is a representative causal factor of organelle damage, namely organelle stress, and influences the phenotypic changes of organ damage caused by disease or aging. For example, organelle stress in the kidney, such as decreased proteostatic activity in the endoplasmic reticulum (ER) and altered energy metabolism in mitochondria, is tightly coupled to glomerular and tubulointerstitial dysfunctions. The ER regulates protein synthesis, folding and degradation via the unfolded protein response (UPR) pathway. Pathogenic ER stress leads to dysregulation of the UPR pathway, and a defective UPR is highly deleterious to renal cell function and viability and is thereby implicated in the pathophysiology of various kidney diseases. Oxidative/glycative stress induced by mitochondrial dysfunction and associated hypoxia is well known final common pathways to end stage kidney disease as well as aging process of the kidney. Abnormal mitochondrial fatty acid metabolism also induced intracellular lipotoxicity. Furthermore, mitochondrial dysfunction is induced by abnormal cilia and aggravates polycystic kidney disease. Our studies and those by others provide a link between the UPR pathway and mitochondrial structure and function, indicating the important role of ER in the maintenance of mitochondrial homeostasis. For example, ER Stress delivers stress response signals to the nucleus via UPR pathway (ER-nucleus crosstalk). ER also regulates fusion and fission of mitochondria and maintains dynamic mitochondrial network (ER-mitochondrial crosstalk).
Restoration of normal ER and mitochondrial function, therefore, holds promise in protecting the kidney from pathogenic stresses as well as ageing. This talk will summarize the molecular mechanisms and pathophysiological roles of organelle crosstalk in kidney disease.

**Analysis of Macromolecular Damage in Diabetic Nephropathy by Imaging Mass Spectrometry.** Paul A. Voziyan1,3,6 (Kerri J. Grove1,5,6, Jeffrey M. Spraggins1,5, Suwan Wang2,5, Paisit Paueksakon6, Raymond C. Harris2,3,6, Billy G. Hudson1,2,3,6, and Richard M. Caprioli1,3,5 (paul.voziyan@vanderbilt.edu)

1Department of Biochemistry, 2Division of Nephrology, 3Department of Medicine, 4Department of Pathology, Microbiology, and Immunology, 5Mass Spectrometry Research Center, and 6Center for Matrix Biology, Vanderbilt University Medical Center, Nashville, TN.

Diabetic nephropathy (DN) leads to a progressive decline in renal function and is the leading cause of end-stage renal disease. One of the mechanisms underlying DN is thought to be renal accumulation of oxidative and glycoxidative post-translational modifications (PTMs), which can cause macromolecular damage. We employed high spatial resolution matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) to determine macromolecular changes in kidneys of eNOS-/- db/db mice, a model of DN. Protein and lipid PTMs, localization patterns, and relative tissue levels were determined in individual renal glomeruli and tubules without disturbing tissue morphology. MALDI TOF protein imaging at ≤ 25 µm and lipid imaging at ≤ 10 µm produced unique ion patterns localizing to the 90 µm glomeruli and 20 µm tubules. The data analysis was facilitated by computationally scanning the FTMS spectra (>5000 peaks) for mass differences of interest between any two
peaks with a tolerance of ≤5 ppm. MALDI images were correlated to tissue histology by overlaying PAS stained and MS images of the same section. A number of protein peaks showed unique localization in DN vs. non-diabetic control groups. The several peaks localized to glomeruli of only the DN mice and had mass shifts consistent with the presence of the carboxymethyllysine and carboxyethyllysine modifications in the DN glomeruli. These peaks were significantly decreased in the DN mice treated with PM, a drug candidate that showed promise in DN clinical trials. Seven Amadori-modified phosphatidylethanolamine (PE) species were found in the cortex of DN kidney using the delta search and confirmed with MS/MS. The most abundant species included Amadori-PE (40:8), Amadori-PE (36:4), and Amadori-PE (36:3). No PE modifications were found in the control tissue. Additionally, high spatial resolution IMS (5 µm) revealed unique lipid expression in renal glomeruli and tubules. Significant increase in the levels of specific glomerular and tubular lipid species from four different classes, i.e. gangliosides, sulfoglycosphingolipids, lysophospholipids, and phosphatidylethanolamines was detected in DN kidneys compared to non-diabetic controls. High-resolution IMS is a powerful tool to detect macromolecular damage associated with DN in individual glomeruli and tubules in the intact tissue and correlate it with renal pathology.

### 4:00-4:15 Break

### 4:15-4:30 Cellular mechanisms of AGE formation Chair: Paul J. Voziiyan

DNA-fructosamine-6-phosphate amadoriase activity of the glycolytic enzyme phosphoglucose isomerase Roumyana Mironova¹, Elitsa Boteva, Konstantin Doychev¹, Vasilena Georgieva¹, Yordan Handzhiyski¹, Evan Gatev², Monika Pischetsrieder³.

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¹Department of Gene Regulation, Institute of Molecular Biology “Roumen Tsanev”, Bulgarian Academy of Sciences, Sofia, Bulgaria. ²Department of Medical Genetics, University of British Columbia, Vancouver, Canada. ³Department of Chemistry and Pharmacy, Faculty of Sciences, Friedrich Alexander University Erlangen-Nuremberg, Erlangen, Germany.

The Maillard reaction has been demonstrated to affect proteins and nucleic acids under normal physiological conditions in both pro- and eukaryotes. Although enzymes (amadoriases) for “repair” of amino acids and proteins modified with Amadori products (APs) have been discovered in a broad range of organisms, mechanisms for repair of APs-lesions in DNA have not been reported yet. In this study, we provide evidence that the glycolytic enzyme phosphoglucose isomerase (PGI) of E. coli and type III yeast PGI catalyze the breakdown of DNA-fructosamine-6-phosphate (DNA-N-Fr6Ph) to DNA and glucose-6-phosphate. We further demonstrate that an E. coli PGI deficient strain exhibits higher spontaneous mutation rate to rifampicin resistance than the PGI proficient ancestral strain. These data specify PGI as DNA-N-Fr6Ph amadoriase (deglycase) and a novel DNA repair enzyme.

### 4:35-4:50 Intracellular accumulation of advanced glycation end products caused by glycolaldehyde induces murine osteoblastic cell apoptosis via the endoplasmic reticulum stress pathway. Ryusuke Suzuki¹,² (Mitsuru Saito¹, Shoutaro Arakawa³,⁴, Jun-ichi Shirakawa³, Miyu Taniguchi³, Yukio Fujiwara³, Ryoji Nagai³, Keishi Marumo⁴)

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It is well known that the accumulation of AGEs in bone matrix directly deteriorates bone strength and induces harmful effects on bone formation by suppressing osteoblast functions. However, it is not clear whether intracellular AGE accumulation affect osteoblastic functions. We have developed the detection system of AGEs in bone cells accurately by LC-MS/MS. The purpose of the present study is to clarify the effect of intracellular AGE accumulation on ER stress and apoptosis in murine osteoblastic MC3T3-E1 cells. Intracellular AGEs was enhanced by incubating the MC3T3-E1 cells with glycolaldehyde (GA), a precursor for AGEs formation and the relationship between intracellular accumulation of AGEs and apoptosis was studied in vitro.

Methods: MC3T3-E1 cells were cultured in α-MEM medium with 10% fetal bovine serum. Cells were treated with 0-500 µM of GA for 8-24 hours after reaching 80% confluence. The AGEs contents in GA-treated MC3T3-E1 cells was measured by LC-MS/MS and immunochemical staining. Next, the viability of MC3T3-E1 cells was evaluated by WST-8 assay. The ER stress and apoptosis were measured by real-time PCR and western blot analysis.

Result: CML content showed the most significant increase among other AGEs in a dose dependent-manner and GA-pyridine was detected in 500 µM GA-treated MC3T3-E1 cells by LC-MS/MS. In immunochemical staining, anti-CML antibody reacted with GA-treated MC3T3-E1 cells. The viability of MC3T3-E1 cells was significantly reduced in a concentration dependent-manner. Real-time PCR analysis showed that the mRNA expression of glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP) was significantly increased by incubation with 500 µM GA. Western blot analysis showed that GA induced the phosphorylation of eukaryotic initiation factor 2 (eIF2α) and increased cleaved caspase3 protein levels.

Conclusion: GA induced the remarkable accumulation of AGEs, especially CML and GA-pyridine. The present study demonstrated that intracellular accumulation of AGEs in osteoblastic cells induced apoptosis via ER stress. Since AGEs are accumulated in accordance with aging and pathogenesis of diabetes, this study provides the possibility that the enhanced accumulation of AGEs in bones decreases the bone formation and results in the induction of bone fragility such as osteoporosis.

Excursion
Physiological relevance of Dietary Advanced Glycosylation End-Products (dAGEs) and Melanoidins Formed through the Maillard Reaction. Vincenzo Fogliano Food Quality & Design group, Wageningen University, The Netherlands. (vincenzo.fogliano@wur.nl)

The consumption of food rich in melanoidins and dietary advanced glycosylation end-products (dAGEs) is harmful or beneficial for human health? More than 100 years after the Maillard Reaction discovery this is still an unanswered burning question. There are conflicting results on their harmful effects in the literature, partly due to a methodological issue in how dAGEs are determined in food. Melanoidins have positive functions particularly within the gastrointestinal tract, whereas the intake of dAGEs has controversial physiological consequences. Most of the in vivo intervention trials were done comparing boiled versus roasted diet (low and high dAGE, respectively). However, these studies can be biased by different lipid oxidation and by different calorie density of foods in the two conditions. The restriction of dAGEs has been proposed as a strategy for alleviating complications of diabetes and metabolic syndrome and, in general, as important for a healthy lifestyle. Although this is plausible, further efforts must be taken to discover solid evidence to elucidate the mechanisms. Evidence suggests that the damages produced by the heavily processed foods used in the Western diet could be due to the increased intake of some dAGEs but also to other concomitant factors. Food technologists and food industries should be aware that the current processing and formulation strategies lead to a high concentration of melanoidins and dAGEs in foods with high energy density and whose consumption is harmful for human health. For this reason, observational studies cannot disentangle the specific physiological effects of melanoidins and dAGEs from the general negative effects linked to heavily processed, refined, calorie-dense foods. The attraction that humans have to cooked foods is linked to the benefits they have had during humankind's evolution. The goal for food technologists is to design low-energy-dense products that can satisfy humans' attraction to rewarding cooked foods.
Protein glycation in plants as a new aspect in Maillard reaction research – possible role in ageing and stress response. Andrej Frolov, 1 Tatiana Bilova, 1,2 Christian Ihling, 3 Tatiana Mamontova, 1,4 Ahyoung Kim, 1 Gagan Paudel, 1 Elena Lukasheva, 1,4 Nadezhda Frolova, 5 Veronika Chantzeva, 2 Claudia Birkemeyer, 5 Galina Smolikova, 2 Sergei Medvedev, 2 Gerd U. Balcke, 6 Thomas Vogt, 6 Alain Tissier, 6 Andrea Sinz, 3 Wolfgang Brandt, 1 Ludger Wessjohann 1

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Glycation is a post-translational modification of free protein amine and guanidine groups with carbonyl compounds. The occurrence of advanced glycation end products (AGEs) during thermal processing of foods is well established. Besides, AGEs are the recognized markers of ageing, and are associated with metabolic and neurodegenerative disorders (e.g. diabetes mellitus and Alzheimer disease) in mammals. During the last decade, AGEs were identified in exhaustive hydrolysates of plant proteins. We present here a comprehensive characterization of the constitutively glycated plant proteome, which was shown to be significantly different from the mammalian proteome [1]. Thereby, we employ an integrated approach, relying on a combination of metabolomics and proteomics methods implementing model peptide-based glycation systems. We demonstrate that ageing of plants is accompanied by the accumulation of glycation products in leaves, seeds and even legume root nodules [2]. These site-specific glycation hotspots indicate a high site specificity of the protein Maillard reaction. Finally, plant experiments with light, drought, heavy metal and gravitational stresses revealed pronounced effects on the patterns of protein glycation both on the qualitative and quantitative levels [3]. Implementation of metabolite profiling and model peptide systems gave access to a direct interpretation of protein AGE patterns in the context of glycation potential of individual metabolites. However, despite relatively high levels of protein glycation, the biological role of this phenomenon in plants still needs to be comprehensively addressed.

Microbial metabolism of glycated amino acids. Michael Hellwig, (Marie Börner, Falco Beer, Tom Boenke, Thomas Henle), Food Chemistry, Technische Universität Dresden, Germany. Michael.Hellwig@tu-dresden.de

Maillard reaction products (MRPs) originating from malt are decisive for the color and aroma of beer. Glycated amino acids such as maltulosyllysine, pyrraline, and MG-H1 have been quantitated in beer in substantial amounts, but little is known about a possible fermentation of individual glycated amino acids by brewer’s yeast (Saccharomyces cerevisiae). Specific microbial metabolites of glycated amino acids have not been known up to now. Individual MRPs (fructosyllysine, maltulosyllysine, pyrraline, formyline, maltosine, and MG-H1) were incubated in the presence of S. cerevisiae in different media, and the stability of the compounds was measured by amino acid analysis and HPLC with UV and MS/MS detections. Metabolites were identified by RP-HPLC-DAD-MS/MS and selected compounds were synthesized independently. In the presence of S. cerevisiae, free glycated amino acids except formyline turned out to be stable in a nutrient-rich medium. Formyline was degraded by 10% to the corresponding α-hydroxy acid. The degradation was dependent on the medium used for yeast cultivation and was strongly enhanced in
minimal medium. When glycated dipeptides were applied instead of amino acids, nearly complete transformation of the MRPs to the respective α-hydroxy acids and higher alcohols was observed. The novel higher alcohols pyrralinol (up to 200 µg/L) and formylinol (up to 50 µg/L) were quantitated in beer for the first time. We conclude that glycated amino acids are metabolized by *S. cerevisiae* through the Ehrlich pathway, a metabolic route important for the formation of key aroma compounds such as phenylethanol and 3-methylbutanol. Glycation of malt protein might therefore have an impact on the formation of these aroma compounds in beer. Interaction of glycated peptides with transport systems might also influence yeast nutrient supply and intracellular signaling.

9:0-9:15

**The inhibitory effect of procyanidins on advanced glycation end products (AGEs) formation in alcohol medium and corresponding mechanisms.** Qian Wu, (Chao Wang), Hubei University of Technology, Wuhan, Hubei, China (qianwill2007@163.com;521035287@qq.com)

Alcoholic beverages can accumulate a large amount of advanced glycation end products (AGEs) during storage and cause food safety problems. Previous studies have shown that procyanidins have a good inhibitory effect on AGEs formation in aqueous solution. However, the effects of alcohol on the formation of AGEs and the inhibition mechanisms of procyanidins in alcoholic medium are still unclear, which make it more difficult to effectively use procyanidins in alcoholic beverages. This project was carried out to investigate the effect of alcohol on fluorescent AGEs and CML formation in model system and beer at 50 °C for 5 days. The inhibition mechanisms of (+)-catechin (CC), (-)-epicatechin (EC) on AGEs formation in alcohol medium were studied as well. The results showed that alcohol promoted AGEs formation and decreased inhibitory abilities of CC and EC significantly (*P* < 0.05). CC and EC could effectively inhibit fluorescent AGEs and CML formation, which were better than the positive control aminoguanidine (AG). The corresponding mechanisms included trapping α-dicarbonyl compounds and forming stable adducts, scavenging free radicals and inhibiting enzymes activities. This study will provide an important basis and theoretical foundation to develop procyanidins as natural AGEs inhibitors in alcoholic beverages.

9:20-9:35

**The Impact of Glycation on the Fatty Acid Binding Capacity of Human Serum Albumin.** Christian Henning² (Christine Stübner², Dariush Hinderberger², Matthias Girndt¹, Roman Fiedler³, Marcus A. Glomb⁴). Institute of Chemistry – Food Chemistry, ¹Institute of Chemistry – Physical Chemistry, ²Department of Internal Medicine II; Martin-Luther-University Halle-Wittenberg, Halle/ Saale, Germany. (christian.henning@chemie.uni-halle.de)

Human serum albumin (HSA), the most abundant protein in blood, is known as an important carrier protein. Binding to HSA enables distribution and availability of various compounds, e.g. pharmaceuticals or long-chained fatty acids, well beyond their solubility in plasma. In the presence of reducing sugars, degradation products thereof and of other reactive carbonyl compounds derived from various pathways, HSA is prone to non-enzymatic chemical alterations and subsequent changes in biological function. Glyoxal is an reactive α-dicarbonyl compound of quantitative importance in vivo. Formed by lipid peroxidation, DNA oxidation, sugar autoxidation and oxidative degradation of glycated proteins, it acts as a potent precursor compound for advanced glycation endproducts. In the present study, HSA was modified with glyoxal under physiological conditions. Reversibly albumin-bound glyoxal was removed by a trapping reaction with free arginine. All relevant advanced glycation endproducts were quantitated by LC-MS/MS. The impact of such post-translational HSA alterations on the transport capacity of long-chained fatty acids was characterized by spin-labelling and continuous-wave electron
paramagnetic resonance spectroscopy. Native HSA possesses a maximum of seven distinct binding sites. With an increasing degree of glycation the results indicated a significant loss of maximal binding sites per protein and a clear change of dissociation constants. Tryptic peptide mapping enabled us to relate these findings to molecular changes at specific binding sites. Further investigation of plasma protein samples of uremic patients as a model for higher glycated HSA vs. healthy subjects gave first insights into the in vivo situation. In conclusion, we were able to link post-translational HSA modification expressed as a well-defined set of AGEs to a specific biological function, the ligand binding capacity. Glycation significantly impaired the fatty acid binding capacity of HSA and thus may cause adverse effects in certain diseases like diabetes or uremia.

| 9:40-9:55 | Comparative evaluation of three different ELISA assays and HPLC-ESI-ITMS/MS for the analysis of \( \text{N}^\varepsilon \)-carboxymethyl lysine in food samples. Armando Gómez-Ojeda\(^a\) (Sarahi Jaramillo-Ortiz\(^b\), Katarzyna Wrobel\(^b\), Kazimierz Wrobel\(^b\) Gloria Barbosa-Sabanero\(^c\), Claudia Luevano-Contreras\(^d\), Maria Pia de la Maza\(^e\), Jaime Uribarri\(^f\), Ma. Dolores del Castillo\(^g\), Ma. Eugenia Garay-Sevilla\(^g\)). \(^a\) Department of Medical Science, University of Guanajuato, Col. Obregón Leon, Guanajuato, Mexico. \(^b\) Department of Chemistry, University of Guanajuato, Centro Guanajuato, Gto., Guanajuato, Mexico. \(^c\) University of Chile, Santiago, Chile. \(^d\) Department of Medicine, The Icahn School of Medicine at Mount Sinai, New York, NY, USA. \(^e\) Food Bioscience Group, Department of Food Analysis and Bioactivity, Institute of Food Science, Research (CIAL), Spanish National Research Council (CSIC), Madrid, Spain. (armando.gomez@ugto.mx)

Advanced glycation end products (AGE) research began at the very beginning of Maillard reaction discovery a century ago, AGE generation in food leads to many different compounds formation, among them \( \text{N}^\varepsilon \)-carboxymethyl lysine (CML). With the endogenous glycation confirmation some decades after the importance of AGE formation in clinical research significantly increase. AGE accumulation in the body has been implicated in the pathogenesis of diseases such as diabetes and cardiovascular disease. Exogenous AGE consumption contributes to body AGE pool, several clinical studies have shown that high consumption of dietary AGEs (dAGEs) is associated with indicators of oxidative stress and inflammation, which may play an important role in causing chronic disease\(^5\), high vs. low-CML diets have been shown to increase levels of serum AGEs, inflammation markers, endothelial dysfunction, and to impair flow-mediated dilation and insulin sensitivity; however the published results on the toxic effects of dAGEs remain controversial. Due to this, it is mandatory to have accurate and easily reproducible standard methods for their quantification in both foods and biological samples, as a first step to elucidate if dAGE are a risk factor for human health. Current research on exogenous advanced glycation end products (AGE) has been carried out mostly on \( \text{N}^\varepsilon \)-carboxymethyl-lysine (CML) determination as a feasible chemical indicator for AGE formation in food, in this regard there is a controversy concerning the most convenient yet reliable method(s) for this task. The purpose of this work was to compare CML determination by three different ELISA assays methods and HPLC-ESI-ITMS/MS in several food items. Among food items tested all four methods showed the same decreasing order in CML concentration: beef, bacon>chicken > fish>dairy products>grain products>fruits/vegetables. Small differences of CML concentrations in food items prepared by different culinary treatment were clearly distinguished by HPLC-ESI-ITMS/MS but these small differences could not always be detected by ELISA. This work demonstrates a reasonable relationship between CML determined by ELISA and HPLC-ESI-ITMS/MS and therefore supports the implementation of ELISA in food CML/AGEs screening.
Reactions of common thermal process contaminants in foods during digestion. Vural Gökmen (B. Aytül Hamzalioğlu). Food Quality and Safety (FoQuS) Research Group, Department of Food Engineering, Hacettepe University, Ankara, Turkey. (vgokmen@hacettepe.edu.tr)

Ingestion of food is considered as the major route of exposure to many contaminants in human health risk assessment. Besides, total amount of a contaminant found in the ingested food does not always reflect the amount that is available to the body. Therefore, determination of the bioaccessibility of a thermal processing contaminant from the matrix, and the fate of ingested processing contaminant during digestion is an important issue for human health. This presentation describes the fate of acrylamide, HMF and dicarbonyl compounds in bakery and fried potato products during in vitro multistep enzymatic digestion process simulating gastric, duodenal and colon phases. The results revealed a significant reduction in the concentrations of acrylamide, HMF and dicarbonyl compounds at the end of digestion. Binary model systems composed of target processing contaminants and amino acids were used to understand the mechanism of their reduction. High-resolution mass spectrometry (HRMS) analyses confirmed Michael type addition of amino acids to acrylamide, HMF and dicarbonyl compounds under simulated digestion conditions. Due to their elimination and formation potential during in vitro digestion process, levels of acrylamide, HMF and dicarbonyl compounds ingested with foods may not directly indicate their absorption rate through gastric, duodenal and colon.

Contribution of glucose and selected amino acids to the formation of α-diketones upon wafer baking. Tomas Davidek*, (Thierry DUFOSSE*, Ondrej NOVOTNY* and Imre Blank**). *Nestec Ltd., Nestlé Research Orbe, Orbe, Switzerland, **Nestec Ltd., Nestle Research Center, Lausanne, Switzerland. tomas.davidek@rdor.nestle.com

The formation pathways of buttery smelling α-diketones from reducing sugars, namely that of 2,3-butanedione, have been the focus of many studies. The majority of these studies were performed in model systems applying aqueous, dry heated and even pyrolytic conditions. The results obtained have shown that formation pathways strongly depend on the reaction parameters such as moisture, temperature, pH and type of amino acid. Contrary to model systems, much less data are available concerning the formation mechanism of α-diketones in real food systems. The formation of α-diketones upon extrusion cooking and upon coffee roasting can be cited as examples. To extend our knowledge regarding real food systems, the generation of 2,3-butanedione and 2,3-pentanedione from glucose and selected amino acids (glycine, alanine, serine and threonine) was studied upon wafer baking. Several labelling experiments were performed employing \(^{13}C_6\)-glucose or \([U-^{13}C]\)-amino acids and the generated α-diketones were analysed by gas chromatography/mass spectrometry. Based on the labelling patterns obtained, the reaction pathways of 2,3-butanedione and 2,3-pentanedione upon wafer baking will be discussed and compared to those observed upon extrusion of cereals and coffee roasting.
Multiresponse kinetic modelling of the formation of α-dicarbonyl compounds in peach nectars. İşıl Gürsul Aktağ (Vural Gökmen)
Hacettepe University, Ankara, Turkey. (isilgrsl@gmail.com)

The α-dicarbonyl compounds (DCs), mainly formed from sugars through caramelisation or Maillard reaction during food processing and storage, are significant precursors of advanced glycation endproducts (AGEs). The aim of this study is to investigate the effect of storage on the formation of DCs in peach nectar based on a multi-response kinetic modeling approach. For this purpose, peach nectars were prepared to simulate the industrial procedure and stored 20 weeks at 4°C, 26°C, and 37°C. Changes in the concentrations of sucrose, glucose, fructose, amino acids, 3-deoxyglucosone (3-DG), glucosone (G), glyoxal (GO), threosone and 5-hydroxymethylfurfural (HMF) were monitored by liquid chromatography and mass spectrometry techniques. To understand the predominant reaction mechanism during storage, a comprehensive reaction equation was generated and the equations were figured out by a mathematical model with setting up differential equations for each reaction steps. To solve the equations and define the reaction rate constants, experimentally observed data was fitted to mathematical model through Athena Visual Studio software version 14.2. The results suggested that free amino acid concentrations showed no change at all temperatures while sucrose decreased and glucose, fructose increased dramatically during storage at 37°C. From this point of view, caramelisation and acid hydrolysis of sucrose were determined as the main chemical reaction responsible for DCs formation. On the other hand, the most abundant DCs in peach nectar are G and 3-DG. At the beginning, G concentration (6.80 mg/L) was found higher than 3-DG (1.59 mg/L), however, towards the end of storage, 3-DG formation increased more than G. The reason for that could be the fragmentation of G to GO via retroaldolisation, since the results showed that GO was determined as well as there was no methylglyoxal as degradation product of 3-DG. The maximum concentration of 3-DG was reported as 71.46 mg/L at 37°C after 14 weeks of storage. In addition, there was no HMF formation at 4 and 26°C temperatures, whereas it increased with storage at 37°C. With this regard, the contribution of 3-DG dehydration on the formation of HMF was found to be more than fructose dehydration through fructofuranosyl cation. Multi-response kinetic modeling is a highly powerful approach to unravel the sophisticated reaction mechanisms in foods. The use of multi-response kinetic modeling in this study provides understanding the most possible pathway of sugar degradation and the fate of the key intermediate DCs as a precursor of AGEs from the point of quality and safety issues in peach nectars.

Understanding changes in aroma generation in model biscuits where inorganic salts have been used to mitigate acrylamide formation. Jane K. Parker (Neslihan Taş, Tolghahan Kocadağlı, Dimitrios P. Balagiannis, Vural Gökmen).
Food Engineering Department, Hacettepe University, Beytepe Campus, Ankara, Turkey. Department of Food and Nutritional Sciences, University of Reading, Whiteknights, Reading, UK. (j.k.parker@reading.ac.uk)

Since the Maillard reaction is the root of both flavour generation and acrylamide formation, a reduction in acrylamide is often associated with loss of flavour. The influence of inorganic salts such as NaCl, KCl and CaCl₂ on the Maillard reaction during food processing has been widely studied, often with regard to mitigation of acrylamide, where the Ca²⁺ ion in particular is known to suppress acrylamide formation via a number of proposed mechanisms. Although in such systems acrylamide, colour, and reactive intermediates have been studied in detail, far less
is known about the inevitable and concomitant changes in the volatile profile, and the impact of these changes on the aroma of real food systems. A much better understanding of the relationship between acrylamide mitigation and volatile formation is required in order to control flavour formation in, for example, low acrylamide baked goods. In this work, a low moisture model dough system comprising wheat flour, glucose and an inorganic salt (NaCl, KCl, CaCl$_2$ or calcium lactate) was heated at different time and temperature combinations and compared to a control with no added salt. Sugars, free amino acids, reactive intermediates, acrylamide and up to 40 volatile aroma compounds (predominantly Maillard reaction products) were fully quantified, and a further 100 aroma compounds were identified and semi-quantified against a single internal standard. GC-Olfactometry was used to determine which of these aroma compounds might be important for flavour, but many other volatiles were also monitored in order to elucidate some of the mechanistic changes in the presence of these salts. Pyrazines, Strecker aldehydes and acrylamide all decreased significantly when CaCl$_2$ was added, indicating a loss of key aroma compounds. In contrast, 2-furfural, HMF, 2-acetylfuran and other related compounds increased significantly when CaCl$_2$ was added. Interestingly, 2-furanmethanol and a group of furans significantly increased when calcium lactate was added, and other volatiles such as maltol, furaneol, 2-acetylpyrrole and 2-acetyl-1,4,5,6-tetrahydropyridine, which are particularly relevant for baked notes, followed a similar pattern. These changes in aroma compounds are not related to pH and will be discussed in terms of three key pathways: Maillard reaction proceeding through 1,2-enolisation of the Amadori Rearrangement Product, Maillard reaction proceeding through 2,3-enolisation, and caramelisation. Finally, we will look at possible routes to acrylamide formation and present a holistic mechanistic picture incorporating the formation of acrylamide, HMF and key aroma compounds.

11:35-11:50 The impact of coffee roasting profile on freshness markers in coffee aroma – towards a universal method to quantify coffee freshness. Anja RAHN (Samo SMRKE, Marco WELLINGER, Chahan YERETZIAN). Zurich University of Applied Sciences, Coffee Excellence Centre, Wadenswil, Switzerland. (samo.smrke@zhaw.ch).

The recent growing demand for high quality specialty coffee puts focus on the core value of specialty coffee, the freshness of coffee. Past studies have found that there are three major factors that contribute to loss of freshness and degradation of coffee aroma: temperature, humidity and oxygen. Coffee is inherently an unstable product and will inevitably become stale during storage. The perceived loss of quality is a consequence of complex chemical processes that lead to aroma degradation, either by loss of aroma by desorption from coffee matrix and degradation by oxidation, or through formation of new compounds. There are currently two approaches how to quantify coffee staleness: (i) either by identifying molecules that are lost or formed during storage and that negatively affect the sensory properties of coffee or (ii) through the definition of freshness indices. The freshness indices were introduced as a simple and robust indicator for assessing coffee freshness. An index that is often applied for monitoring freshness of coffee during storage is the ratio between the GC/MS intensities of the two molecules: methanethiol (MeSH) and dimethyl disulfide (DMDS). These molecules are chosen because MeSH is highly volatile and unstable in oxidative environment and forms DMDS through oxidation. This behaviour makes the MeSH/DMDS ratio very sensitive to temperature and the presence of oxygen during coffee storage. The formation (during roasting) and degradation (during storage) of the compounds included in such freshness indices are not completely understood. The generation
of coffee aroma compounds during roasting through the Maillard reaction is
dependent on various parameters, such as roasting time/temperature profile,
moisture content, and initial composition of green coffee beans. The rate of
formation of molecules used for freshness indices is impacted by these parameters
and the indices have not yet been validated for use as a general tool to measure
coffee freshness. Usually, a freshness index is applied on one particular coffee
(variety or blend), roasted with a defined roasting profile. In this contribution we
explored how modifying the roasting profile changes the resulting freshness
indices and searched for a freshness index that is not impacted by the roasting
profiles. GC/MS analysis of a coffee roasted along four different roasting profiles
was performed and different freshness ratios were evaluated. Furthermore,
statistical analysis was performed by means of machine learning to test if it is
possible to predict coffee freshness independent of roast profile, based on GC/MS
aroma analysis.

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<th>12:00-2:00 Lunch Discussion</th>
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<tr>
<td><strong>Nutrition B</strong></td>
<td><strong>Sources, transit, modulation and impact of dietary glycation products. Chairs: Frédéric J. Tessier &amp; Eric Boulanger</strong></td>
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<td>2:00-2:15</td>
<td><strong>Metabolic Transit of Maillard Reaction Products in Cattle. Thomas Hofmann, (A. Kahler, T. Henle. Food Chemistry, Technische Universität Dresden, Dresden, Germany. [<a href="mailto:thomas.hofmann4@tu-dresden.de">thomas.hofmann4@tu-dresden.de</a>])</strong></td>
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Recently, Schwarzenbolz et al. (J. Agric. Food Chem., 2016, 64, 5071–5078) were
able to show that organic and conventionally produced milk can be distinguished
via analysis of free Maillard Reaction Products (MRPs). This observation is based on
the different feeding regimes of the individual husbandry of cattle. For the
production of organic milk, the higher intake of graze by the cows results in a
reduction in the use of highly processed concentrate feed, while conventional cow
husbandry uses mainly concentrate feed due to the high energy requirements of
the animals. This variation in the different feeding regimes affects the levels of free
MRPs in milk, so that estimations can be made concerning the "MRP supply" via
feed. In this context, the analysis of free MRPs in milk can contribute greatly to the
control of food authenticity (organic/conventional food). In the current study, the
metabolic transit of MRP in dairy cows was investigated. For this purpose, feed and
metabolic products such as milk, faeces and urine from several feeding studies with
two sets of 30 cows were analysed. Using LC-MS/MS and isotopically labeled
standard substances we quantitated MRPs, e.g. CML, pyrraline and MG-H1 in feed
(after enzymatic hydrolysis) and also in milk, faeces and urine of the respective test
groups. Approximately 22% of pyrraline ingested with food and 34% of ingested
MG-H1 could be found in urine and a correlation between ingestion through feed
and release through urine could be observed. In the milk samples, only about 1.3%
of the absorbed pyrraline and 0.2% of MG-H1 were found, but a correlation
between intake via the feed and release into the milk can also be assumed.
Excretion of CML via milk and urine appears to be independent of uptake via feed.
These findings on the metabolic transit of MRPs in feed into cow’s milk allow precise
statements on the feeding of processed (pyrraline- and MG-H1-rich) feed and thus
information on the species-appropriate feeding of cows.

| 2:20-2:35 | **Evaluation of the ingestion and digestion of carboxymethyl-modified albumin by Caenorhabditis elegans. Chantal FRADIN1,2 (Charles PAUL-CONSTANT1,2, Rachel LITKE1,2, Axel GUILBAUD1,2, Michael HOWSAM1,2, Frédéric J. TESSIER1,2, Eric BOULANGER1,2). 1University of Lille, Inserm, CHU Lille, U995 - LIRIC – Lille** |


Context and aim – Carboxymethyllysine (CML)-modified proteins are believed to be more difficult to digest than non-glycated proteins, resulting in a lower absorption rate of their amino acids and modified lysines. In this regard, it might be difficult to properly assess the role of dietary advanced glycation end-products in health. The aim of our study was to compare the in vivo digestibility of a protein, the human serum albumin (HSA), according to its glycation status. Caenorhabditis elegans was chosen to perform this analysis in order to start investigating the effect of dietary CML in aging.

Methods – HSA was incubated with glyoxalic acid (60mM) to produce CML-modified HSA (CML-HSA). Non-modified HSA (Ctrl-HSA) was used as control. The laboratory strain of C. elegans (N2) was used throughout the study. N2 worms were cultured at 20°C during various times in liquid medium (S-medium) containing their bacterial food (Escherichia coli strain OP50) supplemented with CML-HSA or Ctrl-HSA (3 to 50µM of proteins corresponding to 0.06 to 1mM of CML for CML-HSA). After efficient washes to remove the components of the culture medium, worms were homogenized by bead-beating. Peptide-bound CML and HSA recovered from soluble and insoluble worm extracts were detected by western blot analysis with specific antibodies. Free CML was measured in worm extracts by LC-MS/MS. In addition, C. elegans lifespan was measured in presence of CML-HSA and Ctrl-HSA.

Results and discussion – CML-HSA and Ctrl-HSA seemed to be equally ingested by the worms. Non-digested and fragments of digested HSA were detected in the soluble extracts from worms incubated with either CML-HSA or Ctrl-HSA. Fragments of digested CML-HSA carried CML epitopes. The ingestion levels of both proteins were higher during the early adulthood of the worms. Free CML was mainly detected in worms incubated with CML-HSA, with a plateau rapidly reached; suggesting a successful absorption of this modified amino acid. Surprisingly, a small amount of endogenous CML was detected in older worms incubated with Ctrl-HSA, demonstrating that the source of CML recovered from the worms incubated with CML-HSA is mainly the diet. Finally, ingestion of dietary CML impaired significantly the worms’ lifespan on both liquid and solid media.

Conclusion – Dietary CML is efficiently ingested and digested by C. elegans. Qualitative and quantitative detection of CML in the worms allows us to draw a direct link between ingestion of CML and aging.

Comparison of genetic and environmental murine models of type II diabetes and their glycation status using mass spectrometry for the determination of glycation products in vivo. Frédéric J. Tessier1,3 (Axel Guilbaud2,4,5 Michael Howsam1,5 Florian Delguste, Éric Céline Niquet-Léridon,6,7 Eric Boulanger1,3) 1University of Lille, Inserm - Lille Inflammation Research International Center, Lille, France. 2Institut Polytechnique UniLaSalle, Transformations & Agro-Ressources Unit, Beauvais, France. 3Francophone Maillard Reaction Society (FМaRS), Lille, France. 4VF Bioscience, Parc Eurasanté, Loos-lez-Lille, France. (frederic.tessier@univ-lille.fr)

Background and aim - There is a growing body of evidence that glycation plays a key role in the pathophysiological processes associated with diabetes, aging and age-related diseases. Nevertheless, studies of pharmaceutical or lifestyle (e.g. diet) approaches to limit the formation and accumulation of advanced glycation end-products (AGEs) remain inconclusive. Only a few animal models of diabetes have been used to study the anti-glycation potential of drugs and diets. The aim of this study was to compare weight gain, glycaemia, glucose tolerance and glycation
levels in mice from a genetic model with mice from two diet-induced obesity models (DIO).

**Methods** - Fifteen male, C57BL/6 wild-type (WT) mice were compared with 15 db/db littermates for a duration of 12 weeks; in addition, 36 male, C57BL/6 WT mice were either fed a normal diet (ND), a high fat diet (HF) or a high fat high sucrose diet (HFHS) for 28 weeks. Body weight, food intake, fasting glycaemia and oral glucose tolerance were measured at different points during the experiment. After sacrifice, kidneys, lungs, hearts and livers were analyzed for early (furosine) and advanced glycation (free and protein-bound CML) using stable-isotope dilution LC-MS/MS.

**Results** – The body weight of db/db mice at sacrifice was 42.9±5.2g compared with 30.6±1.5 for their controls. At the end of the study, the body weights of HF and HFHS mice were higher compared to that of ND mice (48.2±2.9, 47.3±4.0, 32.0±2.0g at 34 months, respectively). Fasting glycaemia, OGT, furosine and both free and protein-bound CML in all organs tested were significantly higher in db/db mice compared with their controls. Among the DIO models, a slight but non-significant increase of fasting glycaemia was observed while only HF mice developed a significant intolerance to glucose. Furosine concentrations were greater only in the liver proteins of both HF and HFHS mice compared with ND mice. The accumulation of free CML was significantly higher in the kidneys and lungs of mice fed a normal diet compared to that of HF and HFHS mice. The endogenous formation of CML was not affected by diet.

**Conclusion** - Compared to DIO murine models, the genetic db/db model is a more pertinent preclinical model for the study of glycation and testing of new anti-glycation therapies.

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**Effects of a probiotic on glycation in a type 2 diabetes mouse model. Axel Guilbaud** 1,2,3 (Michael Howsam 1,3, Céline Niquet-Léridon 3,4, Florian Delguste 3, Patrice Maboudou 3, Anne Garat 5, Sophie Lestavel 7, Éric Boulanger 1,3, Frédéric J. Tessier 1,3, 1University of Lille, Inserm, Lille Inflammation Research International Center, Lille, France. 2VF Bioscience, Parc Eurasanté, 59120 Loos-lez-Lille, France. 3Francophone Maillard Reaction Society (FMaRS), Lille, France. 4Institut Polytechnique UniLaSalle, EGEAL Unit, Beauvais, France. 5Laboratory of Biochemistry, Biology and Pathology center "Pierre Marie Degand", CHRU Lille Bd du prof Leclercq, 59037 Lille Cedex. 6Univ. Lille, CHU Lille, Institut Pasteur de Lille, EA 4483-IMPECS-IMPact de l'Environnement Chimique sur la Santé humaine, Lille, France. 7University of Lille, Inserm, CHU Lille, Institut Pasteur Lille, Lille, France. (axel.guilbaud@gmail.com).

**Context and aim** – According to a growing body of literature, glycation is one of the principal pathways implicated in the long-term organ damage typical of type 2 diabetes. Many studies have described therapeutic strategies to prevent and reduce the formation of advanced glycation end products (AGEs), but results are mixed. New anti-diabetic therapies using probiotics have been proposed, but no studies have yet demonstrated a protective effect of probiotics on glycation. Our aim was to investigate the effect of a probiotic – *Lactobacillus fermentum* – on: 1) diabetic symptoms (body weight, glycaemia, glycated hemoglobin, oxidative stress, liver steatosis profiles), and; 2) glycation status via LC-MS/MS measurements of furosine and carboxymethyllysine (CML).

**Methods** – Thirty male, wild-type (WT) C57BL/6 mice and 30 db/db littermates were divided into control groups and treated groups. The 2 treated groups (WT & db/db) were administered *L. fermentum* (10¹⁰CFU/mL) by daily gavage for a duration of 12 weeks while the control groups (WT & db/db) received a daily gavage of water. Body weight, fasting glycaemia, glucose tolerance and HbA1c were monitored at
different points during the experiment. After sacrifice plasma, erythrocytes, kidneys, lungs, heart, and liver were sampled. Oxidative stress was measured in erythrocytes; steatosis was assessed by histological sections and measurements of triglycerides in the liver. In addition, ASAT/ALAT were measured in plasma and organs by stable-isotope dilution LC-MS/MS.

Results – After only one week of treatment, weight gain was lower in the treated db/db group. From six weeks of treatment, a better regulation of glycaemia was observed in the treated WT group compared with their controls - while no difference was observed between the 2 db/db groups in this regard, oxidative stress was reduced in db/db group treated with L. fermentum. The preventative effect on steatosis in the treated groups (both WT and db/db) was associated with a lower liver weight, lower levels of triglycerides and ASAT/ALAT compared with controls. Initial results for glycation show lower furosine and free CML levels in the kidneys of WT and db/db groups treated with L. fermentum.

Conclusion – L. fermentum has potential as a therapeutic, in both healthy and type 2 diabetes cases, and we observed several beneficial effects such as reduced weight gain, ameliorated glucose metabolism, reduced oxidative stress, prevention of steatosis and reduced renal formation/accumulation of both early and advanced glycation products.

Effects of advanced glycation endproducts on DNA damage and telomere dynamics. Permal Deo1,2 (Caitlin McCullough1, Wai Mun Lim1, Varinderpal S.Dhillon1, Michael Fenech1). 1School of Pharmacy and Medical Science, University of South Australia, Adelaide, South Australia, Australia. 2Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, Australia. 3Genome Health and Personalised Nutrition Laboratory, CSIRO, Adelaide, South Australia, Australia. (permal.deo@unisa.edu.au)

This study investigated the effect of reducing sugars (glucose and fructose), and advanced glycation endproducts (AGEs) on genome damage in WIL2-NS cells. The study further investigated telomere dynamics. For generation of AGES, sugars (glucose and fructose) (0.1 M) were incubated with bovine serum albumin (BSA) (1 mg/mL) at 37 ± 1 °C for 6 weeks. The levels of lysine and Nε-(carboxymethyl)lysine (CML) in the AGE-BSA model systems were quantified using LC-MS/MS. Cell treatment studies were based on 2 dietary sugars (glucose and fructose) (2.5 – 40 mM) and AGE-BSA (Glu-BSA, Fru-BSA) (200 – 600 µg/mL) in a dose dependent manner for 10 days. DNA damage (cytostatic, cytotoxic and genome damage) and telomeres length were assessed using the cytokinesis-block micronucleus cytome (CBMN-Cyt) assay and quantitative real time – polymerase chain reaction (qRT-PCR), respectively. Higher concentrations of fructose (20-40 mmol/L), exerted a significant genotoxic effect on cells, whereas, the damaging effects of glucose were minimal overall. Similarly, at higher concentrations of AGES (400-600 µg/mL), only some genotoxic effects were observed after the 10 day exposure. qRT-PCR showed that glucose treated cells at higher concentration (20 - 40 mM) showed significant telomere shortening when compared with the controls (p < 0.001). Similarly, Glu-AGE treated cells at a high concentration (600 µg/mL) also showed a significant telomere shortening effect in the cells (p < 0.05). Fructose and Fru-AGE only presented a minimal effect on telomere shortening. Overall, Glu-BSA exerted a significant increase in nitric oxide (NO) (p < 0.01) and tumour necrosis factor – α (TNF-α) (p < 0.05) when the concentration of AGES increased from 200 to 600 µg/mL. In conclusion, this study demonstrates a potential link between reducing sugars, AGES and genomic instability. At high concentrations of both sugars and
AGEs, similar to what might be expected in a diabetic individual, genome damage and telomere shortening is likely to occur. Ultimately, these damage could increase the risk of age related diseases over the long term, such as diabetic complications and dementia. These findings indicate the need for further research into this area and to establish whether this effect is replicated in humans. Furthermore, this study suggests a need to develop preventative therapies for improving dietary habits and T2DM management.

In vitro anticancer effects of a novel RAGE inhibitor on suppression of tumor malignant phenotypes in human fibrosarcoma cells. Seiichi Munesue¹ (Ai Harashima¹, Akihiko Takeuchi¹, Mariko Tanaka¹, Shuhei Kawano¹, Nantaphat Leerach¹, Yu Ooshima¹, Yui Kinoshita¹, Akira Sato², Mika Shindo², Shingo Nakajima², Mana Inada², Sei-ichi Tanuma², Yasuhiko Yamamoto¹). ¹Department of Biochemistry and Molecular Vascular Biology, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Japan. ²Department of Biochemistry, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda, Chiba, Japan. (smunesue@med.kanazawa-u.ac.jp)

Receptor for advanced glycation end-products (RAGE) is recognized as a pattern-recognition receptor and has been implicated in the pathogenesis of malignant phenotypes of cancers, inflammatory reactions, Alzheimer’s disease, and diabetic vascular complications. We have demonstrated that RAGE overexpression in human fibrosarcoma HT1080 cells could promote tumor malignant phenotypes, such as migration, invasion and metastasis. In this study, we identified papaverine as a novel RAGE inhibitor using the COSMOS (conversion to small molecules through optimized-peptide strategy) drug design system. To examine anti-tumor effects of papaverine, RAGE-overexpressing, dominant-negative RAGE-overexpressing and the control HT1080 cells were employed. Addition of papaverine significantly inhibited RAGE-dependent NFκB activations evoked by the exposure of AGE-BSA and high mobility group B1 (HMGB1) in a dose-dependent manner. In addition, papaverine treatment could suppress tumor cell proliferation, migration and invasion of the HT1080 cells in a RAGE-dependent manner. These findings thus suggest that papaverine could function as a RAGE inhibitor and provide new insights into the field of RAGE biology, particularly anticancer therapy.

Advanced glycation endproducts are important features in algorithms for diagnosis and typing of early-stage arthritis. Usman Ahmed,² (Kashif Rajpoot,² Paul J. Thornalley² and Naila Rabbani³). ¹Clinical Sciences Research Laboratories, University of Warwick, University Hospital, Coventry, UK, ²School of Computer Science, University of Birmingham, Birmingham, UK and ³Proteomics Research Technology Platform, University of Warwick, University Hospital, Coventry, UK (ozman1984@gmail.com)

Background and aims: The detection of arthritis at the early stages before irreversible damage to the joint has occurred is vital for effective therapy. There is no blood-based test for detection of early-stage osteoarthritis (eOA) and the anti-cyclic citrullinated peptide antibody (anti-CCP-Ab) test for early-stage rheumatoid arthritis (eRA) requires refinement - sensitivity is 61%. There is also inflammatory joint disease other than RA which is often self-resolving (non-RA). Increased glycated, oxidised and nitrated amino acids (protein glycation, oxidation and nitration free adducts) are found in plasma/serum of early-stage arthritic disease, originating from increased proteolysis in the arthritic joint. We explored the application of these with the bone turnover and resorption marker, hydroxyproline.
(Hyp), and anti-CCP-Ab status as biomarkers for early detection and typing of arthritic disease.

**Materials and methods:** Patients with knee joint eOA, eRA, non-RA and healthy subjects were recruited for the study (n = 186). Plasma/serum was analysed for glycated, oxidized and nitrated amino acids by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry. Data-driven machine learning using support vector machines was employed to develop two-step diagnostic algorithms: step-1 - classifying between healthy controls and early-stage arthritic disease (any type); and step-2 – classifying type of arthritic disease (eOA, eRA or non-RA). The contribution of each feature in the algorithms to classification accuracy was assessed by determining the change in area-under the curve of receiver operating characteristic curve (AUROC) when a feature is omitted from the algorithm and re-trained. For perfect classification, AUROC = 1.0.

**Results:** For optimum discrimination between healthy subjects and patients with early-stage arthritis (any type), features in the algorithm and their relative importance were: CEL > glucosepane (GSP) > Hyp > NFK > MetSO > MG-H1 > 3DG-H > G-H1 > 3-NT > DT > CMA - Algorithm 1 (abbreviations as in the reference below, unless given). This had sensitivity 90%, specificity 83% and AUROC of 0.93; for random selection, AUROC = 0.50. For patients with early-stage arthritis, optimum performance to discriminate between eOA, eRA and non-RA included features: anti-CCP-Ab > 3DG-H > CML > FL > GSP > MetSO >CEL > 3-NT - Algorithm 2. For eOA, this had sensitivity 94%, specificity 96% and AUROC 0.98; for random selection AUROC = 0.33.

**Conclusions:** We conclude that serum/plasma glycated, oxidized and nitrated amino acids may be used for blood-based early-stage detection and typing of arthritis where glycated amino acids are dominant classifying features.

### 4 short oral poster presentations: Medicine, Nutrition, Food (5 min per presentation)

1) **Alena Soboleva:** Formation of glycation products in blood plasma under hyperglycemic conditions: biomarker value and reactivity aspect
2) **Saskia C. van de Zande** Physical activity and Skin autofluorescence in a large population study (LifeLines cohort)
3) **Shoutaro Arakawa:** Comprehensive analysis of Advanced Glycation End-products (AGEs) in human cancellous bone: correlation of AGEs contents in bone, serum and urine
4) **Wan-Ju Yeh:** The improvement of diabetic nephropathy and Alzheimer’s disease-like symptoms through glyoxalase 1 modulation in Drosophila nephrocytes
5) **Kelly Fuller/Haus:** A single high fat meal alters soluble RAGE profiles and peripheral blood mononuclear cell RAGE expression
6) **Jelte Boersema:** Skin accumulation of advanced glycation end products is increased in patients with an abdominal aortic aneurysm
7) **Antonio Dario Troise:** Microemulsions and Amadori compounds: unravelling molecular dynamics from foods to gastrointestinal digestion
Poster Presentations
Comprehensive analysis of Advanced Glycation End-products (AGEs) in human cancellous bone: correlation of AGEs contents in bone, serum and urine. Shoutaro Arakawa1,2, Mitsuru Saito1, Ryusuke Suzuki1,2, Jun-ichi Shirakawa2, Umi Taniguchi2, Ryoji Nagai2, Keishi Marumo1. Department of Orthopaedic Surgery, Jikei University School of Medicine,1 Laboratory of Food and Regulation Biology, 2Tokai University School of Agriculture. Nishi-shimbashi, Minato-ku, Tokyo, Japan. (shotaoru@gmail.com)

Purpose: Accumulation of Advanced Glycation End-products (AGEs) in bone collagen deteriorates the bone quality and strength. Although Pentosidine is used as a surrogate marker for AGEs in bone collagen, each AGE structure might accumulate in a different manner because of its distinct formation pathway. Furthermore, the level of Pentosidine in bone collagen is lower than that of non-crosslinking AGE structure such as CML. Therefore, we have established a detection system of various AGEs including Pentosidine using a LC-time of flight (TOF)-MS. Thus, the primary purpose of this study was to observe the tendencies of AGEs accumulation in human bone, and the secondary purpose was to evaluate whether AGEs analysis in serum and urine could estimate AGEs contents in bone collagen.

Methods: Human cancellous bones were harvested from lateral tibia of 210 cases (mean age: 74) who underwent total knee replacement because of end-stage osteoarthritis. Their serum and urine were also collected one month prior to surgery. Pulverized, delipidated and demineralized bone specimen was reduced, spiked with isotope-labelled internal standards of each AGEs and hydrolyzed to release free AGEs adducts. After purification by solid phase extraction, CML, CEL, MG-H1, CMA, CEA and Pentosidine were determined by LC-TOF-MS. AGEs contents in serum and urine were determined following reduction, hydrolysis and solid phase extraction.

Results: MG-H1 was the most abundant AGE structure in bone collagen. Among cases, contents of all AGEs structures showed moderate to strong correlations (Pearson’s correlation coefficients : 0.5-0.9, p<0.05). MG-H1 contents in bone collagen were weakly correlated with MG-H1 contents in serum. No significant correlation was observed between urine.

Conclusions: In bone collagen, content of one AGE structure potentially represent other AGEs contents. Therefore, the conventional method using Pentosidine as a surrogate marker is considered to be applicable. And CML or MG-H1 can be practical alternatives because they can be determined by ELISA. In a clinical practice, measurement of serum is appropriate to estimate AGEs contents in bone collagen.

Effect of vitamin B6-deficiency on mouse behavior and monoaminergic system. Kazuya Toriumi, Mitsuhiro Miyashita, Yasue Horiiuchi, Kazuhiro Suzuki, Akiko Kobori, Izumi Nohara, Yukiko Shimada, Emiko Hama, Nanako Obata, Masanari Itokawa, Makoto Arai. Department of Psychiatry and Behavioral Sciences, Tokyo Metropolitan Institute of Medical Science (toriumi-kz@igakuken.or.jp)

Schizophrenia is a heterogeneous psychiatric disorder characterized by positive and negative symptoms and cognitive impairment. Recently, we have reported that about 20% of schizophrenia patients show accumulation of pentosidine, one of advanced glycosen end products (AGEs), and lower level of vitamin B6 (VB6), which works as a scavenger against AGEs, in the peripheral blood. Furthermore, our clinical study found that the VB6 level is inversely proportional to the score of PANSS, suggesting that the loss of VB6 might contribute to development of the symptom in schizophrenia. In order to uncover the relationship between VB6 level and schizophrenia, we developed VB6-deficient mice by feeding C57BL/6J male mice with a VB6-lacking diet containing low VB6 at 5mg/100g pellets from 8 to 12 weeks of age, while control mice were fed with a normal diet in which VB6 is contained at 1.4mg/100g pellets. After the feeding for 4 weeks, the plasma VB6 level in VB6-deficient mice decreased to about 3% of that in control mice. Moreover, the body weight of VB6-deficient mice did not increase during the feeding of VB6-lacking diet, leading to significant difference in the body weight between VB6-deficient and control mice. Next, to evaluate the effect of the low VB6 on mouse behavior, we performed...
behavioral tests using VB6-deficient mice. In the social interaction test, VB6-deficient mice showed less interaction compared with the control mice, corresponding to an increased negative symptom-like behavior. These behavioral data suggest that the VB6 deficiency might be associated with the negative symptoms. Finally, to investigate whether the VB6-deficiency affected the function of monoaminergic neuronal systems, the tissue contents of monoamines and their metabolites in various regions of the brains were measured. A marked increase in 3-Methoxy-4-hydroxyphenylglycol (MHPG) was shown in all brain regions compared to that in the control, which is consistent with many clinical reports that increased MHPG level was shown in schizophrenia patients. Furthermore, because of the increased MHPG, the ratio of MHPG to noradrenaline significantly increased in VB6-deficient mice, suggesting that the activities of noradrenergic neuronal systems were increased in VB6-deficient mice. These results suggest that VB6-deficiency might be involved in schizophrenia symptoms via the enhancement of noradrenergic system.

Drug library screening to identify new compounds for inducing ectodomain shedding of RAGE and formation of soluble RAGE. Shuhei Kawano1, Hanae Miyazawa1, Seiichi Munesue1, Ai Harashima1, Mariko Tanaka1, Duong Thi Minh Thoa1, Nontaphat Leerach1, Yu Ooshima1, Yu Kinoshita1, Hiroshi Yamamoto1,2, Yasuhiro Yamamoto1. 1Department of Biochemistry and Molecular Vascular Biology, Kanazawa University Graduate School of Medical Sciences, 13-1 Takara-machi, Kanazawa 920-8640, Japan, 2Division of Molecular Medicine and Therapy, Tohoku University Graduate School of Medicine, Sendai, Japan. (m.mk@igakuen.or.jp)

AIM: The aim of this clinical trial was to obtain proof of concept for high-dose pyridoxamine as a novel treatment for schizophrenia with enhanced carbonyl stress.

METHODS: Ten Japanese schizophrenia patients with high plasma pentosidine, which is a representative biomarker of enhanced carbonyl stress, were recruited in a 24-week, open trial in which high-dose pyridoxamine (ranging from 1200 to 2400 mg/day) was administered using a conventional antipsychotic regimen. Main outcomes were the total change in Positive and Negative Syndrome Scale score and the Brief Psychiatric Rating Scale score from baseline to end of treatment at week 24 (or at withdrawal).

RESULTS: Decreased plasma pentosidine levels were observed in eight patients. Two patients showed marked improvement in their psychological symptoms. A patient who harbors a frameshift mutation in the Glyoxalase 1 gene also showed considerable reduction in psychosis accompanied with a moderate decrease in plasma pentosidine levels. A reduction of greater than 20% in the assessment scale of drug-induced Parkinsonism occurred in four patients. Although there was no severe suicide-related ideation or behavior, Wernicke's encephalopathy-like adverse drug reactions occurred in two patients and were completely suppressed by thiamine supplementation.

CONCLUSION: High-dose pyridoxamine add-on treatment was, in part, effective for a subpopulation of schizophrenia patients with enhanced carbonyl stress. Further randomized, placebo-controlled trials with careful monitoring will be required to validate the efficacy of high-dose pyridoxamine for these patients.

Pyridoxamine, a novel treatment for negative symptoms of schizophrenia. Makoto Arai1, Mitsuhiro Miyashita1, Takashi Dan1, Kazuya Toriumi1, Yasue Horiuchi1, Akiko Kobori1, Kazuhiro Suzuki2, Toshio. Miyata2, Masanari Itokawa1. 1Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan; 2Division of Molecular Medicine and Therapy, Tohoku University Graduate School of Medicine, Sendai, Japan.

RAGE is a multi-ligand and a pattern-recognition receptor. It is implicated in the development of various diseases including inflammation and cancer. Enzymatic ectodomain shedding of RAGE leads to the formation of soluble RAGE (sRAGE), which can function as a decoy-type receptor against RAGE ligands. In this study, we performed a drug screening to identify new chemical compounds for inducing RAGE ectodomain shedding using an FDA Drug Screen-Well Library (BML-J) composed of ~640 chemical compounds. For the screening, we employed C6 glioma cells expressing human RAGE and an NFkB-dependent luciferase system in combination with a sRAGE ELISA. We found 12 potential candidates for activating RAGE ectodomain shedding and sRAGE formation in vitro. These findings let us further investigate in vivo effects of the candidates on the prevention and the treatment of RAGE-related diseases.
Nuclear localization and function of the glycolytic enzyme phosphoglucose isomerase. Elitsa Boteva, Milena Tileva, Elena Krachmarova, and Roumyana Mironova. Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria (el.boteva@gmail.com)

In recent studies we have found that the glycolytic enzyme phosphoglucose isomerase (PGI) of *Escherichia coli* and yeast type III PGI catalyze in vitro the breakdown of DNA-fructosamine-6-phosphate (DNA-N-Fr6Ph) to glucose-6-phosphate (G6Ph) and DNA. DNA-N-Fr6Ph represents an Amadori product (APs) resulting from the non-enzymatic interaction between G6Ph and DNA in the early step of the Maillard reaction. As result of glycation in vivo DNA accumulates both APs and advanced glycation end products with mutagenic potential. Therefore, we hypothesized that the observed DNA-N-Fr6Ph deglycation activity of PGI may be implicated in repair of G6Ph-derived lesions on DNA in vivo. In view of the conserved structure and function of PGI from bacteria to humans, we further reasoned that in order to carry out the proposed DNA repair activity in eukaryotes PGI should translocate to the nucleus. To explore this possibility, we studied the human cell line PC3 for nuclear localization of PGI by a variety of methods including Western blotting, fluorescent microscopy and chromatin immunoprecipitation. All data unambiguously indicated that besides in the cytosol human PGI resides also in the nucleus. In support of this finding, bioinformatics analyses revealed a strong nuclear localization sequence in two of the human PGI isoforms. Further experiments demonstrated that nuclear extracts of PC3 exhibit DNA-N-Fr6Ph deglycation (amadoriase) activity. Taken together these data assign for the first time a particular function to nuclear PGI linked to the repair of G6Ph-modified DNA.

2SC level in the peripheral blood of diabetic mice and patients with diabetic complications is significantly increased as compared with other AGEs. Jun-ichi Shirakawa¹, Kenta Ichimaru¹, Tomo Fukushige², Takeshi Matsumura³, Eiichi Araki³, Yukio Fujiwara³, Ryoji Nagai³, ¹Laboratory of Food and Regulation Biology, Graduate School of Agriculture, Tokai University, Japan; ²Department of Internal Medicine, Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Japan; ³Department of Cell Pathology, Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Japan. (jshirakawa33920@gmail.com)

Main Objectives: S-(2-succinyl)cysteine (2SC) is formed by a Michael addition reaction between cysteine residues in proteins and fumarate, an intermediate of the TCA cycle. Since 2SC is generated from a mitochondrial metabolite, it is considered a useful index of mitochondrial dysfunction that does not require tissue disruption. Although 2SC accumulation in cultured cells and mouse tissue can be assessed by anti-2SC antibody and gas chromatography-mass spectrometry following multiple derivatization of samples, the quantitation of 2SC in peripheral blood is still difficult. In this study, we developed a 2SC detection system in biological samples via liquid chromatography-tandem mass spectrometry (LC-MS/MS) to clarify the relationship between 2SC levels and hyperglycemia in vivo.

Strategy and Methods: Internal standards, [¹³C₅, ¹⁵N₁] 2SC and [¹³C₆] lysine, were added to the culture media, and were passed through the molecular weight cut off filter and solid phase extraction columns. Samples were detected using LC-MS/MS. Similarly, 2SC content in the serum of diabetic animals and patients was also analyzed.

Main Results: LC-MS/MS analysis demonstrated that 2SC is detectable in hydrolysates consisting of 1 L serum and 15 L cell culture medium. Results indicated that 2SC contents in the cells and culture media were increased when adipocytes were incubated under hyperglycemic conditions. In addition, 2SC contents in the serum of STZ-induced type 1 and db/db type 2 diabetic mice were higher as compared with those in control mice. On the other hand, other AGE structures were not increased in type 2 diabetic mice. Furthermore, diabetic patients exhibited lower 2SC content; while significant increase in 2SC content was observed in patients with diabetic complications.

Conclusions: Changes in extracellular 2SC level were reflected in intracellular 2SC concentration. Since 2SC content in the blood can be detected with 1 µL serum, mitochondrial stress may be monitored via peripheral blood sampling. Our results suggested that 2SC monitoring in the human serum would be an effective mean to evaluate diabetic complications.

DELAY OF CELL SENESCENCE AND DECREASED PROTEIN GLYCATION BY CALORIC RESTRICTION MIMETIC EFFECT OF SULFORAPHANE. Mingzhan Xue¹, Florence Hariton¹, NAILA RABBAN², MARK FOWLER¹ and...
Background: Increased cell senescence contributes to the pathogenesis of aging and aging-related disease. Despite evidence of the ability to slow cell senescence in vitro by exposure to low glucose concentrations or dietary bioactive compounds that activate transcription factor Nrf2, the connection between these and the mechanism of delay of cell senescence is unknown. We investigated this in human MRC-5 lung fibroblasts in vitro. Methods: MRC-5 Fibroblasts (European Collection of Animal Cell Cultures, Porton Down, U.K) were passaged every 7 days until senescence. From passage 2, cells were incubated with and without the broccoli-derived Nrf2 activator, R-Sulforaphane (SFN; 1 μM) at the start of each passage. There was no cytotoxicity of SFN at this concentration. Custom quantitative mRNA array (Nanostring method) was performed at passages 3 and 11. Culture medium at the start and end of each passage was analysed for glucose, L-lactate and D-lactate and metabolic fluxes deduced. Cell protein glycation adducts and glycated amino acids in the culture medium were assayed by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry. Results: MRC-5 fibroblasts were cultured to senescence with maximum cumulative population doubling level (cPDL) of 38.7 ± 0.1 (n = 3) at passage 8. Incubation with 1 μM SFN from passage 2 led to delayed senescence and increased maximum cPDL at passage 10 of 40.9 ± 0.3 (treatment effect, P = 2 × 10−5 and time x treatment interaction, P = 0.004; repeated measures). In four independent studies, the increase in cPDL achieved with SFN treatment above control was 2.0 ± 0.3 (P < 0.001). Cell senescence was associated with a progressive increase in rate of glucose metabolism by up to 12-fold, with a similar increase in flux of formation of methylglyoxal (MG) – as judged by increased D-lactate formation, and increasing proteomic and genomic damage. SFN decreased glucose metabolism and suppressed the increased formation of L-lactate and D-lactate on the approach to senescence. SFN increased the expression of thioredoxin interacting protein (TXNIP) which curbs cellular entry of glucose by decreasing levels of glucose transport proteins. This was reflected by decreased Nε-fructosyl-lysine (FL) residue content of cell protein and decreased formation of MG and advanced glycation endproducts (AGEs) with SFN treatment. Conclusion: SFN delayed the senescence of MRC-5 fibroblasts in vitro. It induced a caloric restriction mimetic effect likely mediated by down regulation of glucose entry into cells through increased expression of TXNIP and reflected in decreased early and advanced protein glycation.

Prostate cancer malignancy is enhanced by glyctoxins. Chi-Hao Wu1,2 and Shih-Hong Khoo2. 1Bachelor’s and Master’s Degree Programs of Nutritional Sciences, School of Life Sciences, National Taiwan Normal University, Heping East Road, Taipei, Taiwan; 2School of Nutrition & Health Sciences, Taipei Medical University, No. 250 Wuxing St. Taipei 11031, Taiwan (chwu@ntnu.edu.tw)

Advanced glycation end products (AGEs), also known as glyctoxins, both endogenous and exogenous sources have been implicated as a catalyst of aging, cancer, diabetes, Alzheimer's and other complications in body. Prostate cancer (PCa) has been known for its indolence, recent epidemiology data suggests an accelerating decrease in patients’ diagnosis age with aggressive forms of PCa, thus a plausible relationship between westernizing diet and PCa malignancy was proposed for further examination. Of note, patients with cancer have increased levels of AGEs and the role of receptor for AGEs (RAGE) in cancer malignancy is unclear. Studies have shown RAGE contributes to tumor carcinogetic microenvironment, thus it is reasonable to deduce AGE-RAGE axis as a pivotal role in cancer malignancy. Current study aims to explore the role of AGE-RAGE interaction in cell and animal models supported by clinical-based evidence of a possible molecular modulation of autophagy in drug resistance and PCa malignancy. Present results showed AGEs were able to induce proliferation, metastasis, and invasion in different PCa cell lines. DU145 and PC3 cells were engineered to overexpress and or silence RAGE, genetically RAGE overexpression PCa cell lines exhibited decreased sensitivity to chemodrugs (mitoxantrone and doxetaxel). The results revealed that RAGE may have downregulated apoptosis and upregulated autophagic activity through canonical PI3K/Akt/mTOR and ERK pathways. Next, we verified such phenomenon through two sets of animal model: a xenograft animal model monitored through bioluminescence imaging and established a long-term AGEs-fed diet model on ICR mice. Results further suggest RAGE may play a vital role in tumorigenesis while long-term ingestion of dietary AGEs may trigger prostate neoplasia in mice.
Clinical data and histologic samples of 72 PCa patients were analyzed to reveal a significant association between increased RAGE expression and diminished survival rate. In conclusion, present study demonstrated solid evidence for glyctoxins involvement in the tumorigenesis and malignancy of PCa.

**Methylglyoxal modification of peroxiredoxin 6 as a biomarker for diabetic complications.** Tomoko Oya-Ito1,2, Yuji Naito3, Tomohisa Takagi4, Keisuke Shima5, and Yoshikazu Yoshikawa1. 1 Department of Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kaji-i, Japan 2 Department of Nutrition, Shubun University, Aichi, Japan, 3Shimadzu Corporation, Kyoto, Japan. oya.t@shubun.ac.jp

Methylglyoxal (MG) is an endogenous highly reactive dicarbonyl degradation product formed from triose phosphates during glycolysis, and forms stable adducts primarily with arginine (Arg), lysine (Lys) and cysteine (Cys) residues of proteins. An antioxidant protein peroxiredoxin 6 (Prx6) was found as the major MG-adducted protein in red blood cells (RBCs) from diabetic patients. Peroxidase activity of recombinant Prx6 was inhibited by MG modification in a concentration-dependent manner. Mass spectrometry analysis of peptide fragments of MG-modified recombinant Prx6 identified residues Arg-24, Arg-41, Arg-132, Arg-106, Arg-219, and Lys-63 as the modification site in the protein. Arg-132 is situated in the catalytic center of peroxidase activity. Prx6 contains one conserved reactive residue Cys-47 in its active site. The decrease in the number of free thiols in Cys residue upon incubation with MG was observed. These results suggest that the decrease in peroxidase activity is due to modification of active-site residues in Prx6. The level of MG modification on Prx6 was correlated to both fasting glucose concentration (p<0.0001) and postprandial glucose concentration (p<0.0001) in diabetic patients. This modification level was more sensitive than HbA1c against fasting glucose concentration (p<0.0003) and postprandial glucose concentration (p<0.0001). It was revealed that the level of MG modification on Prx6 does not change with time since diagnosis of diabetes. Moreover this modification level was higher in the hyperlipidemia patients than in the healthy control subjects. Therefore, it was suggested that protein modification by MG plays an important role in the development of diabetic complications.

**Novel α-Oxoamide AGES as Oxidative Stress Markers.** TIM BALDENSPERGER8, Alexander Zipprich1, Marcus A. Glomb8, 4Institute of Chemistry – Food Chemistry, 1Department of Internal Medicine I; Martin-Luther-University Halle-Wittenberg, 06120 Halle/Saale, Germany; tim.baldensperger@chemie.uni-halle.de

The highly reactive α-dicarbonyl compounds glyoxal and methylglyoxal are major precursors of post-translational protein modifications in vivo. Model incubations of N\(^{\text{\text{-t-Boc}}}\)-lysine and either glyoxal or methylglyoxal were used to further elucidate the underlying mechanisms of the N\(^{\text{\text{-carboxymethyl}}}\)lysine (CML) and N\(^{\text{\text{-carboxyethyl}}}\)lysine (CEL) reaction cascades. After independent synthesis of authentic reference standards, we were able to detect glyoxal specific N\(^{\text{\text{-glyoxylyl}}}\)lysine and methylglyoxal specific N\(^{\text{\text{-pyruvoyl}}}\)lysine for the first time by HPLC-MS\(^{\text{2}}\) analyses. In contrast to CML and CEL the formation of these two novel α-oxoamide AGES was highly accelerated at pH 9.6 compared to 7.4. Moreover they were exclusively formed under aerated conditions, suggesting them as potent markers for oxidative stress. Analogous to the well-known Strecker degradation pathway leading from amino acids to Strecker acids, the oxidation of an enaminol intermediate is suggested as the key mechanistic step. Artifact formation of α-oxoamide AGES was ruled out by incubations containing the corresponding carboxylic acids and α-hydroxamido AGES. Finally a highly sensitive work-up for the determination of AGES in tissues was developed. In support of our hypothesis, the levels of N\(^{\text{\text{-glyoxylyl}}}\)lysine and N\(^{\text{\text{-pyruvoyl}}}\)lysine in rat liver indeed correlated with liver cirrhosis and ageing.

**Reduced HDL function in children and young adults with type 1 diabetes correlates with glycation by 3-deoxyglucose.** Daniah Abdulrahman M Alamoudi1, Martin Heier2,3, Ingebjørg Seljeflot2,4 Kristian F. Hanssen2,5 Knut Dahl-Jørgensen2,7, Michael Oda9, Hanna Dis Margeirsdottir2,5 Sabah Pasha1, Paul J Thornalley2 and Naila Rabbani1,9. 1Clinical Sciences Research Laboratories, University of Warwick, University Hospital, Coventry, UK, 2Faculty of Medicine, University of Oslo, Oslo, Norway, 3Oslo Centre for Biostatistics and
Background and aim: Patients with type 1 diabetes mellitus (T1DM) are at increased risk of cardiovascular disease (CVD). Measures of high-density lipoprotein (HDL) function provide a better risk estimate for future CVD events than serum levels of HDL cholesterol. Functional assessments are: HDL apoA-1 exchange (HAE) – a measure of its ability to release lipid-poor apolipoproteinA-1, an essential step in reverse cholesterol transport. The objective of this study was to evaluate if glycation markers correlate with HDL function in T1DM patients after 5 year follow up compared with healthy control subjects.

Materials and methods: Participants in the atherosclerosis and childhood diabetes study (Oslo, Norway) were examined after 5 years follow-up. Randomly selected patients with T1DM with normal renal function and age 18.9 ± 2.8 years (n = 40), grouped by low HAE (33.6 ± 4.0 %/mg/dl, n = 20) and high HAE (70.7 ± 5.8 %/mg/dl, n = 20), were included in this study; 20 healthy control subjects with similar follow-up were also studied. Protein glycation free adducts were quantified in 10kDA ultrafiltrate of plasma by stable isotopic dilution analysis by LC-MS/MS. SPSS was used for statistical analysis: Mann-Whitney U significance test and Spearman correlation.

Results: Plasma FL, CML CEL, 3DG-H, CMA and glucosepane free adduct concentrations were increased in patients with T1DM, with respect to healthy controls: Nε-fructosyl-lysine and advanced glycation endproducts (AGEs): + 77%, P<0.001; CML, +43% and CEL, +22%, P<0.05; 3DG-H, +34%, P<0.01; and CMA, +47% and glucosepane, + 96%, P<0.001. Plasma 3DG-H free adduct concentration was increased in T1D patients with HDL of low HAE, compared to T1D patients with HDL of high HAE: median (quartiles); 151 (121-194) versus 116 (84 - 131) nM; P = 0.03. Plasma 3DG-H free adduct concentration of T1D patients with HDL of low HAE was also increased compared healthy controls 151 (121-194 versus 96 (74 -123) nM; P = 0.0006. In diabetic patients, plasma 3DG-H free adduct concentration correlated negatively with HAE: r = -0.48, P = 0.003; and in all subjects, plasma glucosepane free adduct concentration correlated negatively with HAE/HDL ratio: r = -0.29, P = 0.03.

Conclusions: There was a strong negative association of plasma 3DG-H free adduct concentration with HAE functional activity of HDL in patients with T1DM. Dicarbonyl stress may be a driver of HDL functional impairment in T1DM and thereby contribute to risk of CVD.

Formation of glycation products in blood plasma under hyperglycemic conditions: biomarker value and reactivity aspect. Alena Soboleva,1, 2 Duc Viet Nguyen,1, 3 Tatiana Mamontova,1, 3 Ekaterina Nikitina,1 Gregory Mavropulo-Stolyarenko,1, 2 Maria Vikhnina,1, 3 Julia Shumilina,1 Vasily Stefanov,1 Tatiana Grishina,1 Tatiana Karanova1, 5 and Andrej Frolov1, 5 Saint-Petersburg State University, Biological Faculty, Department of Biochemistry, Russian Federation, 2 Leibniz Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Germany, 3 University of Leipzig, Faculty of Chemistry and Mineralogy, Institute of Analytical Chemistry, 4 Federal Almazov North-West Research Centre, Russian Federation. 5 The First Pavlov State Medical University of St. Petersburg, Russian Federation. (st023185@student.spbu.ru)

Protein glycation is usually referred to as a non-enzymatic modification of lysyl and arginyl residues underlined by interaction with reducing sugars and dicarbonyl products of their degradation. Resulting early glycation products (i.e. Amadori and Heyns compounds) are readily involved in further reactions, leading to formation of advanced glycation end products (AGEs). In human organism, these compounds demonstrate clearly pro-inflammatory effects, underlying sub-clinical inflammation, which, in turn, leads to development of atherosclerotic changes in blood vessels and onset of type 2 diabetes mellitus (T2DM). Currently, glycated blood proteins (hemoglobin and human serum albumin) are the recognized markers of this disease. However, this information are not sufficient for effective glycemic control. Therefore, during the recent decade, individual glycation sites (IGS) were demonstrated to be promising T2DM markers, potentially providing a possibility for more reliable control of sugar levels in blood. In our experiment, the patterns of potential biomarkers were compared in cohorts (n = 20) of T2DM patients (non-smoking women 45-75 years old with the HbA1C levels of...
Glycative stress and sleep quality. Yoshikazu Yonei, Mari Ogura, Wakako Takabe, Masayuki Yagi Anti-Aging Medical Research Center & Glycative Stress Research Center, Doshisha University, Kyoto, Japan (yyonei@mail.doshisha.ac.jp)

Introduction: Glycative stress is a life-threatening risk factor we are facing to nowadays. My presentation this time is about the relation between the sleep quality and glycative stress that becomes a hot topic recently.

Method: Melatonin plays a key role for keeping a high quality sleep. It is reported that in vitro actions of melatonin include anti-oxidative and AGE-breaking. This time effect of melatonin administration is elucidated on plasma glucose changes, especially focused on postprandial hyperglycemia. The sleep quality was evaluated by various points of view; melatonin actions, glucose level changes during sleep, and effect of breakfast-skipping and comfortable mats.

Results: The sleep quality plays an important role to keep our health, also associated with glycative stress. Less quality caused hyperglycemia while sleeping, affecting the appetite of next morning. If we skip breakfast, it stimulates the secretion of anti-insulin hormones, i.e. glucagon, thus causing postprandial hyperglycemia in the lunch time. Less sleep quality means less secretion of melatonin. Melatonin did not inhibit in vitro AGE formation and not modify the AGEs/RAGE interaction in the macrophage cell line. However it was revealed that melatonin enhances AGE breaking (Glycative Stress Res 3(1): 38-43, 2016). Our clinical study shows that melatonin administration improves sleep quality and ameliorates postprandial hyperglycemia in the next day. We also have conducted clinical studies using mats with “A Distinctive 4-Layer 3-Dimensional Structure” which may improve sleep quality. Significant improvements were observed in GH/IGF-I secretion, oxidative stress, immunological function and lipid metabolism.

Conclusion: It is already known that glycative stress can be reduced through an appropriate diet, lifestyle. The sleep quality seems also important to reduce glycative stress though various mechanisms including AGE breaking action of melatonin.

Study on glycated-hemoglobin metabolism of macrophages in tumor microenvironment. Yukio Fujiwara, Cheng Pan, Takenobu Nakagawa, Yoshihiro Komohara, Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan. fuji-y@kumamoto-u.ac.jp

In recent years, we have been conducting research on compounds that inhibit tumor progression by regulating the activation of tumor-associated macrophages (TAM) involved in tumor progression, and on molecules that control the activation of TAM. In this study, we clarified that CD163, a hemoglobin scavenger receptor on macrophages, plays an important role in tumor development. Furthermore, we recently obtained interesting results on CD 163 mediated metabolism of glycated hemoglobin, a early maillard reaction product. Glycated hemoglobin-haptoglobin complex induced inflammatory cytokines production via CD163. We will introduce its role of individual major plasma carbohydrates in formation and stability of detected protein-bound hexosamines. Indeed, among such sugars, fructose and mannose might impact in hexosamine formation, whereas autoxidation of ascorbic acid and arabinose could compromise their stability. Therefore, we incubated model synthetic peptide (Ac-AFGSASGA-NH₂) and model protein (human serum albumin, HSA) in presence of individual sugars or their mixtures at 37ºC during 0, 7, 14, 21 and 28 days. The experiments with peptides revealed different reactivity of plasma sugars and kinetics of formation AGES. Experiments with HSA indicated, that at some sites, abundance of hexosamines could be modulated by non-glucose sugars. The reported study was funded by RFBR according to the research project № 18-34-00927.

Glycoxalase 1 OVEREXPRESSION ASSOCIATED MULTI-DRUG RESISTANCE IN CANCER CHEMOTHERAPY. Hafsia Abbas,¹ Mingzhan Xue,¹ Naiila Rabbani¹² and Paul J. Thornalley¹

7.5-10%, not undergoing insulin therapy) and age-matched normoglycemic controls. IGS were analyzed by the LC-based bottom-up proteomic approach, relying on protein tryptic digestion, boronic acid affinity chromatography (BAC) and solid phase extraction prior to RP-HPLC-QqTOF-MS. Biomarker potential of IGS was addressed by label-free relative quantification. Statistical analysis relied on the Mann-Whitney test and linear discriminant analysis (LDA). Totally, 51 differentially glycated protein lysine residues were identified, among them 42 individual glycation sites worked as biomarkers of type 2 diabetes. In the next step, we addressed the role of individual major plasma carbohydrates in formation and stability of detected protein-bound hexosamines. Indeed, among such sugars, fructose and mannose might impact in hexosamine formation, whereas autoxidation of ascorbic acid and arabinose could compromise their stability. Therefore, we incubated model synthetic peptide (Ac-AFGSASGA-NH₂) and model protein (human serum albumin, HSA) in presence of individual sugars or their mixtures at 37ºC during 0, 7, 14, 21 and 28 days. The experiments with peptides revealed different reactivity of plasma sugars and kinetics of formation AGES. Experiments with HSA indicated, that at some sites, abundance of hexosamines could be modulated by non-glucose sugars. The reported study was funded by RFBR according to the research project № 18-34-00927.
Background and aims: Overexpression of Glo1 induces anti-cancer multi-drug resistance (MDR) in human tumour cell lines and human tumour cells in primary culture. It may be linked to increased GLO1 gene copy number and elevated transcriptional activity of Nrf2 via antioxidant response element (ARE)-linked up-regulation of Glo1 transcription. We hypothesize that the cytotoxicity of anticancer drugs is mediated, in part, by inducing increase of MG to cytotoxic levels. This may be achieved by drug-induced increased MG formation and/or decreased MG metabolism; the latter achieved by drug-induced direct or indirect Glo1 inhibition. The aim of this study is to characterise the level of MDR to clinical anti-cancer drugs by Glo1 overexpression in a model human tumour cell line in vitro and investigate the MDR mechanism for drugs where it is most marked.

Materials and methods: HEK293 cells were stably vector-transfected to overexpress Glo1 and with empty vector as control. The effect of anticancer drugs on growth and toxicity of HEK293 cells in three conditions (wild-type, Glo1-overexpression and empty vector) was studied in vitro and median growth inhibitory concentration (GC50) values determined. The effect of cell permeable Glo1 inhibitor, S-p-bromobenzylglutathione cyclopropyl diester (BBGCp2) on the potency of anticancer drugs was also studied. The glyoxalase system and dicarbonyl metabolism were characterised by measuring cellular activities of Glo1, Glo2, MG reductase and MG dehydrogenase. The flux of formation of D-lactate – a surrogate indicator of flux of MG formation, glucose consumption and net L-Lactate formation were measured in HEK293 cells cultures by end-point enzymatic assays.

Results: Stable transfectant HEK293 cells overexpressing Glo1 had 4 fold increase in Glo1 activity, compared to empty vector transfectant controls. Glo1 was the major fate for MG metabolism in HEK293 cells. Doxorubicin, mitomycin C, paclitaxel, mechlorethamine and methotrexate had the highest resistance conferred by Glo1 overexpression: MDR was 16-fold, 15-fold, 8-fold, 7-fold and 7-fold, respectively. BBGCp2 potentiated the cytotoxicity of anti-cancer drugs. There was an increase in flux of formation of D-lactate and L-lactate and consumption of D-glucose in HEK293 cells treated with mechlorethamine, doxorubicin, paclitaxel and methotrexate, compared to untreated cells. However, cells treated with mitomycin C had a decrease and increase in D-lactate and L-lactate formation respectively.

Conclusions: Stable increased Glo1 expression in HEK293 cells conferred MDR to clinical anticancer drugs. In most cases, MG was increased by drug-induced increased glycolysis and increased flux of MG formation. At the GC50 values, anticancer drugs were not direct Glo1 inhibitors.

COMPARISON OF PLASMA AND SERUM ADVANCED GLYCATION ENDPRODUCTS LEVELS: A MULTIVARIATE APPROACH. Manuel Suárez1, Mingzhan Xue3, Naira Rabbania1-3 and Paul J. Thornalley4

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Background and aim: Advanced glycation endproducts (AGEs) are a group of compounds, formed by glycation of proteins. AGEs have been linked to the onset and progression of a wide range of diseases such as diabetes, cardiovascular disease, chronic kidney disease, arthritis, autism and cancer. Their quantification in blood samples is gaining increasing attention as a suitable procedure for disease diagnosis and prediction of progression from the early-stages. Estimation of glycation free adducts (glycated amino acids) is often preferred due to the limited pre-analytic processing required – usually preparation of sample ultrafiltrate. Although AGEs are quantified in serum and plasma, a direct comparison of the estimates obtained and the potential effect of serum preparation is lacking. The aim of this study is to compare Nε-fructosyl-lysine, AGEs, oxidation and nitration free adducts in plasma and serum and to confirm their suitability and analytical equivalence in clinical studies.

Methods: Human volunteers (n = 6) were recruited at University Hospital, Coventry. UK: 4 female, 2 male; age 47.8 ± 15.8 years; BMI 25.9 ± 4.0 kg/m². Venous blood samples were collected with informed consent. Ethical approval was given by the East Midlands Regional Ethics Committee UK; reference 16/EM/0065. Serum and
plasma (with EDTA anticoagulant) were prepared. Ultrafiltrate (10k Da cut-off) was prepared from the samples by microspin ultrafiltration (14,000 g, 4 °C, 1 h) and kept at -80 °C until analysis. It was then assayed for concentrations of glycated, oxidized and nitrated amino acids (glycation, oxidation and nitration free adducts) by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry using an Acquity-Xevo-TQS LC-MS/MS system (Waters, Elstree, U.K.). Analytes determined were: glycation adducts - FL, CML, CEL, CMA, G-H1, MG-H1 and 3DG-H, glucosepane and pentosidine; oxidation adducts – DT, NFK, AASA and GSA; nitration adduct 3-NT (see references for acronyms). The evaluation of the impact of the whole set of compounds was performed with Metaboanalyst using multivariate analysis such as principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and hierarchical heatmaps.

**Results:** No significant difference in the concentration of glycated, oxidized and nitrated amino acids was observed between plasma and serum. Multivariate analysis showed that samples from the same person group together. This confirms the suitability of both fluids for assay of glycation, oxidation and nitration free adducts.

**Conclusion:** The analysis of plasma or serum for Ne-fructosyl-lysine, AGE and trace-level oxidation and nitration free adducts may be performed and used with analytical equivalence in clinical studies.

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The improvement of diabetic nephropathy and Alzheimer’s disease-like symptoms through the glyoxalase 1 modulation in *Drosophila* nephrocytes. Wan-Ju Yeh, Chih-Chiang Chan, Yung-Ming Chen, Shuei-Liong Lin

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The progression of chronic kidney disease is closely related to Alzheimer’s disease. Advanced glycation end products (AGEs), one form of uremic toxin, have been proposed to deteriorate diabetic nephropathy (DN), as DN patients with higher circulating AGEs levels. However, whether AGEs causally related to the impairment of brain function in DN patients remains unknown. The present study aimed to investigate the interaction between DN and AD using *Drosophila* model to interrogate the molecular action of AGEs. The *Drosophila* nephrocyte shares functional and structural similarities with mammalian podocytes and proximal tubules. Results from the methylglyoxal (MG) feeding study showed that, treatment of MG to wild-type flies led to smaller body size and a delay in development. The lethal dose of MG had been identified as 60 μM, no surviving fly was found when fed with MG of higher concentrations. Additionally, excessive dietary MG disrupted the nephrocytic function and stimulated reactive oxygen species formation. Interestingly, the impairment of nephrocytic function led to elevated levels of circulating MG, which ultimately caused the CEL accumulation in brain. This study also established a dual-expression system of *Drosophila* for exploring the interaction of DN and AD. The fly strain that expressed human αβ in brain and glyoxalase (GLO)1 in nephrocyte had been genetically produced by Lex-LexAop system and GAL4-UAS system, respectively. Our results revealed that nephrocytic-specific knockdown of GLO1 impaired the neuromuscular activity on day 21 in the following of a decrease nephrocyte function on day 7. In conclusion, GLO1 may act as a protective gene against DN, and subsequently delay the development of AD.

**Glycation of optin reduces its anti-angiogenic activity: Relevance to diabetic retinopathy.** Ahmeda Benjama, Mark Slevin and Nessar Ahmed. School of Healthcare Science, Manchester Metropolitan University, Manchester, United Kingdom (N.Ahmed@mmu.ac.uk)

Diabetic retinopathy affects around 60% of diabetic patients within 20-years of diagnosis. Diabetic retinopathy is characterised by uncontrolled angiogenesis affecting the retina of the eye. Build-up of tissue advanced glycation endproducts (AGEs) have been implicated in diabetic complications including retinopathy. Indeed, methylglyoxal, a reactive dicarbonyl compound found in retinal cells can rapidly form AGEs that are not only detectable in the retina but are involved in the pathogenesis of diabetic retinopathy. Opticin, a 45KD protein located in the vitreous and retina of the eye inhibits angiogenesis, and could offer protection against retinopathy. In this study, the effect of glycation on opticin-induced inhibition of angiogenesis *in vitro* was determined together with the associated intracellular signalling. We also investigated whether opticin undergoes glycation in the retina of diabetic mice. Opticin was glycated *in vitro* by incubating with methylglyoxal in phosphate buffer, pH 7.4 at 37°C for up to 24-hours. The extent of glycation of opticin was quantified by detecting a shift in mass...
with increasing glycation following mass spectrometry. Angiogenesis was assessed by examining the proliferation and migration of cultured aortic endothelial cells in the wound-healing assay. Intracellular signalling changes of p-ERK1/2 following exposure to glycated and native opticin were analysed using Western blotting. Immunohistochemistry coupled with fluorescence microscopy were used to examine eye sections from diabetic mice in order to detect any co-localization of opticin and AGEs. Glycation of opticin reduced its anti-angiogenic ability and was associated with an increase in p-ERK1/2 expression. Increased co-localization of opticin and AGEs was detected in the eyes of diabetic mice. Glycation of opticin impairs its anti-angiogenic properties and such an effect in vivo may in part explain the uncontrolled angiogenesis seen in patients with diabetic retinopathy. Glycation of opticin deserves more attention in order to understand the underlying cellular and molecular mechanisms in diabetic retinopathy so that an effective treatment can be developed.

N5-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) is increased in skin biopsies of patients with systemic sclerosis: a potential role of AGES in the pathogenesis? I.M. Atzeni1, I. Groendijk2, A.J. Stel2, A.J. Smit3, G.F.H. Diercks3, J. Westra3, D.J. Mulder3. 1Department of Internal Medicine, Division of Vascular Medicine, University of Groningen, Groningen, The Netherlands. 2Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. 3Department of Pathology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. (i.m.atzeni@umcg.nl).

Background and aims: Systemic sclerosis (SSc) is a rare auto-immune disease hallmarked by progressive fibrosis and generalized vasculopathy, leading to severe internal organ complications and premature mortality. Vasospastic attacks of the digital arteries known as Raynaud’s phenomenon (RP) are usually the first presenting symptom. Insidiously, more skin and organ involvement evolves, which is irreversible due to the underlying fibrotic processes. Therefore, it is of importance to study pathways that precede complications, which can be easily identified and modified early in the course of the disease. Ligand-RAGE interactions lead to pro-inflammatory mechanisms. AGES are important ligands and are increased in SSc and putatively associated with profibrotic processes.

Patients and methods: 12 skin biopsies of SSc patients and one healthy control were compared by immunohistochemistry. SSc patients were selected based on 2013 EULAR/ACR clinical criteria from which skin biopsies have been previously taken for clinical practice. These biopsies included 6 of affected skin (median age 56 years, 1 male) and 6 of unaffected skin (median age 58 years, 2 males). Distribution of N5-(carboxymethyl)lysine (CML) and N5-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) was obtained with anti-AGE-antibodies. The intensity was assessed semi-quantitatively on epidermal cells, skin adnexa, endothelium, inflammatory cells, dermal fibroblasts and muscle. These were graded as follows: 0 (no staining), 1 (weak), 2 (moderate) and 3 (strong) intensity.

Results: Affected skin of SSc patients showed severe thickening of the dermis due to collagen deposition. MG-H1 staining was more intense on all skin structures in SSc patients compared to our healthy control. CML staining showed no differences between SSc patients and control. While comparing affected and unaffected skin, MG-H1 showed more pronounced presence on endothelium, inflammatory cells and dermal fibroblasts in affected skin and more presence on skin adnexa in unaffected skin.

Conclusion: These preliminary results show that MG-H1 accumulation is present in skin of patients with SSc and co-localizes with cells involved in its pathogenesis. Interestingly, this was not observed for CML. Additionally, preliminary results from our group also showed that in vitro stimulation of human dermal fibroblasts with AGE-BSA showed strong inflammatory responses (data not shown). Therefore, the AGE-RAGE axis will be further investigated in inducing a profibrotic milieu leading to fibrotic differentiation of fibroblast preceding complications in systemic sclerosis. The role of RAGE will be investigated using a specific RAGE inhibitor

Skin accumulation of advanced glycation end products is increased in patients with an abdominal aortic aneurysm. Boersema JP, de Vos LC, Links TP, Mulder DJ, Smit AJ, Zeebregts CJ, Lefrandt JD. 1 Division of Vascular Medicine, Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. 2Division of Vascular Medicine, Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. 3Division of Endocrinology, Department of Internal Medicine, University of Groningen, University Medical Center Groningen,
OBJECTIVE: Advanced glycation end products (AGEs) are implicated in the pathogenesis of cardiovascular disease. Accumulation of AGEs is driven by oxidative or glycemic stress and can be assessed by skin autofluorescence (SAF). SAF is increased in patients with peripheral artery disease (PAD) and independently associated with mortality and major adverse cardiovascular events in these patients. PAD and abdominal aortic aneurysm (AAA) share several risk factors. Inflammation is an important process in AAA formation and increases levels of oxidative stress. We therefore hypothesized that SAF would be increased in AAA patients compared with controls.

METHODS: A case-control study was performed in 248 AAA patients and 124 controls without AAA or PAD matched for age and presence of diabetes mellitus. SAF was noninvasively assessed with the AGE Reader (Diagnotics Technologies BV, Groningen, The Netherlands).

RESULTS: SAF was higher in AAA patients than in controls: 2.89 ± 0.63 vs 2.68 ± 0.63 arbitrary units (P = .003). PAD comorbidity was associated with increased SAF within the AAA patient group (P = .01). After correction for known factors influencing SAF (age, current smoking, hypertension, and estimated glomerular filtration rate), PAD comorbidity remained an independent determinant of SAF. Logistic regression analysis of the total cohort showed an unadjusted odds ratio (OR) of 1.74 (95% confidence interval [CI], 1.20-2.51) for the presence of AAA with each unit increase of SAF and an adjusted OR of 1.78 (95% CI, 1.22-2.60) after correction for cardiovascular comorbidity (cerebrovascular disease and coronary artery disease). After additional correction for sex, current smoking, hypertension, and use of lipid-lowering drugs, this significance was lost (adjusted OR, 1.53; 95% CI, 0.94-2.48).

CONCLUSIONS: Skin accumulation of AGEs, measured by SAF, is increased in patients with AAA compared with controls without AAA or PAD, independent of the presence of coronary artery disease and cerebrovascular disease. In AAA patients, SAF is closely associated with the presence of PAD and cardiovascular risk factors.


AGEs in extracellular matrix enhance the TGFβ2-mediated mesenchymal transition of lens epithelial cells. Mi-Hyun Nam1 and Ram H. Nagaraj1,2,*. 1Department of Ophthalmology, School of Medicine and 2Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado, Anschutz Medical Campus, Aurora, CO. (mi-hyun.nam@ucdenver.edu).

The capsule secreted by the lens epithelium is the thickest basement membrane (BM) in the human body. The proteins in the BM are generally long-lived and are targets for post-translational modification during aging. Our previous study showed that with aging, AGEs accumulate in the human lens capsule and promote TGFβ2-mediated epithelial-to-mesenchymal transition (EMT) of lens epithelial cells (LECs), which could play a role in fibrosis associated with posterior capsule opacification (PCO). We have also shown that αB-crystallin is essential for the TGFβ2-mediated EMT in LECs. We have now investigated the signaling mechanisms by which AGEs in extracellular matrix (ECM) enhance the TGFβ2-mediated EMT in LECs. We found that ECM-AGEs upregulated the non-canonical pathway of TGFβ2 signaling by promoting phosphorylation of ERK, AKT, and p38 MAPK during EMT. This EMT response was strongly suppressed by the AKT specific inhibitor LY294002, which also suppressed TGFβ2-induced upregulation of nuclear Snail and inhibited phosphorylation of GSK3β. αB-Crystallin was upregulated in an AKT-dependent manner during AGEs/TGFβ2-mediated EMT in LECs. The absence of αB-crystallin in LECs suppressed TGFβ2-induced GSK3β phosphorylation, resulting in lower nuclear Snail levels. Taken together, these results show that ECM-AGEs enhance the TGFβ2-mediated EMT response through activation of the AKT/Snail pathway, in which αB-crystallin plays an important role as a linker between the TGFβ2 and AGE-mediated signaling pathways.

α-Dicarbonyls are increased in the left ventricle during myocardial infarction in humans and overexpression of glyoxylase-1 reduces infarct size in a rat model of myocardial infarction. Chimhanda T1, Hanssen N, Brouwers O.1,3,4, Krijnen P.A.J.1, Miyata T.3, Stehouwer C.D.A.1,4, Niessen H.W.M.2, Schalkwijk C.G.3,4,1 Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, the Netherlands.
Myocardial infarction (MI) is a major cause of death in Western society. The outcome of MI is partly dependent on inflammation and endothelial dysfunction following initial ischaemic-reperfusion. We previously described the presence of advanced glycation end products (AGEs) on the activated endothelium of small intramyocardial blood vessels in MI patients. Recent work found that AGEs contribute to negative cardiac remodelling and dysfunction post-MI [2]. Together this indicates a substantial role for glycation in ischemic-reperfusion injury after MI. We hypothesised that dicarboxyls, the major precursors in AGE formation, and AGEs are increased in MI patients and that reducing dicarboxyls by glyoxylase-1 (GLO1) overexpression decreases infarct size and is protective for the outcome in MI.

Methods and Results: In post-mortem tissue from MI patients (n=20), we found an increase of the AGE precursors methylglyoxal (MGO) and glyoxal (GO), and the AGE Nε-(carboxy-methyl) lysine (CML) in the left ventricle (LV) tissue as compared to the control right ventricle (RV) from the same individuals, in MI patients who died during the first phase of an infarct. To further elaborate the role of dicarboxyls in MI, wild type (WT, n=16) and GLO-1 overexpressing (n=16) female Wister rats underwent a 40 min ligation of the left anterior descending coronary artery. Rats were sacrificed 1 or 28 days after reperfusion. GLO-1 overexpression, as confirmed by enzyme activity, inhibited MI-induced CML accumulation and resulted in a 56% smaller infarct size after 28 days reperfusion (p<0.05). Furthermore, 1 day after MI, GLO-1 overexpression reduced circulating biomarkers for cardiac injury, cTnI/T and FABP3, whose levels remained lower 28 days after infarction. The fibrotic area within the infarct area was higher in GLO-1 animals than wild-type animals (31.7% and 15.4%, respectively; p<0.05), indicating improved wound healing. Human endothelial cells cultured in a hypoxic environment (I/R, 0% O₂, 24 hrs) demonstrated reduced GLO-1 activity compared to normoxia-cultured cells (p<0.001) and coincided with an increase in AGES. Specific siRNA down-regulation of GLO-1 and expression profiling showed that differentially expressed genes were those predominantly involved in endothelial cell injury and activation, most of these showed similar differential expression in arrays obtained from human clinical MI.

Conclusion: Our work indicates that accumulation of α-dicarboxyls contributes to infarct size and poor prognosis following myocardial infarction. Thusly, the quenching of α-dicarboxyls during reperfusion could be an interesting intervention to improve the outcome of MI.

Pathophysiologlcal link between diabetes and colorectal cancer: effect of diabetic microenvironment, metformin and 5-fluorouracil on glyoxalase 1 protein level. Lukáš Pácal, Katarína Chalásová, Erik Kročka, Alžbeta Višocká, Katefina Kaňková. ‘Department of Pathophysiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic. paci@med.muni.cz

Background and aims: Epidemiologic studies showed that (i) type 2 diabetes mellitus is associated with increased risk of certain cancers including colorectal cancer (CRC) and that (ii) CRC clinical outcome is worse in diabetics compared to non-diabetic subjects. On the other hand, antidiabetic treatment, specifically metformin, was associated with better prognosis and also increased efficacy of standard chemotherapeutic agents in CRC. Despite the indirect evidence of anticancer effects of metformin and sporadic evidence of its benefit as an adjuvant to standard chemotherapy mechanisms are not fully understood yet. One of links explaining adverse role of diabetes in cancer incidence and outcomes might involve increased dicarboxyl stress with subsequent up-regulation of glyoxalase 1 (GLO1). While increased GLO1 level or activity in diabetic patients may be beneficial, in some tumors, including CRC, a higher level of GLO1 may confer invasive, metastatic potential and diminish tumor sensitivity to therapy. So far it is unclear whether metformin might play a direct role in regulating cancer sensitivity to therapy and whether GLO1 might mediate such effects. Aim of the project was to study the effect of (i) diabetic microenvironment, (ii) metformin and (iii) first line CRC cytostatic agent 5-fluorouracil (5-FU) on GLO1 protein level in two colorectal cancer cell lines in vitro.

Materials and methods: HCT116 (wildtype p53+/+ and deleted p53−/−) and DLD1 (wildtype AGR2+/+ and deleted AGR2−/−) were cultured 30 hours in either 5.5 mmol/l glucose (NG) or 25 mmol/l glucose (HG) with or without the
addition of metformin (500µmol/l) or 5-fluorouracil (5-FU, 5µmol/l). Protein level of glyoxalase was determined using Western blotting with specific antibody.

**Results:** Expression of GLO1 is CRC-type resp. mutation-specific differing between HCT116 and Dld1 cell lines as such and between wild-type and KO AGR2 Dld1 but HCT116 lines. Moreover, while glucose does not affect GLO1 protein level in neither of the wild type cell lines, HG affects GLO1 level in conflicting manner – increasing it in HCT116 p53+/− and decreasing it in Dld1 AGR2−/− (results are not statistical significant though). Furthermore, in the case of treatment, the GLO1 level was decreased by 5-FU in Dld1 AGR2+/+ cells but not in AGR2−/− or any HCT116 cells. Finally, we did not find the effect of metformin on GLO1 protein level in any of the cultivating conditions.

**Conclusion:** We observed cancer cell-specific differences in GLO1 expression. AGR2 might be an important player in GLO1 regulation mediating also the effect of glucose and 5-FU on GLO1 expression. On the contrary, we were not able to document effect of metformin those settings. The results therefore hypothetically support the possible involvement of dicarbonyl stress in CRC biological and clinical behaviour but limit the evidence for metformin direct effects. (This study was supported by the grant GA16-14829S from the Czech Grant Agency)

**METABOLIC DRIVERS OF DICARBONYL STRESS INDUCED BY HIGH GLUCOSE CONCENTRATION IN HUMAN PROXIMAL TUBULAR EPITHELIAL CELLS IN CULTURE.** Ohoud Al-Ghamdi,¹ Mingzhan Xue,² Paul J Thornalley³ and Naila Rabbani¹. ¹Clinical Sciences Research Laboratories, University of Warwick, University Hospital, Coventry, UK and ²Proteomics Research Technology Platform, University of Warwick, University Coventry, UK. O.Algamdi@warwick.ac.uk

**Background and aim:** Dysfunction of renal proximal tubular epithelial cells (PTECs) in hyperglycemia in diabetes is associated with dysfunction linked to the development and progression of diabetic kidney disease. This is characterised by increased production of transforming growth factor-β leading to tubulointerstitial fibrosis, abnormal salt and glucose transport and increased cell senescence. Glyoxalase 1 (Glo1) catalyses the metabolism of the reactive glycating metabolite, methylglyoxal (MG). Overexpression of Glo1 prevented the development of experimental diabetic kidney disease in mice, even when limited to PTECs and vascular endothelial cells. It therefore appears that dicarbonyl stress induced by hyperglycemia in PTECs may be influential in development of diabetic kidney disease. In this study we sought to investigate the metabolic drivers of increased MG, precursor of major increased advanced glycation endproducts (AGEs) in diabetic kidney disease, by studying human PTECs in primary culture in low and high glucose concentrations.

**Materials and methods:** Human PTECs were incubated in primary culture with 7 mM (model normoglycemia) or 25 mM glucose (model hyperglycemia) for 4 days in quadruplicate, changing medium after 2 days. Glucose consumption and flux of formation of L-lactate and D-lactate were measured by enzymatic assays over the initial 2 days of culture; the latter is a measure of flux of MG formation. The activities of Glo1 and glyoxalase 2 (Glo2) were assayed by spectrophotometric assay after 4 days.

**Results:** When human PTECs were incubated with high glucose concentration in vitro for 4 days there was a decrease of Glo1 activity, with respect to low glucose control: 350 ± 19 versus 249 ± 19 mU/mg protein; - 29%, P<0.01. Glo2 activity was unchanged: 39.3 ± 12.3 versus 39.3 ± 2.2 mU/mg protein. There was a 3-fold increase in glucose consumption by PTECs in high glucose cultures: glucose consumption (nmol/day/10⁶ cells) – 18.2 ± 1.4 versus 5.7 ± 1.0; P<0.001. This was associated with an increase in flux of formation of D-lactate – 2.70 ± 0.12 versus 2.21 ± 0.32; + 22%, P<0.05 – and increase in net flux of formation of L-lactate - 293 ± 12 versus 260 ± 0.12; + 13%, P<0.05.

**Conclusion:** Human PTECs suffer down regulation of Glo1 activity and increased in flux of MG formation in model hyperglycemia in primary culture. These are likely metabolic drivers synergising to increase the concentration of MG in PTECs in diabetes. Overexpression or Glo1 inducer therapeutics may correct this and prevent and alleviate diabetic kidney disease.

**Total Synthesis of Pentosidine in Multi-Gramm Scale.** John Fontan*, Philippe Maetz*, Raimund Maier*, Miriam Sandner*, Haixiang Zhang*, and Thomas Bruckdorferab* ¹Iris Biotech Laboratories GmbH, Adalbert-Zoellner-Str. 1, D-95615 Marktredwitz, Germany
The extracellular matrix in most tissues is characterized by progressive age-related stiffening and loss of proteolytic digestability that are accelerated in diabetes and can be enhanced by the non-enzymatic reaction of reducing carbohydrates and extracellular matrix proteins. Non-enzymatic reaction between reducing sugars and amino acids called the Maillard reaction was first reported in the field of food chemistry more than 100 years ago. This reaction also progresses slowly primarily from glucose with proteins in vivo, and an early product called the Amadori product is converted into advanced glycation end-products (AGEs). AGEs are continuously formed under normal circumstances, but more rapidly under a variety of stresses, especially oxidative stress and hyperglycemia. They serve as markers of stress and act as toxins themselves. Pentosidine is typical of the class and its fluorescence allows it to be seen and measured easily. Because it is well characterized, it is often studied to provide new insight into the biochemistry of AGE compounds in general. Derived from ribose, a pentose, pentosidine forms fluorescent cross-links between the arginine and lysine residues in collagen. Although it is present only in trace concentrations among tissue proteins, it is useful for assessing cumulative damage to proteins by non-enzymatic browning reactions with carbohydrates. In vivo, AGEs form pentosidine through sugar fragmentation. In patients with diabetes mellitus type 2, pentosidine correlates with the presence and severity of diabetic complications. Due to its high pharmacological interest increasing supply and large scale production becomes a necessity with high importance. We describe here a new synthetic process, which allows to produce pentosidine on multi-gram scale by a very economic process. It nicely can be characterized and yields with superior purity.

Convergence of Plasma Glycation Product Levels with Conventional (CONV) Treatment at the End of the Intensive (INT) Glycemic Control Period in the DCCT Study. David R. Sell, Bernadette T. Fokkens, the DCCT/EDIC Research Group, and Vincent M. Monnier. Departments of Pathology and Biochemistry, Case Western Reserve University, Cleveland, Ohio, USA. Division of Vascular Medicine, Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. (drs7@case.edu).

Metabolic memory represents the durable long-term effects of INT glycemic therapy on long-term complications after hemoglobin A1C levels have risen. Our previous studies showed that age-related processes such as AGE accumulation and insolubilization of protein like collagen are accelerated in T1D and are related to the progression of diabetic complications. In the present longitudinal study, a total of 182 individuals with T1D, age 13-39 years at baseline, were randomly assigned and treated for up to 10 years with either INT (n=102) or CONV (n=80) for glycemic control as part of the DCCT study. A1C levels were ~ 7% and 9% in the INT and CONV groups, respectively. At study end, CONV individuals were trained in INT, all subjects were encouraged to continue INT, and diabetes care was returned to their own providers. All individuals were then observed for as long as 25 years after DCCT end during which A1C levels were nearly identical ~ 8%. Plasma and urine samples were taken at three different time points, TP1, TP2, TP3, corresponding to approximate years 4, 8 and 17 from onset of the DCCT study. Skin biopsies were surgically obtained at years 8-9 (i.e., close to TP2) and insoluble collagen samples were prepared by extraction. Fourteen markers for AGE formation and oxidation were measured by liquid chromatography-mass spectrometry (LC-MS) in digests of plasma proteins and collagen as well as the 10KD filtrates of urine for each individual at each TP (TP2 for collagen). Results: Three markers emerged as the most important in explaining treatment levels at TPs. Levels of fructose-lysine, glucosepane and CML were all significantly (P<0.0001) decreased in plasma proteins at both TP1 and TP2 in INT vs. CONV, but increased at TP3 after A1C rose in the original INT. In comparison, levels in CONV were significantly (P<0.01) decreased in plasma proteins at TP3 (vs. TP1 and TP2) converging with levels observed in the INT group at TP3. Levels were highly correlated with hemoglobin A1C at each TP in plasma proteins (fructose-lysine, glucosepane, CML, r=0.395-0.601, P<0.0001) and urine (fructose-lysine, glucosepane, r=0.307-0.403, P<0.0001; CML, P>0.05). Levels significantly correlated with collagen insolubility at TP2, but not at TP1 and TP3: plasma proteins (TP2), r=0.193-0.273, fructose-lysine (P<0.0001), CML (P<0.001), glucosepane (P<0.012); urine (TP2), r=0.177, fructose-lysine, glucosepane (P<0.034), CML (P>0.05). Overall, convergence of CONV with INT was indicated by a nonsignificant (P>0.05) difference in levels between these treatments at TP3.
**Advanced Glycosylation end Products and cardiovascular risk in Mexican young population with metabolic syndrome.** Preciado-Puga Mónica del Carmen1, (Garay-Sevilla Ma. Eugenia1, Gómez-Ojeda Armando1, Muñoz-López Daniela Beatriz2, González-Yebra Ana Lilia4 Macías-Cervantes Maciste Habacuc2, García-Espitia Jorge Arturo2). 1Universidad de Guanajuato, Campus León. Puente Milenio 1001, Predio San Carlos, León, Gto, México. (mdc.preciadopuga@ugto.mx).

**Introduction.** The Metabolic Syndrome (MS) is a set of metabolic abnormalities considered a risk factor for developing cardiovascular diseases and is a public health problem in México. Carboxymethyllysine (CML) is one of the Advanced Glycosylation end Products (AGE) that have been related with increase in cardiovascular risk in people with SM. The Castelli I index is a ratio useful in the assessment of vascular risk which could be used in Mexican population to evaluate the cardiovascular risk related to the level of AGEs in serum.

**Objective:** To correlate the cardiovascular risk using the Castelli I index with CML in serum (sCML) of patients with positive components of the MS.

**Methods:** A transversal study was conducted in Mexican shoe workers in central Mexico. MS was diagnosed using the harmonized criteria for which blood pressure (>130/90 mmHg), waist circumference (>90 cm in men, >80 in women), triglycerides (>150 mg/dl), HDL (<40 mg/dl in men, <50 mg/dl in women) and fasting glucose (>100 mg/dl) were measured. The Castelli I index was calculated as follows: total cholesterol/high density cholesterol and sCML was measured with MyBiosource® ELISA. Statistica V13 was used for the statistical analysis. Descriptive statistics, U-Mann Whitney, Kruskall Wallis and Pearson correlation were performed using a value of \( p \leq 0.05 \) as significant. The protocol was approved by the local ethics committee.

**Results:** 271 shoe workers participated in the study (68.6% men and women 31.4%), age 38±11.9 years. We found statistically significant difference between the groups with \( n=138 \) and without SM \( n=133 \) in sCML (0.4 - 8.94 ng/mL), median 1.475 vs (0.24 - 5.7 ng/mL), median 1.3). A statistical difference was found between the people with 5 positive components of MS and the other groups with 4, 3, 2, 1 and 0 positive components in sCML. We found a positive correlation between sCML and the Castelli I index \( (r=0.183051, p <0.05) \). \( (H=13.13864, p=0.000) \) and Castelli I index \( (H= 113.3854, p=0.000) \).

**Conclusions:** We found differences between group and with without MS in the level of sCML, while cardiovascular risk and sCML levels were as high as the number of positive MS components increased, supporting that cardiovascular risk is related with the level of AGEs in circulation. Modifications in lifestyle could reduce the number of positive MS components reducing the cardiovascular risk through the AGEs level among other factors within this population. (Financial support PRODEP UGTO-PTC-475)

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**Posters in Food**

**The Reduction Mechanism of Catechins on Ne-(carboxymethyl) lysine.** Yuting Li1, Lin Li1,2,a,b,c,*, Bing Li1,2,a,b,c,*, Jie Zhu2, Xiaozhen Liu1, Yi Hu5, Xia Zhang2,a. 1 School of Chemical Engineering and Energy Technology, Donggwan University of Technology, Donggwan, China. 2 School of Food Science and Engineering, South China University of Technology, Guangzhou, China. 3 Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, Guangzhou, China. (liyt@dgut.edu.cn)

Dietary advanced glycation end products (AGEs) have been paid more and more attention since their relationship with many diseases has been proved. In order to reduce the hazard of dietary AGEs, the AGEs levels in foods should be cut down. Besides inhibition effect, additives may also have the potential elimination effect on AGEs which is ignored by food researchers. In this study, the elimination and reduction mechanism of Ne-(carboxymethyl) lysine (CML, a typical AGE) by catechins was investigated. The efficiency of EC and EGC at 80 and 100°C in a glucose-lysine model system at pH 5.0, 7.0 and 8.0 to mimic different food processing conditions by HPLC-MS. 0.001-5% of EC and EGC were found to
reduce CML concentration by up to 45.5 ± 1.0% and 51.0 ± 1.7% respectively in the model system. EC and EGCG could reduce the glyoxal levels and increase the fructosamine levels in the model systems by up to 25.3 ± 1.2% and 24.5 ± 0.9% respectively, indicating the inhibition effect on CML formation. In addition, the quinones of EC and EGCG were found to trap CML by a Michael addition or Schiff base addition to form catechin-CML adducts under neutral (pH 7.0) and alkaline conditions (pH 8.0), which may be an additional mechanism by which CML concentration is reduced. Adducts formed via the reaction of catechin quinones with CML in the catechins additional glucose-lysine system were measure by UPLC-ESI-MS/MS. These achievements could be supplementary materials for the existing inhibition mechanism of CML, and proved that catechins could reduce the hazardous compounds levels produced during the Maillard reaction. They enriched the theories in food safety research.

Inhibition of acrylamide by glutathione in model system and cookies. Yuchen Zhu a, b & Fang Chen a, b

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Acrylamide (AA, CAS No. 79-06-1), a neurotoxin, genotoxin, and potential carcinogen (IARC, 2007), is commonly found in starch-rich foods (Friedman, 2003). Many strategies have been designed to inhibit the formation of AA during food processing, such as reducing the contents of precursors, optimizing process parameters, using novel processing methods, and adding some AA inhibitory agents (Palermo, Gokmen, De Meulenaer, Ciesarova, Zhang, Pedreschi, et al., 2016). Reduced glutathione (L-γ-glutamyl-L-cysteinyl-glycine, GSH) is a major antioxidant exists in cellular redox reactions and thioether formation in both plants and animals (Wu, Fang, Yang, Lupton, & Turner, 2004). Studies reported that GSH can participate in the Maillard reaction and inhibit the formation of AA (Casado, Sanchez, & Montano, 2010; Claeyts, Vleeschouwer, & Hendrickx, 2005). The inhibition effect of GSH on AA was possibly attributed to the sulfhydryl group of GSH. However, no direct evidence for the inhibition of AA by GSH has been reported and the inhibition mechanism has not been detailed investigated. Thus, the inhibition effect of GSH on AA was investigated in the present study. Different amounts of GSH were added into the model systems and cookies to investigate the inhibition effect of different GSH level on the formation of AA. The variances of main compounds involving in AA inhibition were monitored in both model systems and cookies using UPLC-MS/MS. Results showed that GSH could significantly inhibit AA formation in both model system and cookies. In the meantime, the addition of GSH showed no significant inhibition effect on cookies texture, while increased the surface lightness of GSH added cookies. Possible inhibition pathways of GSH on AA formation were proposed. The major inhibition pathways were attributed to the competitive reaction between Asn and GSH with the available sugar moiety as well as the declined pH. Besides, the competitive reactions between Asn and GSH degradation products (i.e., glynine and cysteine) and the elimination reaction between AA and GSH or cysteine also played some minor roles in the inhibition of AA. The present work provides a better understanding of the inhibition pathways of AA by GSH and suggests that GSH could be used as a potential inhibitor to decrease AA formation in foods.

A novel compound isolated from the reduced ribose-tryptophan Maillard reaction products on the expression difference of mainly inflammatory factors. Dan Qin a, Bing Li b, c, *, Lin Li b, c, a, *, Ming Wu b, Di Zhao a, Xia Zhang a, c, Ling Chen a, c, Guoqin Liu a, c, Xiaoxi Li a, c, a School of Food Science and Engineering, South China University of Technology, Guangzhou, 510640, China, b School of Chemical Engineering and Energy Technology, Dongguan University of Technology, Dongguan, China, c Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, Guangzhou, China (danqin.scut@hotmail.com).

In this study a compound of 549.24 Da named BF-5 was purified from the ribose-tryptophan Maillard reaction products by solvent extraction and reverse phase high performance liquid chromatography. The anti-inflammatory assay indicated that BF-5 could also significantly reduced the production of NO, TNF-α, and IL-6 secreted by lipopolysaccharide- induced RAW 264.7 cells. RT2 profile PCR arrays showed that BF-5 could downregulate 38 inflammatory cytokines and upregulate 5 inflammatory cytokines. The structure of BF-5 was identified as 2-(1-carboxy-2-(1H-indol-3-yl)ethyl)-1-(1,2,3-trihydroxypropyl)-1,2,3,4,6,7,12,12b-
octahydropyrazino [1',2'-1,2] yrido [3,4'-b] indole-6-carboxylic acid by means of LC-MS/MS and 1D- and 2D-NMR analyses. The compound showed higher anti-inflammatory activity and less toxic to the normal human liver cells LO2 than dexamethasone. This research suggested the potential utilization of BF-5 as an attractive candidate for a functional therapeutic agent.

**Tagatose formed lower level of N\(^\circ\)-(carboxymethyl)lysine in low-pasteurisation condition.** Ho-Young Park, Mi-Jin Oh, and Yoonsook Kim. Division of Functional Food Research, Korea Food Research Institute, Jeollabuk-do 55365, Republic of Korea. kimyus@kfri.re.kr (Y. Kim)

Dietary advanced glycation end products (AGEs) are involved in the pathogenesis of diabetic complications, atherosclerosis, and kidney disease. In this study, we investigated the formation of \(N^\circ\)-(carboxymethyl)lysine (CML), a well-known AGES, from the reaction of casein from bovine milk with different reducing sugars (glucose, tagatose, and xylose) at various sugar concentrations and heating temperatures (75 and 120 °C). The concentration of CML was measured using an enzyme-linked immunosorbent assay. Additionally, SDS-PAGE was performed in order to observe the changes in the molecular weight of casein. The results revealed that tagatose led to a lower CML concentration at 75 °C than glucose or xylose, thus no significant differences at 120 °C. We concluded that it would be more appropriate to use tagatose rather than glucose or xylose as a sweetener in pasteurized dairy products.

**Mitigation of Acrylamide Formation in Potato Chips by Vacuum Baking.** Burçe Ataç Mogol, Kübra Akkurt, Vural Gökmen. Hacettepe University, FoQuS Research Group, Department of Food Engineering, 06800 Beytepe, Ankara, Turkey. (burcea@hacettepe.edu.tr)

After the discovery of acrylamide in a variety of foods, it took great attention both by authorities and consumers since it had already been classified as “probable human carcinogen” in class 2A by the International Agency for Research on Cancer\(^6\). Researchers started to investigate the ways to minimize the acrylamide formation in foods, especially in bread, bakery products, breakfast cereals, coffee and potato products (crisps, French fries) due to their relatively high concentration of acrylamide. These studies mainly based on controlling the precursors and the Maillard reaction, the responsible reaction for the formation of acrylamide. Decreasing process effect (thermal load) is one of the most effective ways to control Maillard reaction, thus acrylamide formation. However, decreasing the thermal load by decreasing process temperature or time might not result in the same product tested. Therefore, different alternative processing techniques have been recently introduced for acrylamide mitigation without altering the characteristics of the end product. In our previous study, vacuum baking, either alone or in combination with conventional baking, eliminated the acrylamide formation in biscuits\(^5\). The decrease in the boiling point of water under vacuum provides low-temperature baking in vacuum oven while maintaining the texture of the biscuit. Based on this, this study aimed to investigate the potential of the vacuum baking in decreasing the acrylamide formation in baked potato chips. With this regard, potato dough was prepared from potato flakes, and the dough was rolled into the diameter of 3 cm and the weight of 3.00±0.05 g. The chips were then baked either in the conventional oven (CB) at 180-190 or 200°C for different times (2-8 min) or in a vacuum oven (VB) at 120-160°C for 6-14 min (pressure 10 mbar). The amount of acrylamide in CB-chips increased by increasing temperature or time reaching to a concentration up to 1246±70 ng/g. On the other hand, vacuum baking mitigated the acrylamide formation in the chips baked at 120, 130, and 140°C. VB-chips baked at 160°C for 6 and 7 min contained acrylamide at a concentration of 77.8 ng/g and 86.3 ng/g, respectively. The reason is that the effective conductive heating in vacuum oven leads to acrylamide formation during prolonged heating.

In conclusion, vacuum baking was found to be an effective alternative technology to mitigate/reduce the acrylamide formation in baked potato chips.

**Fermented Maillard reaction products of whey protein and galactose improve intestinal barrier function and reduce inflammation in dextran sulfate sodium-induced mice's colitis.** Yu-Jin Jeong, Da Hyun Kim, Su-Hyun Chun, and Kwang-Won Lee. Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, 02841, Republic of Korea. yjinny1227@korea.ac.kr
Maillard reaction products (MRPs) which are the complex mixture of amino acids and reducing sugars were verified as anti-inflammatory and anti-oxidant chemical compounds. It has been reporting that milk proteins which were fermented by lactic acid bacteria were alleviating colon diseases. We investigated that fermented MRPs improved symptoms of colon diseases. Inflammatory bowel diseases (IBDs) belongs to autoimmune diseases and is hard to recover completely. Although there are various causes of occurring autoimmune diseases, diets and imbalance in bowel epithelial barrier are the main reasons in case of IBD. Generally, the function of the intestinal epithelial barrier which is an important factor in IBD protects lamina propria mucosae from environmental factors. The recent studies have reported the effects of various samples based on food compositions on IBDs. This study examined the improving intestinal epithelial barrier function and anti-inflammatory effects in IBD animal model. We administrated orally 14 consecutive days for confirming the precautionary effects of galactose-whey protein Maillard reaction products (Gal-WPI MRPs) on colitis. To inducing injury in colitis of mice, we supplied 2% dextran sulfate sodium (DSS) in drinking water on 7 -14 days. After sacrifice, the colon of DSS induced mice was used for analysis. We determined effects of Gal-WPI MRPs by colon length, body weight change, the permeability of intestine and biological factors. Gal-WPI MRPs have effects on anti-oxidative activity and ameliorate immune cell infiltration into colon tissue. Also, we measured the mRNA expression of cytokines such as interleukin (IL) -1β, IL-6, and tumor necrosis factor-α (TNF-α) and tight junction protein such as occludin, E-cadherin, and matrix metalloproteinase-9 (MMP-9) in DSS induced colitis to know about anti-inflammatory effect and intestinal permeability of Gal-WPI MRPs treated mice colon. Our data showed the high efficacy in mRNA level of tight junction protein and level of FITC-dextran particularly. In this study, we determined that protecting intestinal barrier by Gal-WPI MRPs is caused by maintaining the tight junction protein which connected tightly to intestinal and tightly connected barrier improve IBD.

Interactions of Coffee and Bread Crust Melanoidins with Hydroxycinnamic and Hydroxybenzoic Acids in Aqueous Radical Environment. Ecem Evrim Çelik a,b,c Jose Manuel Amigo Rubio d, Mogens Larsen Andersen d, Vural Gökmen a. a Food Engineering Department, Hacettepe University, 06800 Beytepe, Ankara, Turkey b Chemometrics and Analytical Technology, Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg C, Denmark. c Ingredients and Dairy Technology, Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg C, Denmark. ecemevrim@hacettepe.edu.tr

This study aimed to investigate the interactions of coffee and bread crust melanoidins with 20 different hydroxycinnamic and hydroxybenzoic acid (HCA/HBA) derivatives containing different numbers of –OH and –OCH3 groups localized at different positions on their aromatic ring. The mechanism of the interactions of melanoidins with other antioxidant compounds was explained with a structural approach. Experimental studies were carried out in DPPH radical medium by monitoring the absorbance in the presence of melanoidins and HCA/HBA derivatives separately and in mixtures. Design of Experiment tools were utilized to construct the experimental matrices for melanoidins + HCA/ HBA mixtures studies. PCA (Principal Component Analysis) and ASCA (Anova Simultaneous Component Analysis) were applied on the multivariate data obtained to extract the meaningful information needed. Results are given in terms of percentage of inhibition values, which were calculated with respect to the absorbance of the DPPH radical itself. Area under the curve (AUC) values calculated from the plots of time versus inhibition % for coffee and bread crust melanoidins and HCA/HBA derivatives were ranged between 6532±97-19106±85, 3997±102-7565±159 and 1678±81-22486±119, respectively. The antioxidant/ pro-oxidant properties of HCA/ HBA derivatives were changed according to their –OH and –OCH3 contents. Synergistic interactions were revealed for both coffee and bread crust melanoidins with HCA/HBA derivatives in general, with some exceptions. The significance of the concentrations of coffee and bread crust melanoidins on radical scavenging activity was clearly centered from the scores plots obtained with PCA and the p values obtained by ASCA. Phases the of radical scavenging reactions were also revealed from the loadings plots.

3-Desoxyglucosone and Methylglyoxal derived Hydroimidazolones of Creatine in Meat. Stephanie Treibmann, Franz Spengler, Jürgen Löbner, Julia Degen, Thomas Henle, Chair of Food Chemistry, Technische Universität Dresden, Bergstr. 66, Dresden, Germany stephanie.treibmann@chemie.tu-dresden.de
Creatine is an amino compound, which is linked to the energy metabolism in vertebrate muscle. Dicarbonyl compounds such as methylglyoxal and 3-desoxyglucose (3-DG) are formed from sugars or glycolysis by-products. Recently it was shown that creatine reacts rapidly with methylglyoxal to form methylglyoxal-derived hydroimidazolone of creatine (MG-HCr) [1]. In muscle no dicarbonyl compounds were found, although they are prevalent in many other foodstuffs [2]. Therefore it was the aim of this study to investigate the reaction of creatine with 3-DG and the occurrence of 3-DG and methylglyoxal derived hydroimidazolones of creatine in meat. From incubation mixtures consisting of 3-DG and creatine, a new hydroimidazolone of creatine, namely \( N^{-(4\text{-butyl-1,2,3-triol-5-oxo-1-imidazolin-2-yl})}\text{sarcosine (3-DG-HCr)} \), was isolated and characterized by NMR spectroscopy. To quantitate MG-HCr and 3-DG-HCr, raw and fried meat and meat products were homogenized and defatted. After adding isotopically labelled standards, samples were extracted, deproteinized, and analyzed via hydrophilic interaction chromatography coupled to tandem mass spectrometry. MG-HCr and 3-DG-HCr were quantitated in meat samples. While minor amounts were found in raw samples, the concentrations increased up to 100 fold during frying. The content and ratio of MG-HCr and 3-DG-HCr varies in different meat products and depends on manufacturing conditions and ingredients. These results prove that creatine acts as an efficient scavenger for dicarbonyl compounds.

The Maillard Reaction of Fructose in Sweeteners and Bakery Products. Stephanie Treibmann, Anne Hellwig, Michael Hellwig, Thomas Henle, Chair of Food Chemistry, Technische Universität Dresden, Bergstr. 66, Dresden, Germany. stephanie.treibmann@chemie.tu-dresden.de

The Maillard reaction between fructose and amino compounds, which leads to Heyns compounds as the first stable intermediates, has been hardly investigated up to now. McPherson and Krause have shown that fructose-induced modifications of proteins may play a role in fructose-containing bakery products as well as in diabetic complications. The purpose of this study was to investigate the fructose pathway of the Maillard reaction in foodstuffs via quantification of protein-bound Heyns compounds and other Maillard reaction products. Heyns compounds, Amadori compounds and protein-bound AGEs were quantitated via RP-HPLC coupled to tandem mass spectrometry after cleanup procedures and enzymatic hydrolysis. Following cleanup procedures and derivatization with o-phenylenediamine 1,2-Dicarbonyl compounds were quantitated via RP-HPLC with UV detection. Protein-bound Heyns compounds were quantified in bakery products in concentrations up to 287 mg/kg. The profiles of 1,2-dicarbonyl compounds varied in products with fructose and glucose. Therefore a new mechanism of 1,2-dicarbonyl generation from fructose was proposed. Thus, the fructose pathway of the Maillard reaction in foods was further characterized and its relevance in foodstuffs could be shown.

Glucose Fragmentation Pathways during Dicarbonyl Compounds Formation. Haiping Qi a, Lin Li a,b,c *, Bing Li a,c *, Zhi Li Liang a, Xuehui Wu a, Xia Zhang a,c, Zhenbo Xu a,c, Jianyu Su a,c. a School of Food Science and Engineering, South China University of Technology, Guangzhou, China. b School of Chemical Engineering and Energy Technology, Dongguan University of Technology, Dongguan, China. c Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, Guangzhou, China. (bli@scut.edu.cn).

Dicarbonyl compounds exist widely in commonly consumed foods. They were considered to be associated with formation of many AGEs. In this study, the source of carbon backbone of dicarbonyl compounds (glyoxal, GO; methylglyoxal, MGO; glyceraldehyde, GLA) formed in a glucose–lysine reaction system was explored. In order to obtain information about carbon skeleton fragmentation pathways of glucose in the process of dicarbonyl compounds (GO, MGO, GLA) formation, carbon atoms of glucose were traced based on stable isotope. Moreover, determination the weightings of labeled and unlabeled dicarbonyl compounds. The change of dicarbonyl compounds contents derived from glucose with high heat treatment condition (100-220°C) were investigated. It was shown that the peak levels of GO occurred at 160°C. The total amounts of GLA accumulated gradually with elevated temperature, as well as total amounts of MGO. The content weightings of GO, MGO and GLA were independent of temperature change (160-220°C), individually. The formation of GO and MGO were closely related to the fragmentation of C1-C2 of glucose. There was a minor portion of GLA derived from C1-C2 fragmentation.
Primary investigation of the occurrence of hydroxymethylfurfural (HMF) in a range of smoked food products
Bouzalakou-Butel Laura-Artemis¹, Provatidis Pantelis¹, Keith Sturrok² and Alberto Fiore³. ¹ Division of Food & Drink School of Science, Engineering & Technology. Abertay University Dundee, UK. ² Division of Science, School of Science Engineering and Technology. Abertay University Dundee, UK. (a.fiore@abertay.ac.uk)

The 5-hydroxymethylfurfural (HMF) is a chemical compound produced in foods through many different pathways. In the past few years, studies revealed its potential mutagenic, carcinogenic cytotoxic properties [1]. Determination of HMF started as an indicator of the extent of thermal processing and as a quality indicator. It has been identified in a variety of food products such as bread, breakfast cereals, juices, milk and honey [2]. In contrast with the other thermal processes that the formation of HMF occurs inside the product, food smoking fulfils the conditions that result to form HMF not only in the product (hot smoking), but also from the pyrolysis of the wood matrix that is used for smoking, hence contamination of the product [3][4]. Until now, there are no studies examining the relation between the smoking procedure and HMF contamination of smoked food. This study is a primary investigation using HPLC-UV to measure HMF levels in three categories of smoked food products; cheese, processed meat, and fish. HMF was discovered in all three product categories supports our prediction there is contamination from the smoke utilised in the process and production of HMF during cooking during the process. The results ranged from 1 ppb (Metsovone traditional Greek smoked cheese) to 4ppm (Hot-smoked ready to eat mackerel). Subsequently, only for smoked cheese products, was there found a correlation between HMF and phenolic compounds analysed thought SPME-GCMS. Samples that were higher in HMF concentration were also higher in syringol and cresols. It will be interesting to further explore the smoking procedure’s effect on the HMF formation together with mitigation strategies that reduce HMF formation while not altering the flavour of the smoked products.

Explorative investigation on the anti-glycative activity of rapeseed by-product extract. Marta Navarro⁴, Bruna de Falco⁵, Francisco J. Morales⁴, Despina Daliani⁵, Alberto Fiore⁵, ⁴ Institute of Food Science, Technology and Nutrition, Madrid, Spain, ⁵ School of Science, Engineering & Technology, Division of Food & Drink, University of Abertay, Bell Street, Dundee, Scotland, United Kingdom (a.fiore@abertay.ac.uk)

Formation of Advanced Glycation End-products (AGEs) in biological systems are increased during hyperglycaemia due to higher the levels of circulating glucose, as well as carbonyl reactive species. AGEs are causative factor of common chronic diseases. Since synthetic AGE-inhibitors exerts unwanted side effects and polyphenols act a potent antiglycative agents, vegetables (fruits, seeds and related by-products) are good candidates for searching natural inhibitors. The aim of this research is to explore the suitability of a polyphenol-rich rapeseed cake extract (RCE) in the decrease the AGEs formation in an in vitro model. Total Phenolic Content, anti-oxidant, anti-glycative activity, specific inhibition of AGEs (pentosidine and argpyrimidine), and methylglyoxal trapping capacity of the rapeseed cake extract were evaluated. The metabolomic profile of the extract was also analysed through GC-MS. Different phenols, amino acids, carbohydrates, organic acids and fatty acids are identified in the RCE by GC-MS. Results confirms the high concentration of polyphenols correlated with the antioxidant capacity and anti-glycative activity in a dose dependent manner. Rapeseed cake extract (3.7 mg mL⁻¹) reduced significantly the formation of free fluorescent AGEs and pentosidine up to 27.60%. The anti-glycative activity of the extract is likely due to the high concentration of sinapinic acid in its metabolic profile, and the mechanism of action is mediated by methylglyoxal trapping. Results pointed out the promising high potential in using Rapeseed Cake extract as a food supplement to ameliorate the formation of AGEs. Then, rapeseed cake extract should be consider a suitable candidate for the prevention of glycation-associated complications of age-related pathologies.

Investigation of Lipid Derived Amino Acid Modifications in Fried Foods. Yeşim Karademir, Mecit Halil Oztop
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Non-enzymatic browning reactions have particular importance in terms of both formation of characteristic food flavors and some potentially toxic compounds resulted mainly from amino acid modifications. Although typical reaction products have been pronounced prevalently for Maillard reactions, assessment of lipid derived protein modifications in foods is still a matter due to the lack of a common known marker. In this study lipid protein interactions were investigated in potato chips prepared in sunflower oils at different thermoxidation levels (180 °C-0, 6, 12, 18, 24 h). 2,4-decadienal was selected as the precursor carbonyl compound. In addition to 2,4-decadienal, 2-pentylpyridine and acrylamide were quantified in chips samples as reaction products. High resolution mass spectroscopy analyses confirmed the formation of a novel intermediate, Decadien-1-amine in potato chips for the first time. Overall results revealed that the significant increases were observed in Decadien-1-amine and 2-pentylpyridine amounts when the 6 h oxidized oil was used whereas 2,4-decadienal had also the highest concentrations in both chips and oils. On the other hand, acrylamide formation was not significantly influenced by heat treatment of the oil. It was concluded that lipid oxidation might have far more important role in non-enzymatic browning reactions than previously thought by the identification and measurement of more specific reaction products.

Newer aspects of Tryptophan chemistry in scavenging of carbonyl compounds. Raheleh Ghassem Zadeh, Varoujan Yaylayan, McGill University, Department of Food Science and Agricultural chemistry, Ste Anne de Bellevue, Quebec Canada (raheleh.ghaszemzadeh@mail.mcgill.ca)

Among the essential amino acids tryptophan has been shown to have the highest inhibitory activity towards early and advanced glycation of BSA and highest reduction in the levels of lysine modification compared to the control experiments. During thermal processing of foods tryptophan is also known to scavenge aromatic aldehydes such as benzaldehyde and vanillin and aliphatic aldehydes including sugars through Pictet-Spengler reaction to generate tetrahydro-β-carbolines and similar to intact tryptophan they also show activity as free radical scavengers and as antioxidants. However, their thermal stability and degradation products under processing conditions have not been studied. Commercially available 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole was pyrolyzed at 250°C as a model tetrahydro-β-carboline and the degradation products were analyzed through GC/MS. The analysis have indicated the formation of tryptamine and 1,2,3,4-tetrahydrocyclopenta[b]indole consistent with a reverse Pictet-Spengler reaction. In addition, indole, 2-ethenyl-2H-indol and 3-methylindole were also detected; the latter two compounds can arise from the degradation of tryptamine. The identification of 2,3-dimethylindole in the reaction products also confirmed the release of formaldehyde from the reverse Pictet-Spengler process and its subsequent capture by 2-methylindole. Subsequently, various tetrahydro-β-carbolines were pyrolytically generated in situ from the interaction of tryptophan with various aldehydes and analyzed through GC/MS and isotope labeling technique to confirm the generality of the reverse Pictet-Spengler reaction. Furthermore, aqueous mixtures of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole; tryptophan and phenylacetaldehyde and phenylacetaldehyde/indole in 1:1 relative molar ratios were heated in closed reactors at 220°C for 2 h, and the samples were analyzed using qTOF-MS/MS, isotope labeling and commercial standards to identify the formation of tryptamine and the capture of phenylacetaldehyde by indole. The analysis have indeed indicated the formation of tryptamine and 2,3-bis[(E)-2-phenylethenyl]-1H-indole. This study supports the use of tryptophan as a potential carbonyl scavenger in food processing acting not only through Pictet-Spengler and its reverse reaction but also through its various degradation products such as indole and 3-methylindole.

Glycation of seed proteins during accelerated ageing. Maria Vikhnina, Tatiana Mamontova, Elena Lukasheva, Marie-Louise Heymich, Sabrina Gensberger-Reigl, Tatiana Bilova, Wolfgang Hoehenwarter, Monika Pischetsrieder, Sergei Medvedev, Galina Smolikova, Andrej Frolov. 1Department of Biochemistry, St. Petersburg State University, 2Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, 3Food Chemistry Unit, Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg, 4Department of Plant Physiology and Biochemistry, St. Petersburg State University, and 5Proteome Analysis Research Group, Leibniz Institute of Plant Biochemistry. vikhnina@gmail.com
In the modern world, seeds of crop plants represent one of the major sources of daily consumed foods. Among them, cereals, legumes, and oilseed rape dominate in European agriculture, tremendously impacting in global protein consumption and production of biodiesel. Importantly, both quality and food safety of seeds can be affected by prolonged storage. Moreover, even a short exposure of seeds to high temperatures and humidity results in loss of their viability and quality, i.e. events, accompanying seed ageing. Although protein glycation was reported to accompany such accelerated ageing of seeds, corresponding products, as well as protein targets and potential effects in heterotrophic consumers, are still unknown. Therefore, here we characterize the changes in seed glycated proteome, accompanying accelerated ageing of pea seeds, and address possible effects on mammalian cells. For this, proteins were isolated from pea seeds by phenol extraction, quantitatively reconstituted in presence of detergents, hydrolyzed by trypsin, and resulted digests were analyzed by nanoHPLC-Q-Orbitrap-MS. Identification of glycated peptides with affected sites therein and annotation of modified proteins relied on SEQUEST search against a combined legume sequence database, whereas label-free relative quantification was performed by LCGuan software. To assess the overall glycation levels, reconstituted total protein fraction was subjected to exhaustive enzymatic hydrolysis. Resulting monomers were derivatized with N²-(5-fluoro-2,4-dinitrophenyl)-L-valine amide (L-FDVA) and analyzed by UHPLC-LIT-Orbitrap-MS. The analysis revealed significant age-related alteration in AGE patterns, although some products demonstrated decrease in their abundance. To explain this, additional metabolomic experiments are being carried out. The authors thank Russian Science Foundation (project No. 17-16-01042) for financial support.

**Effect of Roasting on Early Glycation and Advanced Glycation End Products of Hazelnuts.** Neslihan Göncüoğlu Taş, Vural Gökmen. Food Quality and Safety (FoQuS) Research Group, Department of Food Engineering, Hacettepe University, Beytepe Campus, Ankara, Turkey (neslihangoncuoglu@gmail.com).

Hazelnuts (*Corylus avellane* L.) are consumed especially in snacks and confectionaries after a roasting process is applied. Roasting conditions, 100-160°C for 10-60 min, and the composition of hazelnuts (presence of reducing sugars, amino acids, monounsaturated fatty acids) are suitable for proceeding of series of reactions concurrently. Maillard reaction, caramelization and lipid oxidation are the main reactions taking place during roasting of hazelnuts. With the contribution of these reactions; desirable characteristics like color, flavor and texture are gained by hazelnuts although some undesirable changes like loss of amino acids, formation of 5-hydroxymethylfurfural, early and advanced glycation products are inevitable. In order to understand the effect of roasting process on the progress of Maillard reaction, roasting of Turkish Tombul hazelnuts was performed at 140, 150, 160 and 170°C for 15, 30, 45 and 60 min. As an early glycation marker of Maillard reaction, N-ε-fructosellysine was measured after being converted to furosine by an acid hydrolysis (8N HCl, 110°C for 23 h). Lysine analysis of roasted hazelnuts was also performed by using the same acid hydrolysate to understand the modifications in side chains of protein-bound lysine. N-ε-carboxymethyllysine (CML) was determined as an advanced glycation marker of Maillard reaction. For the analysis of CML, a borohydride reduction and an acid hydrolysis step were applied. After evaporation of acid and addition of water, the samples were passed through hydrophilic-lipophilic balance cartridges and then taken into vials for LC/MS-MS analysis. Furosine content of hazelnuts reached to its maximum level after 30 min of roasting at 140°C (7.5 mmol furosine/mol lysine) and 15 min of roasting at 150, 160 and 170°C (7.9, 8.4, and 8.2 mmol furosine/mol lysine). However, furosine content decreased with increased roasting times due to degradation of N-ε-fructosellysine or reactions of amino acids and sugars other than Maillard reaction. Modification of lysine did not show a similar trend with fur as it continuously increased during roasting, being faster at the 15 min of roasting. CML showed an increasing trend during roasting at 140 and 150°C (2.5 and 3.6 mmol CML/mol lysine after 60 min) while it showed a slow decrease during prolonged roasting at 160 and 170°C. Maximum CML concentration, 5.2 mmol/mol lysine, was reached after roasting at 170°C for 30 min. Determination of fate of Maillard reaction with early and advanced glycation markers at various roasting temperatures and times could help optimization of roasting process from the viewpoint of food safety and health.

**Quality-driven design of heat-treated food: exploring reactivity in bakery products.** M. Cepeda-Vázquez, V. Camel, D. Blumenthal, B. Rega *. UMR Ingénierie Procédés Aliments, AgroParisTech, Inra, Université Paris-Saclay, 91300, Massy, France. (barbara.rega@agroparistech.fr)
A current issue for food safety authorities, industry and research, is the occurrence of furan in food. This heat-generated toxicant may be produced from food constituents via several reaction pathways (such as the Maillard reaction) along with numerous other compounds that contribute to food key quality attributes for consumers (e.g. aroma or color). Developing safe and appealing heat-treated food is therefore an emerging challenge for which reactivity must certainly be considered. In this regard, this work offers a novel and holistic approach in which the relationship between reactivity and quality is first explored (using an optimal design of experiments and principal component analysis), and then used as a means for food product optimization (through a desirability function). More precisely, formulation and processing conditions are considered together as factors impacting food safety and quality in a largely consumed baked good, namely cake. While key aroma marker generation, produced via several reaction pathways is studied along with physical properties and sensory evaluation, furan mitigation and consumer liking are used as optimization criteria. Our findings show that formulation and baking factors all contribute to the formation of furanic compounds either directly or through their interactions. However, when considering the nature of sugar, furan, furfural and 1-hydroxy-2-propanone are predominantly formed from glucose, either via caramelization or Maillard reaction, while pyrazines would be majorly formed from sucrose via sugar hydrolysis and further Strecker degradation. Overall, high sucrose content (with respect to glucose), low temperature and short baking times yield sponge cakes with a minimal furan content and a maximal liking score in the studied product range. Whole egg content (in relation to egg white) may be set in line with preference or by applying different optimization approaches. This work is undoubtedly an important step towards the development of novel strategies for quality-driven design of heat-treated food by considering the link between reactivity and quality.

**A solid food model for the study of Maillard reaction kinetics in realistic baking conditions.** Jeehyun Lee*, Stéphanie Roux, Catherine Bonazzi, Barbara Rega. UMR Ingénierie Procédés Aliments, AgroParisTech, Inra, Université Paris-Saclay, 91200 Massy, France. (jeehyun.lee@agroparistech.fr)

In baked foods, thermal reactions like Maillard and caramelization reactions occur leading to a multitude of quality-related compounds like aroma or health-related compounds. In solid foods the complexity of process and formulation steps has a profound impact on reactivity as it particularly depends on the interdependency of physical and chemical parameters. Unravelling the exact mechanisms and tracking the relevant reaction markers is therefore a real challenge if we want to master food reactivity. Therefore studying complex Maillard reactions in a food model under strictly controlled physical, structural and chemical characteristics will be of paramount help to verify the hypotheses formulated by many decades of results obtained in simple model systems (far from real foods) or in real products (with limited understanding on specific paths). This study shows how an inert model imitative of a sponge cake can be implemented to quantitatively follow Maillard reaction markers (precursors, intermediates and products) during the baking process. Targeted precursors (free amino acids and reducing sugars) were added to an unreactive model and submitted to controlled process conditions. Quantitative methods using on-line-TD-GC/MS and UHPLC-CAD/DAD/MS have been developed and applied to follow more than 20 reaction markers (e.g. fructose, glucose, dicarbonyls, pyrazines, Strecker's aldehydes, acids) throughout the baking process in order to obtain kinetics information at different product temperatures which vary during baking. This approach makes it possible to explain the individual and synergistic role of precursors together with different physical factors under controlled thermal conditions. The results show the apparent difference in the rate of furfurals and pyrazines generation in different models. They also provide insight about the dependency of governing reactions on several factors like time, temperature, and moisture content. These data will be further used for modelling by joining stoichio-kinetic, mass and heat transfer information. Hence this approach provides an excellent opportunity to understand, model and control the kinetics of thermal reactions contributing to the generation of process-induced compounds in solid food. This knowledge will in turn help to design the overall quality parameters of the product.

**Kinetic Modelling of Acrylamide Formation during the Finish-Frying of Sweet Potato Fries with Variable Maltose Content.** Dimitrios P. Balagiannis,† Jane K. Parker,† Jeremy Higley,‡ Gordon Smith,‡ Bronislaw L.
Multiresponse kinetic modelling is a contemporary technique to model, study and compare the kinetics of complex chemical reactions, such as the Maillard reaction. Acrylamide, a probable carcinogen, is generated during food processing. Two related pathways have been proposed - a specific amino acid pathway which occurs directly between glucose and asparagine, and the generic pathway which is assisted by the formation of Maillard-generated reactive intermediates. Even though acrylamide formation has been studied extensively in white Irish potatoes, less attention has been given to sweet potatoes. In sweet potato fries the concentration of glucose and fructose is much higher than in traditional fries made from white Irish potatoes. The former also has a high concentration of maltose, which is absent in the latter. Raw sweet potatoes generally do not contain any maltose, however when heated, thermally stable amylase becomes active, and maltose is formed due to the degradation of starch. Using the multiresponse kinetic modelling approach, we studied the formation of acrylamide during the frying of sweet potato strips made from a standard variety and a low maltose variety of sweet potatoes. The limited amount of acrylamide precursors in the low maltose variety resulted in the generation of low levels of acrylamide. We will discuss the kinetics of acrylamide formation in sweet potatoes and compare them with the generation of acrylamide in white potatoes. In white potatoes our kinetic model suggests that the Maillard reaction contributes significantly to acrylamide formation. In sweet potatoes, where sugars are in excess, our kinetic model estimates that Maillard reaction of glucose with free amino acids is less favoured. On the contrary, the sugar driven fructose pathway and the specific amino acid pathway of maltose are favoured. Comparison of the precursor profiles of the two potato types reveals that the ratio of reducing sugars (RS) to free amino acids (FAAs) is in white potatoes is 0.7:1, while in sweet potatoes it is 15:1. This variation explains the differences in the acrylamide formation kinetics between the two potato types which is controlled by the RS:FAAs ratio.

Microemulsions and Amadori compounds: unravelling molecular dynamics from foods to gastrointestinal digestion. Antonio Dario Troise*, Paola Vitaglione, Department of Agricultural Sciences, University of Naples, Federico II. (antoniodario.troise2@unina.it)

In emulsions, environments with various properties and polarities promote the partitioning and segregation of the reactants and reaction products resulting as a versatile modulator of chemical reactions. In the case of the Maillard reaction (MR), the presence of lipid droplets, dispersed in an aqueous environment can suppress the formation of potentially toxic molecules and can enhance the formation of desired molecules. The basic principle is that microemulsions are able to tune the reactants location thus mastering the formation of Maillard reaction products (MRPs) with consequences at three levels: food quality, flavor, taste perception and gastrointestinal bioaccessibility. Moving from the reactivity of precursors and MRPs in emulsion systems, the MicroREACT project aims at clarifying the perception of tastants and the bioaccessibility of MRPs during gastrointestinal digestion in vitro. Reactants location can modulate the reaction mechanisms in complex food systems during processing and storage. In emulsions containing amino acids, sugars and lipids, tangled reactions may concur to the formation of molecules that impact the quality of foods. The use of hydrocarbon side chain amino acids, such as leucine, alanine and aromatic side chain amino acids along with unsaturated lipid droplets demonstrated that the side chain of amino acids can be anchored to the lipid droplets and can control the reaction rates of Amadori compounds formation in the intermediate stage of the MR. In this study two model systems were developed and compared: the presence of unsaturated triacylglycerols influenced the reaction rates leading to the formation of taste active molecules arisen from glucose and alanine. This approach offered the chance to unravel the MR in foods and to evaluate how lipids and surfactants droplets can influence the formation of MRPs. Data, that will be presented at the conference, represent the basis to evaluate the impact of molecule partitioning both
The relationship between the content of MELANOIDINS AND COLOUR, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF POLISH HONEYS. Małgorzata Starowicz; Anita Osztaszyk; Anna Majkowska. 1Department of Chemistry and Biodynamics of Food, 2Sensory Laboratory, 3Microbiological Laboratory, Institute of Animal Reproduction and Food Research, Tuwima Street 10, 10-748 OLSZTYN, POLAND (m.przygodzka@pan.olsztyn.pl)

The objective of this study was to describe the relationship between melanoidins content and color, antioxidant capacity, and antimicrobial properties of Polish-originated honeys. For the characterization of 18 Polish honeys (buckwheat, lime, rape, multifloral, heather, acacia), were measured spectrophotometrically. To determine antioxidant activity, samples of honeys were analysed using photochemiluminescence assay in mode of antioxidant capacity in water-soluble substances (PCL ACW) and ferric reducing antioxidant potential (FRAP) assay. Further, analysis of antimicrobial properties against E. coli and B. subtilis were performed using micro-dilution assay in a 96-well microplate format. The honey colour was measured considering three parameters L*, a*, b* of the CIELAB system and by sensory panel. According to obtained results from PCL and FRAP assays, buckwheat, heather and lime honeys have the highest ability to scavenge free radicals. Moreover, light-coloured honeys (multifloral, lime, rape, acacia) have lower content of melanoidins than dark-coloured ones (heather, buckwheat). The highest antibacterial activity was observed in buckwheat and heather, and multifloral honeys. From the principal component analysis revealed that all variables (colour, PCL, FRAP, melanoidins content and antimicrobial properties) are strongly correlated between each other. It can be concluded that melanoidins strongly contributed on such parameters of honey as appearance, antioxidant potential and antimicrobial features. However, all of these properties depend on honey type, geographical origin, and/or producer.

Effects of NaCl, KCl, and CaCl$_2$ on the Formation of $\alpha$-Dicarbonyl Compounds and Furfurals and the Development of Browning in Cookies during Baking. Tolgahan Kocadağlı, Vural Gökmen

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Sodium salts are commonly added to cereal products mainly for flavor (sodium chloride) and technological purposes (sodium bicarbonate). There is an increasing demand for sodium reduction in staple foods due to the adverse health effects in higher consumption. Additionally, alkali and alkaline earth metal salts, especially calcium, are added to thermally treated foods to mitigate acrylamide formation. Even though there is considerable knowledge about the effect of sodium, potassium and calcium on furfurals and acrylamide formations during heating of foods, effects of cations on the formation of $\alpha$-dicarbonyl compounds in foods are not known. In this study, it was aimed to understand the formation of glucosone, 1-deoxyxylosone, 3-deoxyxylolosone, glyoxal, methylglyoxal, diacetyl, 5-hydroxymethyl-2-furfural, 2-furfural, and browning development in cookies formulated with NaCl, KCl, and CaCl$_2$ in the presence or absence of leavening agents NaHCO$_3$ and NH$_4$HCO$_3$. Cookies were formulated according to AACC Method 10-54 with certain modifications. Analysis of $\alpha$-dicarbonyl compounds was performed with LC-MS after obtaining their quinoxaline derivatives. The presence of 1.5% NaCl, 1% KCl, and 1% CaCl$_2$ on flour basis had no effect on $\alpha$-dicarbonyl compounds, except 1-deoxyxylosone increased in the presence of KCl and CaCl$_2$. The increase in 5-hydroxymethyl-2-furfural formation in the presence of NaCl, KCl, and CaCl$_2$ was not related to the 3-deoxyxylolosone formation and pH changes. Data showed that the catalyzing effect of cations should occur on the pathways comprising cyclic intermediates either from sucrose degradation or from fructose dehydration. NaCl, KCl, and CaCl$_2$ increased browning in cookies. Moreover, model reaction systems with glucose supported that NaCl, KCl, and CaCl$_2$ enhance browning by increasing furfurals in caramelization. NaCl, KCl, and CaCl$_2$ decreased browning intensity in a heated glucose–glycine system. From the food quality point of view, cations enhance desired consequences of caramelization in cookies. Additionally, from the view of food safety, sodium reduction can be obtained by replacement with potassium without sacrificing the desired consequences of caramelization in cookies. However, it should be considered that they both increase the amount of HMF. Another aspect from the food safety point of view,
although the addition of calcium to foods to mitigate acrylamide formation may result in an increment in HMF but not α-dicarbonyl compounds.

**Posters in Nutrition**

**Assessment of Bioaccessibility/Cellular uptake of furosine, HMF, and CML in GLYCATED WHEY PROTEIN ISOLATES using in vitro digestion and Caco-2 and PSI cells as model.** Kwang-Won Lee*, Hee-Ra Lee, and Min Cheol Pyo. Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, 02841, Republic of Korea-mail: kwangwon@korea.ac.kr

To study the bioaccessibility and cellular uptake of glycated whey protein isolates (GWPI), we used in vitro digestion/Caco-2 or pig small intestinal (PSI) cell uptake model. The GWPI were prepared with glucose and whey protein isolates using dry-heat method for 7 d. The content of furosine, hydroxymethylfurfural (HMF), and carboxymethyllysine (CML) in GWPI were analyzed by HPLC. As GWPI were digested in vitro, the bioaccessibility of GWPI increased in the order of oral stage, gastric stage, and intestinal stage. In Caco-2 cell monolayer, cellular uptake (sum of retention and transport) of furosine, HMF, and CML was 11.2%, 41.7%, 46.6%, respectively. In PSI cell model, cellular uptake of furosine, HMF, and CML was 59.5%, 68.2%, 73.2% respectively. Also, PSI cells gave more uptake of furosine, HMF, and CML in GWPI than Caco-2 cells did. These in vitro digestion and cellular uptake models can serve as preliminary screens to estimate a dietary exposure to exogenous Maillard reaction products.

**The receptor for Advanced Glycation End products induced Matrix metalloproteinases in kidney proximal epithelial cells via ERK/JNK/NF-κB pathways.** So-Ra Jeong, HeeEun Kim, and Kwang-Won Lee. Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, 02841, Republic of Korea. (sopong1004@korea.ac.kr)

Advanced glycation end products (AGEs) are formed by reducing sugars and oxidation of proteins in non-enzymatic process. AGEs accumulation is associated with pathophysiological disease such as atherosclerosis, cancer, diabetic nephropathy. The receptor for AGE (RAGE) mediates inflammatory signals leading to tissue acute or chronic inflammatory cytokine. Mitogen-activated protein kinase (MAPK) signaling pathway is responsible for initiating inflammation in cells. NF-κB activated a range of signals, including activation of MAPK pathway process. AGEs play a significant role in MAPK pathway signal mediated MMPs in Normal rat kidney (NRK-52E) cells. Therefore, in this study, we compared the toxicity of AGEs from different sugars and determined which AGEs had the highest toxicity. We have produced AGEs derived from several analogs of sugars and have identified which signals in the body cause renal damage in each of the AGEs. We evaluated the effects of AGEs mediates systemic renal dysfunction. We suggest that AGEs through its RAGE directly interaction increases MMPs expression via the ERK, JNK and NF-κB pathways.

**Immune-enhancing effect of glycated beta-lactoglobulin.** Su-Hyun Chun, Hee Joon Yoo, and Kwang-Won Lee. Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, 02841, Republic of Korea. (harry1107@korea.ac.kr)

The aim of this study was to confirm to immunomodulatory of glycated beta-lactoglobulin and identification of glycation site. Beta-lactoglobulin as a major compound of whey protein has high essential amino acid and functional properties Glycation, called Maillard reaction, occurs during cooking or storage of foods and recently improves the functional properties of proteins. In this study, beta-lactoglobulin was glycated with lactose by heating at 55°C for 24 h. To confirm the immunomodulatory of beta-lactoglobulin, we measured nitric oxide products and cytokines on the RAW 264.7 cells. To identify the glycated site of beta-lactoglobulin, we applied to the method of in-gel trypsin digestion and a matrix-assisted laser desorption/ionization coupled with time-of-flight tandem mass spectrometer analysis. On the result of this study, we found the glycation site of beta-lactoglobulin having an immune-enhancing effect. Therefore, we suggested that glycated beta-lactoglobulin could be a potential material as an immune enhancer in the foods.
The AGE-RAGE axis in metabolically healthy obese and normal-weight adolescents. Ma. Eugenia Garay-Sevilla*, Sofia Torres-Graciano, Ma. Etzel Villegas-Rodríguez, Antonio E. Rivera-Cisneros, Katarzyna Wrobel, Jaime Uribarri†. 1Department of Medical Science. Division of Health Science. University of Guanajuato Campus. León, Guanajuato. México. 2Universidad Autónoma de Guadalajara and Universidad del Fútbol y Deporte, Pachuca Hidalgo México. 3 Department of Chemistry, University of Guanajuato, Guanajuato, Guanajuato. México. 4Department of Medicine. The Icahn School of Medicine at Mount Sinai, New York, New York. (marugaray_2000@yahoo.com)

Background: Obesity is associated with low-grade inflammation. Advanced glycation end products (AGEs) are among many factors known to contribute to elevated inflammation and it had been expected them to be increased in obese subjects, however circulating AGE levels were found to be decreased in a group of obese adolescents compared to their lean counterparts. A study in adolescents with obesity showed less sRAGE and greater insulin resistance and cardiovascular risks.

Aim: To determine the relationship between circulating advanced glycation end products (AGEs), AGE receptors, markers of inflammation, and insulin resistance in metabolically healthy obese and normal-weight adolescents.

Methods: We recruited 79 adolescents with normal-weight and 68 with obesity from schools in the city of Leon, Mexico and grouped them into metabolically healthy (HOMA-IR <3.0) or unhealthy (HOMA-IR >3.0). We measured carboxymethyllysine (CML), soluble AGE receptor (sRAGE), expression of AGE receptors, TNF-α, IL-6, insulin resistance (HOMA-IR) and Triglycerides/HDL-C index

Results: We found higher triglycerides, TG/HDL-C index, HOMA-IR, TNF-α and IL-6 in the metabolically healthy obese (MHO) group and found correlation between HOMA-IR and BMI, TG/HDL-C index and IL-6, and between TG/HDL-C index with BMI and TNF-α. No correlation was found between markers of obesity and circulating levels of CML or sRAGE. A small group of metabolically unhealthy obese (MUHA) (n=12) had higher CML (15.5 ± 2.7 U/ml, p<0.028) and sRAGE (3123 ± 1363.6 pg/ml, p<0.001) than the MHO group.

Conclusions: HOMA-IR and TG/HDL-C index was associated with BMI and markers of inflammation; CML and sRAGE did not show any association with obesity or inflammation in MHO. These parameters, however, were higher in a group of metabolically unhealthy adolescents, independent of BMI.

A single high-fat meal alters soluble RAGE profiles and peripheral blood mononuclear cell RAGE expression. Kelly N.Z. Fuller, Rudy J. Valentine, Prabhakaran Kumar, Bellur S. Prabhakar, Jacob M. Haus. 1Department of Kinesiology and Nutrition, University of Illinois at Chicago, IL, USA. 2Department of Kinesiology, Iowa State University, IA, USA. 3Department of Microbiology and Immunology, University of Illinois at Chicago, IL, USA. 4School of Kinesiology, University of Michigan, Ann Arbor MI USA. (jmhaus@umich.edu)

A high-fat diet can induce chronic low-grade inflammation and metabolic diseases such as diabetes and atherosclerosis. The receptor for advanced glycation end products (RAGE) plays a critical role in inflammatory signaling in metabolic disease and the soluble form of the receptor (sRAGE) can mitigate these effects. However, less is known about the role of RAGE in postprandial inflammation and the effect of exercise in this context. Thus, we aimed to determine the effects of a single high-fat meal (HFM) with and without prior aerobic exercise on peripheral blood mononuclear cell (PBMC) RAGE biology. Healthy male participants (n=12) consumed a HFM on two separate occasions, one without prior exercise and one 16-18hrs following a bout of aerobic exercise. Total soluble RAGE (sRAGE) and endogenous secretory RAGE (esRAGE) were determined via ELISA and cleaved RAGE (cRAGE) was calculated as the difference between the two. Further, isolated PBMCs were analyzed for RAGE, ADAM10, TLR4 and MyD88 protein expression and ADAM10 activity. The HFM significantly (p<0.01) attenuated circulating sRAGE, esRAGE, and cRAGE by 9.7%, 6.9%, and 10.5% respectively. While the HFM increased PBMC RAGE protein expression by 10.3% (p<0.01), there was no meal effect on PBMC TLR4, MYD88, or ADAM10 protein expression, nor ADAM10 activity (p= 0.2, 0.7, 0.3, 0.2 respectively). There was also no effect of exercise on any experimental outcomes. These findings suggest that PBMCs are involved in postprandial inflammation via RAGE activation. However, due to no change in ADAM10, it is unlikely that PBMCs directly influence sRAGE production following a HFM.
Methanolic fractions of *Petalostigma banksii* fruits attenuates $N^\epsilon$-carboxymethyllysine formation. 
Emma L. Jaunay$^1$, Susan J. Semple$^{1,2}$, Bradley S. Simpson$^{1,2}$, David J. Claudie$^3$ and Permal Deo$^1$.

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Advanced glycation endproducts (AGEs) are modified proteins formed during non-enzymatic glycation. The pathologies of AGEs associated with diabetes, cardiovascular disease, Alzheimer’s and recently, cancer, highlight increasing demand for inhibitors to prevent their formation and associated cell damage. Due to previous issues with synthetic AGE inhibitors, such as side effects in human use, compounds originating from plant and food sources have growing research interest. *Petalostigma banksii*, a native Australian plant, produces a fruit which is traditionally used to treat inflammatory conditions in the medicine practices of the Northern Kuuku I’yu people whose homelands are in Cape York Peninsula, Queensland, Australia. A collaboration between the Chuulangan Aboriginal Corporation and the University of South Australia has allowed the scientific research of this native species. The objectives of this study were to investigate the effects of *P. banksii* fruit extracts on antiglycation and inhibition of $N^\epsilon$-carboxymethyllysine (CML) formation. AGE inhibition was also correlated with phytochemical content and antioxidant activities. A crude ethanolic extract of the fruit of *P. banksii* was partitioned into three fractions, methanol (MeOH), dichloromethane (DCM) and hexane fractions. An optimised in vitro glucose and bovine serum albumin (Glu-BSA) model of endogenous AGE formation was treated with various concentrations of *P. banksii* fruit extract fractions. The inhibition of protein-bound fluorescent AGE was determined using fluorescent spectrometer. CML was quantified via liquid chromatography-mass spectrometry/mass spectrometry LC-MS/MS. Total phytochemical content (total phenolic and flavonoid content) and antioxidant activities (ferric reducing antioxidant power (FRAP) assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition assay and 2,2’-azinobis (3-ethylbenzothiazolesulphonic acid) (ABTS) radical scavenging activity assay) were determined for each *P. banksii* extract fraction. The *P. banksii* MeOH fraction showed significant protein-bound fluorescent AGE inhibition of 80.64 ± 0.99 % at a concentration of 31.25 µg/mL. The most significant finding of this study was the dramatic reduction of CML in Glu-BSA model system with *P. banksii* MeOH fraction treatment. Levels of CML were reduced by the MeOH fraction treatment from 3.57 ± 0.22 mmol CML/mol Lys to 0.04 ± 0.01 mmol CML/mol Lys and this was comparable to a positive control, aminoguanidine. Total phenolic content was significantly correlated with DPPH radical inhibition assay ($r^2=0.714$, $p<0.05$) and weak but significant with protein-bound fluorescent AGE inhibition ($r^2= 0.407$, $p<0.05$). Further investigation into the antiglycation potential of *P. banksii* fruit is warranted.