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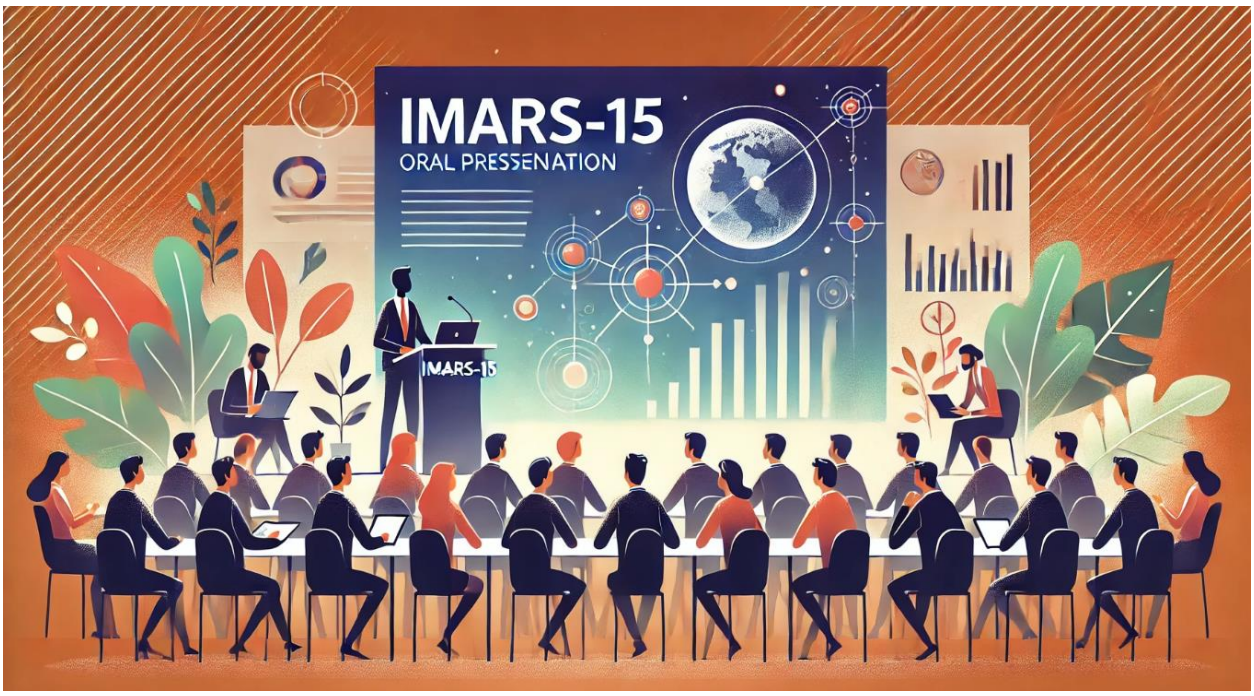
Oral Presentations



Abstract 1: Targeting RAGE using an oral antagonist to prevents Type 1 diabetes via attenuation of effector T cell responses in a preclinical model

I. Buckle [1], A. Fotheringham [1], R. Whiddett [1], P. Chandrashekar [1], C. Du [1], F.L. Henriques [1], J. Loaiza-Naranjo [1], D. McCarthy [1], S. LeBagge [1], C. Valcarce [2], K. Herold [3], J.M. Forbes [1]

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Introduction:

Type 1 diabetes (T1D) has rising incidence and there are limited approved preventative therapies. The receptor for advanced glycation end products (RAGE) is expressed on islet and immune cells pertinent to T1D development and RAGE-RAGE ligand targeting therapies successfully slow T1D onset, protect b-cell function, mass and increase protective T regulatory cells (T_{reg}) in preclinical studies. However, these previous approaches have limitations for clinical translation and hence a small molecule oral RAGE antagonist (oRA) under development for another indication was examined for efficacy to prevent and/or delay T1D onset.

Materials and methods:

Female NOD*ShiLt* mice (n=25/group) were randomised to oRA (3mg/kg q.d. oral gavage) or placebo (Veh) from day 50-64, then 3x weekly to day 150 of life. Mice were followed until day 225 for diabetes incidence and studied prediabetes (N=7-10/group) at either day 64 (for immune cell phenotyping in blood and local lymphoid tissues; pancreatic islet infiltration (insulitis) and insulin content, autoimmunity (IAA+), Legendplex for plasma cytokines and at day 92 for b-cell function by OGTT.

Results:

oRA reduced T1D incidence by day 225 (60% vs Veh 94%; P=0.02) and significantly delayed diabetes onset (median survival 29 weeks vs Veh 17 weeks). oRA showed equivalent efficacy in both high and low titre insulin autoantibody (IA+) groups.

Pre-diabetes at day 64, insulitis was reduced by oRA therapy (P<0.001), while local pancreatic lymph node (PLN) proportions of $CD8^+$ T cells were lower (P=0.004, vs Veh) with increases in $CD4^+$ T cell proportions in PLN and spleen. The proportion of splenic T_{regs} was also greater in oRA treated mice (P=0.01 vs Veh). At day 92 prediabetes, mice treated with oRA had better glucose tolerance during OGTT when compared to placebo (P_{adj}=0.049). Furthermore, a decrease in serum levels of pro-inflammatory cytokines was positively correlated with the length of treatment.

Discussion and conclusion:

These preclinical data suggest therapeutic potential for oRA to delay T1D. Future studies will better characterise immune cell subsets, antigen presentation, autoantibodies and b-cell damage and the ability of oRA to modulate human T and DC activity.

Abstract 2: RAGE-antagonism by Azeliragon counteracts diet-induced adiposity in mice through improvement of lipid metabolism and reduction of inflammation

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Introduction:

The receptor for AGE, RAGE, is a key player in obesity and the related metabolic inflammation that leads to diabetes. We have recently demonstrated that the genetic deletion of RAGE in mouse models of obesity is effective in prevention of insulin resistance and inflammation. However, the pharmacological antagonism of RAGE has been poorly investigated. Azeliragon is a small molecule that blocks the extracellular binding site of RAGE. Azeliragon has been tested in a phase 3 clinical trial in Alzheimer's disease (AD) with no adverse effect in long-term treatment and effective in reduction of inflammation in a type 2 diabetic subgroup of AD patients. The present study aims to evaluate the potential efficacy of Azeliragon in prevention of diet-induced adiposity and metaflammation in mice.

Materials and methods:

C57BL/6NHsd male mice were fed a standard diet (SD, n=10) or a western diet (WD, n=20) for 16 weeks. After the first 4 weeks of dietary intervention, a subgroup of WD mice was treated with 3mg/kg of daily oral Azeliragon administration (WD+AZ, n=10). Body weight and food consumption were monitored weekly. Body composition was measured every 2 weeks with Time Domain Nuclear Magnetic Resonance. The last week of dietary and treatment protocol, mice underwent oral glucose and insulin tolerance tests. Then mice were euthanized, blood, liver and visceral adipose tissue (VAT) were collected and frozen in liquid nitrogen. Hormonal and inflammatory profiles were evaluated in plasma by multiplex immunoassay.

Results:

After 16 weeks of WD, mice displayed a typical picture of metabolic inflammation compared to SD mice, characterized by markedly increased body weight, with significantly higher fat mass and lower lean mass, insulin resistance and hyperinsulinemia, and increased pro-inflammatory markers, paralleled by reduced anti-inflammatory cytokines. Interestingly, the daily administration of Azeliragon in the last 12 weeks of WD significantly slowered body weight increase (38.3 vs. 42.6 g, $p<0.05$), without affecting food intake, and prevented fat mass accumulation (35.6 vs. 43.2% of BW, $p<0.05$) and lean mass loss (58.0 vs. 48.3% of BW, $p<0.05$), preserved insulin sensitivity (ITT AUC: 6047 vs. 7640, $p<0.05$), reduced insulin (2.8 vs. 4.6 ng/mL, $p<0.05$), and restored the anti-inflammatory cytokine IL-10 (67.3 vs. 49.5 pg/mL, $p<0.05$), while reducing the pro-inflammatory cytokines and chemokines IL-6 (13.5 vs. 17.9 pg/mL, $p<0.05$), GM-CSF (1.16 vs. 1.43 ng/mL, $p<0.05$), and CXCL1 (19.5 vs. 38.2 pg/mL, $p<0.005$), in comparison to untreated WD mice. Preliminary western blotting analysis on liver proteins indicate that Azeliragon by antagonizing RAGE modulated lipogenesis activation and stimulated mitochondrial biogenesis and metabolism in comparison to untreated WD mice.

Discussion and conclusion:

The results of the present study provide new knowledge on the benefits of RAGE antagonism on adiposity and metaflammation providing unexplored molecular mechanisms underlying RAGE signalling in different tissues in obesity. Overall, these findings suggest the potential efficacy of Azeliragon treatment in humans as strategy to reduce obesity and prevent type 2 diabetes.

Abstract 3: Plasma advanced glycation end-products, sRAGE, and bone mineral density in girls with anorexia nervosa

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Introduction:

Anorexia nervosa (AN) is a severe psychosomatic eating disorder characterized by a persistent restriction of energy intake associated with a distortion of body image. AN onset is common during childhood and adolescence when most bone mass is acquired. We showed that girls with AN display higher levels of AGE-associated plasma fluorescence (AGE-Fl) than age-matched controls and that their bone mineral density (BMD) and bone turnover marker levels are inversely related to those of AGE-Fl (doi: 10.1007/s00431-021-04199-5). As AGE-Fl is an unspecific marker of AGE accumulation, we focused on chemically defined AGEs and sRAGE in the present study.

Materials and methods:

A total of 102 girls with the restrictive subtype of AN diagnosed according to the 5th Edition of The Diagnostic and Statistical Manual of Mental Disorders and 29 age-matched (14.0±2.7 vs. 14.8±1.9 years, p=0.158) healthy controls (CTRLs) were included. Plasma CML, MG-H1, sRAGE, and esRAGE were determined using ELISA methods, in girls with AN before the initiation of realimentation. The cleaved RAGE (cRAGE) concentration was calculated as sRAGE minus esRAGE. In girls with AN, the DEXA method was used to determine BMD; osteocalcin, amino-terminal propeptide of human procollagen type I (PINP), carboxy-terminal telopeptide of type I collagen (CTX), and insulin-like growth factor-1 (IGF-1) were analyzed by electrochemiluminescence immunoassays.

Results:

Compared with controls, girls with AN had lower body weight standard deviation scores (SDS, 0.3±1.0 vs. -1.8±1.0; p<0.001). Compared with the controls, the girls with AN presented with higher CML levels: 233 (210; 316) ng/ml vs. 355 (275; 508) ng/ml and MG-H1 levels: 0.8 (0.7; 0.9) µg/ml vs. 1.1 (0.8; 1.5) µg/ml, both p<0.001. Both groups displayed similar sRAGE (AN: 2.3±0.9; CTRL: 2.2± 0.8 ng/ml, p=0.42), esRAGE (0.48±0.21 vs. 0.45±0.18 ng/ml, p=0.50), and cRAGE (AN: 1.8±0.8; CTRL: 1.7± 0.9 ng/ml, p=0.49) levels. Multivariate regression using the OPLS model indicated that the lumbar spine BMD z-score in girls with AN was significantly and directly associated with SDS BMI, IGF-1, uric acid, and CTX levels and inversely associated with esRAGE. Conversely, esRAGE levels were significantly and inversely associated with BMI SDS, IGF-1, and the BMD z score of the lumbar spine and were positively associated with disease duration and CTX levels. Neither the variables characterizing bone turnover nor the BMD z scores showed significant correlations with the CML or MG-H1 levels.

Discussion and conclusion:

The pathological mechanisms leading to increased levels of CML and MG-H1 in girls with restrictive AN remain unclear. Enhanced oxidative stress, low body fat content, or intestinal dysbiosis might contribute to this phenomenon. Longitudinal studies are needed to confirm whether esRAGE might be a marker of bone turnover in patients with AN.

Abstract 4: Association between liver fat accumulation and glyoxalase I activity loss, independent of serum dicarbonyls, in humans

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Introduction

The underlying molecular mechanism(s) responsible for the development of metabolic associated fatty liver (MAFL) and its progression to later stages of liver diseases remain(s) unclear. The loss of glyoxalase 1 (Glo1), which leads to an increase in methylglyoxal (MG) and dicarbonyl stress, has been implicated in a variety of diseases, including those related to obesity. This study aims to determine whether changes in the glyoxalase system are present in individuals with increased liver fat. Furthermore, we investigate whether any dicarbonyl biomarkers in serum are associated with liver fat content.

Materials & methods

During liver surgery, 30 individuals with a BMI range of 24.6 – 29.8 kg/m^2 underwent intraoperative liver biopsies, confirmed as normal by a pathologist. Following overnight fasting, biopsies and corresponding blood samples were collected. Whole-body insulin sensitivity was assessed using HOMA-IR. Liver biopsies were analyzed for total triglyceride content, glyoxalase 1 and 2 mRNA, protein expression, and activity. Liquid chromatography-tandem mass spectrometry determined dicarbonyl content and protein-bound glycation/oxidation biomarkers in liver and serum.

Results

The activity of liver Glo1 was inversely correlated with HOMA-IR and liver triglyceride content but not with BMI. Despite the reduction in Glo1 activity, no associations were found with elevated liver dicarbonyls or glycation markers. A sex dimorphism was observed regarding Glo1, with females exhibiting significantly decreased liver Glo1 protein expression and activity, and increased liver MG-H1 content compared to males. Furthermore, no association was observed between dicarbonyl serum concentration and liver fat content.

Discussion & conclusion

This study demonstrates that Glo1 activity is not correlated with any liver protein-bound glycation and oxidation biomarkers, suggesting that Glo1 is not the limiting enzyme for dicarbonyl detoxification. However, our findings indicate that increasing liver fat, even within the low and physiological range, is associated with the loss of Glo1 activity, particularly concerning sex differences.

Abstract 5: Investigating the effects of methylglyoxal stress on immune response to tumors

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Introduction:

Cancer metabolism, and particularly the Warburg glycolytic switch, is an essential aspect of tumorigenesis that has a significant yet often overlooked consequence: the production of methylglyoxal (MG), a very reactive dicarbonyl with high protein glycating capacity notably leading to the formation of advanced glycation end products (AGEs). The accumulation of this oncometabolite sustains specific pro-cancer functions. We have previously demonstrated that MG stress supports breast tumor growth and metastasis *in vivo*.

Materials and methods:

Two breast cancer murine models were used: MMTV-PyMT mice with spontaneous metastatic breast cancer and 4T1 breast cancer model. We performed immunohistochemistry to assess the accumulation of MG adducts during disease progression from adenoma to carcinoma MMTV-PyMT lesions. In both models, tumor-bearing mice were treated with carnosine, a potent MG scavenger dipeptide, to assess the immune landscape in primary tumors and lung metastases using multiplex flow cytometry analysis. We performed a cytokine array for the detection of one hundred cytokines and chemokines in conditioned medium of 4T1 cells under MG treatment. We generated 4T1 cells stably depleted for glyoxalase 1 (GLO1), the primary MG-detoxifying enzyme, resulting in an endogenous MG stress model. We injected GLO1-depleted cells to mice to evaluate their tumor growth, metastatic burden and tumor immune microenvironment. We tested for the first time the combination of carnosine with anti-PD1 immunotherapy in 4T1 model.

Results:

We showed a significant cytoplasmic accumulation of MG AGEs from adenoma to late carcinoma lesions indicating the occurrence of MG stress during breast cancer progression in MMTV-PyMT model. In good accordance, we observed a significant reduction of lung metastatic foci upon mice treatment with carnosine. FACS screening and characterization of the whole immune cell population in MMTV-PyMT experimental tumors demonstrated a significant decrease of granulo-myeloid derived suppressor cells (gMDSCs), an immunosuppressive cell subtype, upon carnosine treatment. Similar results were seen in 4T1 breast cancer syngeneic model. MG-stressed breast cancer cells favored primary tumors and high metastatic burden with increased g-MDSC infiltration in the tumoral microenvironment. Mechanistically, this was partly due to changes in cytokine production under MG stresswhich pointed to higher levels of cytokines specifically associated with immunosuppressive cell recruitment, such as CXCL1 and VEGF. Finally, the combination of carnosine with anti-PD1 antibodies enhanced their individual effectiveness at inhibiting tumor growth and metastasis in the 4T1 grafting model.

Discussion and conclusion:

Taken together, our data point to a novel function of MG in the recruitment of immunosuppressive cells, that could be reversed using carnosine and potentially targeted to enhance the susceptibility of breast tumors to immunotherapy.

Abstract 6: Loss of NAMPT and SIRT2 but not SIRT1 Attenuate GLO1 Expression and Activity in Human Skeletal Muscle

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Introduction:

Glyoxalase I (GLO1) is the primary enzyme for detoxification of the reactive dicarbonyl methylglyoxal (MG). Loss of GLO1 in preclinical models promotes accumulation of MG resulting in a recapitulation of diabetic phenotypes. Skeletal muscle is perhaps the most important tissue for preventing insulin resistance and diabetes development. We previously demonstrated attenuated GLO1 protein in skeletal muscle from individuals with type 2 diabetes (T2D). However, whether GLO1 attenuation occurs prior to T2D and the mechanisms regulating GLO1 abundance in muscle are still unknown.

Materials and methods:

We analyzed GLO1 expression and activity in skeletal muscle tissue biopsies from 15 lean healthy individuals (LH, BMI: 22.4±0.7) and 5 individuals with obesity (OB, BMI: 32.4±1.3). GLO1 protein was attenuated by 26±0.3% in OB compared to LH muscle (p=0.019). We also observed similar reductions for GLO1 activity (p=0.102). NRF2 and Keap1 expression were equivocal between groups despite a 2-fold elevation in GLO1 transcripts in OB muscle (p=0.008). GLO1 knock-down (KD) in human immortalized myotubes promoted the downregulation of proteins involved in skeletal muscle contraction and organization indicating the importance of GLO1 expression for skeletal muscle function. Prior observations in human liver tissue suggested hyperacetylation of GLO1 may preempt its degradation. Thus, to explore this potential mechanism in skeletal muscle, we assessed the effects of histone deacetylases, SIRT1 and SIRT2 KD on GLO1 in human immortalized myotubes.

Results:

SIRT1 KD had no effect on GLO1 protein abundance or activity whereas, SIRT2 KD attenuated GLO1 protein abundance by 28±0.29% (p<0.0001) and GLO1 activity by 42±0.12% (p= 0.0150). KD of NAMPT (the rate limiting NAD^+ resynthesis enzyme) also resulted in attenuation of GLO1 protein (28±0.069%, p=0.003), activity (67±0.09%, p=0.011) and transcripts (50±0.13%, p=0.049). Neither the provision of the NAD^+ precursors NR nor NMN were able to prevent this attenuation in GLO1 protein. However, NR did augment GLO1 specific activity (p=0.022 vs NAMPT KD). Notably, none of these perturbations appeared to alter GLO1 acetylation status. In addition, SIRT1, SIRT2 and NAMPT protein levels were all equivocal in skeletal muscle tissue biopsies from individuals with obesity and lean individuals.

Discussion and conclusion:

These data implicate NAD^+-dependent regulation of GLO1 in skeletal muscle independent of altered GLO1 acetylation and provide rationale for exploring the possibility of NR supplementation as an effective treatment strategy to rescue attenuated GLO1 abundance and activity in conditions such as obesity.

Abstract 7: The Food Glycation Database: an on-line tool to investigate the role of processing and dietary AGEs nutritional implications

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Introduction:

The role of the Maillard reaction (MR) in food quality requires accurate quantitation of glycation compounds. The Glycation Database (GD) offers a comprehensive resource for non-enzymatic glycation compounds, emphasizing Amadori and Heyns compounds, dietary advanced glycation end-products (d-AGEs), and volatile compounds.

Materials and methods:

The GD includes chemical characteristics, food processing type, time and temperature, food storage, and distinctions between free and protein-bound contents. It comprises more than 8,200 content values for 50 different compounds (including CML, HMF, MGO) in over 3,200 foods, accessible via a user-friendly online tool. Entries are derived from 151 peer-reviewed publications accessed through databases such as Reaxys, PubMed, and SciFinder. Data are based on quantification of analytically distinct molecules through liquid and gas chromatography, mass spectrometry, UV, fluorescence, and NMR. Stored in a relational MySQL database hosted on an Oracle Linux server, and accessible through at https://glycation.abertay.ac.uk. The web interface allows searching for various food and compound types, exploring their links, and publications source.

Results:

The GD combines the intrinsic chemical nature of glycation compounds with their concentration in foods, providing an outlook on their in vivo effects. The GD has been developed to provide a centralized repository of food-related data on d-AGEs. The database consists of five main tables: Food items, Food products, Category, Food specification, and Food Compounds. It contains a vast array of compounds found in foods documented to contain free or bound d-AGEs, providing average values of specific compounds when there are multiple entries. It includes information on molecular formula and weights, compound structures, identification numbers, and spectra from well-known chemical databases. Food data are categorized into four sub-categories: food items, food products, category, and food specification. Composition data are categorised into foods and compounds. The GD can be interrogated using the main search bar, searching individual columns, and sorting data in ascending or descending order to switch between compounds and food. The GD also allows the insertion of new data, editing and deletion of existing entries, making it a valuable tool for expanding and maintaining data.

Discussion and conclusion:

This chemistry-oriented GD is a valuable tool for targeted metabolomic studies, defining glycation markers through a data-driven selection of optimum biomarker combinations. It links food to MR products, distinguishes between similar ingredients, and evaluates processing effects on d-AGEs. It could help to build food frequency questionnaires and distinguish between free and bound d-AGEs. The GD provides a centralized platform for researchers, industry, and healthcare professionals to access and analyse d-AGEs data. By leveraging molecule-specific analysis, it offers insights into food glycation markers, potentially improving research.

Abstract 8: Analysis of advanced glycation end products in differently processed pet foods by ultra-high performance liquid chromatography tandem mass spectrometry and association of dietary intake with plasma and urine advanced glycation end products in healthy dogs

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Introduction:

Advanced glycation end products (AGEs), a subset of Maillard Reaction Products (MRP's) formed during thermal processing of foods, play a role in inflammatory and degenerative diseases of human beings. Traditional pet foods are manufactured by thermal processing methods that were inspired by human food production such as cereals and canned goods that may increase dietary AGE (dAGE) content. It is possible that dAGEs may negatively affect pet health since pets are fed these traditional ultra-processed foods for the duration of their lifespan. The aim of this study was to quantify AGEs in pet foods and to determine the influence on canine plasma and urine total AGE concentration. The hypothesis was that diet, plasma, and urine AGEs concentrations would differ between different thermally processed diets.

Materials and methods:

Ultra-high-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) was used to quantify AGEs. Carboxymethyllysine (CML), carboxyethyllysine (CEL), and methylglyoxal hydroimidazolone-1 (MG-H1) were measured in four differently processed diets: ultra-processed canned wet food (WF) and dry food (DF); moderately processed air-dried food (ADF), and minimally processed mildly cooked food (MF). These diets were then fed to 8 apparently healthy laboratory colony dogs divided into pairs for 4 weeks in a Latin square crossover experimental design over a total period of 16 weeks. At the end of feeding each diet, plasma concentration of CML, CEL, MG-H1, glyoxal hydroimidazolone-1 (GH-1), and argpyrimidine (AP), and urine concentration of CML, CEL, and lysinoalanine (LAL) were measured.

Results:

Total dAGEs (mg/100 kcal as fed) was highest in WF. Total plasma AGEs (nM/50 microliter) were significantly highest with WF when compared with the other diets; however, ADF was significantly higher than MF. Urine CML (nmol AGEs/mmol creatinine) was significantly highest with DF than WF and MF. There were no significant differences in total urine AGEs.

Discussion and conclusion:

Different methods of thermal processing of pet foods result in varied dietary AGE content that influenced total plasma AGE but not urine concentration in healthy dogs in this study. Dietary AGE intake may have implications for pet health and disease as they do for human beings.

Abstract 9: Dietary Maillard reaction products N-ε-carboxymethyllysine, N-ε-fructosyllysine and pyrraline in mice: absorption, distribution and their effects on inflammation and oxidative stress

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Introduction:

Endogenously formed Maillard reaction products (MRPs) are suspected to activate the receptor for advanced glycation end-products, RAGE, and subsequently trigger the NFκB-mediated pro-inflammatory signalling cascade. The contribution of individual dietary MRPs to the endogenous pool and their effects on inflammation is currently under debate. The aim of this study was to assess the absorption and distribution of dietary, protein-bound fructosyllysine (FL), carboxymethyllysine (CML) and pyrraline in mice and analyze the promotion of inflammation and oxidative stress in various organs and tissues.

Materials and methods:

C57BL/6N mice of two age groups (12 weeks “young” or 72 weeks “aged”) were each assigned in four sub-groups with eight animals. The control group received a standard diet enriched with native casein (0.4 %). Experimental groups received a standard diet enriched with modified casein, whereby lysine residues were modified with isotope-labeled [^{13}C]fructosyllysine, [^{13}C]carboxymethyllysine or [^{13}C]pyrraline. The diets were given ad libitum and the intervention lasted 30 days. The concentration of free [^{13}C]MRPs in mouse organs was analyzed with UPLC-MS/MS. IL-6 concentration in plasma was analyzed with a commercial ELISA. Glutathione analysis in whole blood was performed via HPLC-UV. Expression of IL-6, RAGE and NQO1 in tissues was analyzed with Western Blotting. Additionally, RAGE and NF-κB were analyzed with immunofluorescence staining in intestinal and renal tissue sections.

Results:

Dietary intake leads to an organ-specific increase of the free [^{13}C]-labeled MRPs. Brain, heart and stomach showed low amounts, whereas kidneys, colon and intestine showed the highest accumulation of MRPs. Pyrraline accumulated strongly in the kidneys, while for CML and FL the highest amounts were detected in the intestine and colon. The varying distribution patterns indicate structure-specific effects during resorption and distribution. Aged animals show lower concentrations of free [^{13}C]-labeled MRPs in organs but not in plasma compared to younger animals. Young animals receiving the [^{13}C]FL-diet had higher colonic IL-6 expression and lower GSH content in whole blood. In aged animals, the MRP-enriched diets had no effects on inflammation and oxidative stress.

Discussion and conclusion:

A diet rich in MRPs fed for 30 days led to an increase of MRPs in organs. The effect on inflammation was organ-, MRP- and age-specific. Thus, studies on the biological effects of glycated proteins require an individual consideration of defined structures.

Abstract 10: On the effect of methylglyoxal on the structure, function and aggregation propensity of alpha-synuclein

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Introduction:

Human alpha-synuclein (aS) is an intrinsically disordered protein mainly expressed in the cytoplasm of neurons of the substantia nigra. Its main biological function is related to its ability to regulate the traffic of synaptic vesicles (SVs) carrying dopamine. Besides its biological role, aS tends to aggregate by forming oligomers that then cluster into amyloid fibrils. These fibers, finally clump into Lewy bodies (LBs), whose accumulation is one of the main hallmarks of Parkinson's disease (PD). The fact that diabetes mellitus increases the risk to develop PD, and the detection of methylglyoxal-derived advanced glycation end products (AGEs) in LBs isolated from PD brains of diabetic people, let the scientists to assume that glycation could be behind the link between diabetes and PD. To get mechanistic insights on this hypothesis, we have studied the effect of methylglyoxal (MG) on the structure, function and aggregation propensity of aS.

Materials and methods:

We have used recombinant aS and purified MG. Since glycation yielded a heterogeneous mixture of aS molecules, we also produced a synthetic aS where all its 15 Lys were replaced by N^{ε}-(carboxyethyl)lysine (CEL). CEL is the main MG-derive AGE found in LBs. We used mass spectroscopy in combination with enzymatic digestion to characterize the MG-derived AGEs formed on aS. Then, we used CD, fluorescence, NMR and molecular dynamics simulations to assess the effect of MG, and the formation of CEL, on the conformation of aS. Moreover, we also studied the effect of MG and CEL formation on the ability of aS to bind and fuse SVs. Finally, we used dynamic light scattering, size exclusion chromatography, atomic force microscopy and steered molecular dynamics simulations to determine the effect of MG and CEL formation on the aggregation propensity of aS.

Results:

Incubation of aS with MG induced the crosslinking of some Lys through the formation of MOLD. However, MG did not induce any remarkable change in the radius of aS, nor induced its structuration. In contrast, CEL extended the conformation of the N-terminal domain as a result of the loss of transient N-/C-terminal long-range contacts. MG, and the synthesis of CEL on aS, decreased the ability of aS to bind SVs and to induce their fusion into bigger vesicles. MG and CEL formation completely inhibited the fibrillization of aS and even its oligomerization propensity. Nevertheless, CEL was not able to disassemble pre-existing amyloid fibrils.

Discussion and conclusion:

The formation of CEL and others MG-derived AGEs drastically changed the electrostatic potential of aS. This, slightly modified the averaged conformational ensemble of aS, but completely depleted aS of one of its most important biological function, that is bind and fuse SVs. Surprisingly, MG completely inhibited aS aggregation, thus proving that AGEs found on LBs must be formed in a later event after aggregation. Our results indicate that the understanding of the effect of glycation on the development of PD needs to be faced beyond its impact on the aggregation of aS, specially on the function of aS.

Abstract 11: Changes in the exo-loop residue T107 alters the activity and function of Glyoxalase 1

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Introduction:

The glyoxalase pathway is responsible for the detoxification of reactive dicarbonyls that are generated as a by-product of cellular metabolism. Glyoxalase 1 (GLO1) is the rate-limiting enzyme of the glyoxalase pathway. Reduced activity of GLO1 leading to dicarbonyl stress is associated with accelerated ageing and age-related dysfunction. Despite the increased burden of AGEs, in ageing animals there is a reduction in GLO1 activity. At the same time, the expression of *Glo1* mRNA is unaltered. We hypothesise that post-translational modification of GLO1 is increased with age and contributes to reduced GLO1 activity. We have previously used *in silico* modelling to show that phosphorylation of Threonine-107 (T107), a key residue of an exo-loop adjacent to but not within the catalytic domain, potentially induces a change in the structure of GLO1, including altered accessibility and binding of substrate to the catalytic domain.

Materials and methods:

We produced and purified several GLO1 variants. Ni-NTA affinity purified GLO1 variants were assessed for enzymatic activity using the GLO1 activity assay and their structural changes were analysed using the Small Angle X-ray Scattering (SAXS) beamline at the Australian Synchrotron (ANSTO).

Results:

Ni-NTA affinity-purified GLO1 T107 variants demonstrated varied catalytic-activity compared to wild-type GLO1. We confirm that selective mutation at T107 alters the structure of GLO1, as demonstrated by SAXS, and consistent with our *in silico* modelling. In particular, phospho-mimetic residues at position 107 display a difference in protein dimensions and activity when compared to wild-type GLO1. Additionally, a synthetically generated phosphorylated T107 purified protein variant indicates that phosphorylation at T107 does alter the activity of GLO1 compared to wild-type unphosphorylated GLO1.

Discussion and conclusion:

These findings support the critical nature of the residue at position 107, and its modification, in maintaining GLO1 structure and thereby function. These results have interesting implications for the role of GLO1 activity in age-related decline and in ageing *per se*.

Abstract 12: Glyoxalase-1 overexpression attenuates arterial stiffness in a mouse model of diabetes

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Introduction:

In diabetes, arterial stiffness is increased and associated with cardiovascular disease. The cause of arterial stiffening in diabetes is not completely understood, but it may involve increased formation of advanced glycation endproducts (AGEs) inducing arterial wall cross-links. Methylglyoxal (MGO), a potent glycolysis-derived intermediate, is a major precursor in the formation of AGEs. MGO is detoxified by the glyoxalase-1 enzyme encoded by *Glo1*. We hypothesize that increased MGO and MGO-derived AGE formation play a role in diabetes-associated arterial stiffening, and have studied this using a transgenic *Glo1* overexpressing mouse model.

Materials and methods:

Three groups (n=10 -12/ group) of nine-week-old C57BL/6J mice were studied: wild-type control, wild-type diabetes, and *Glo1*-overexpressing diabetes (*Glo1*/diabetes). Diabetes was induced by 50 mg/kg streptozotocin (STZ) injections for five days. Fasting glucose was measured every 4 -5 weeks. MGO was measured in plasma and urine with ultra-performance liquid chromatography tandem mass spectrophotometry. After 13 weeks of treatment, tail-cuff blood pressure (BP) and non-invasive carotid-femoral pulse wave velocity (cfPWV) were measured prior to euthanasia. Then, the thoracic descending aorta was harvested (~ 6 mm) and used for *ex vivo* biaxial quantification of passive arterial biomechanics. In addition, two-photon microscopy collagen images were obtained at *in vivo* axial stretch and 100 mmHg pressure, to determine collagen helix angle orientations.

Results:

Glucose levels in the diabetes and *Glo1*/diabetes groups were higher than control (both $p<0.0001$). MGO was increased in diabetes in plasma and urine (1.3-fold, $p<0.01$ and 2.4-fold, $p<0.0001$, respectively) and *Glo1* overexpression decreased MGO in urine (1.25-fold, $p<0.01$). There was no difference in systolic BP (110±4 vs. 104±3 mmHg, mean±SEM, $p=0.26$) and in cfPWV (2.60±0.14 vs. 2.55±0.11 m/s, $p=0.80$) between control and diabetes. However, there was a 1.2-fold increase in *ex vivo* PWV based on *in vivo* systolic BP in diabetic mice ($p<0.01$), which was attenuated 1.1-fold by *Glo1* overexpression ($p<0.05$). Collagen fibres in the diabetes groups showed a longitudinal orientation, while in the *Glo1*/diabetes group they were predominantly circumferentially oriented.

Discussion and conclusion:

In this animal model of diabetes, *Glo1* overexpression attenuates arterial stiffness accompanied by notable effects on collagen structure, indicating that MGO contributes to diabetic arterial stiffening. Targeting MGO may provide a relevant novel approach to prevent arterial stiffening in diabetic mice.

Abstract 13: Clinical validation of plasma glycated amino acid-based blood test for early-stage osteoarthritis

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Introduction:

Osteoarthritis is a common degenerative joint disease in the elderly and overweight which greatly impairs quality of life. Key sites of impact are the joints of the knee and hip. Detection of early-stage osteoarthritis (eOA) offers opportunities for lifestyle and therapeutics interventions to alleviate symptoms and prevent severity progression. We previously reported the discovery phase of a blood test for eOA of the knee based on the quantitation of plasma glycated and oxidized amino acids released by proteolysis of cartilage and other proteins of the joint in eOA. Herein, we sought to validate this blood test in a large, independent subject cohort with application to eOA of the hip.

Materials and methods:

Subjects with eOA of the hip (n=110; 39±12 years) and healthy control subjects (n=120; age 33±9 years) were recruited at Meridan Hospital, Coventry, UK and Qatar Biobank, Doha, Qatar. Criteria for eOA were: new onset hip pain, normal radiographs of the symptomatic hip and arthroscopic examination showing macroscopic findings classified ≤ grade II Outerbridge scale. For healthy control subjects, inclusion criteria were no history of joint symptoms, arthritic disease or other morbidity. Exclusion criteria were a history of injury or pain in either hip, taking medication and any abnormality of the hip. Blood samples were collected after overnight fast with EDTA anti-coagulant. The concentrations of glycated and oxidized amino acids and hyp in plasma were determined by stable isotope dilution analysis liquid chromatography-tandem mass spectrometry. Classifier algorithms were trained and tested by 5-fold cross-validation using the Support Vector Machine method with plasma hyp and 14 glycated and oxidized amino acid analytes.

Results:

Minimum algorithm features for optimum classification of eOA and healthy controls were: N_{ω}-carboxymethylarginine, glyoxal, methylglyoxal and 3-deoxyglucosone-derived hydroimidazolones G-H1, MG-H1 and 3DG-H, and glucosepane. Accuracy was 95%, sensitivity 96%, specificity 94% and area-under-the-receiver operating characteristic plot AUROC 99%. Positive and negative predictive values, PPV and NPV, were 94% and 97%, respectively. Positive and negative likelihood ratios, LR+ and LR-, were 21.4 and 0.04, indicating strong, often convincing evidence of the presence and absence of eOA.

Discussion and conclusion:

This blood test may be optimally applied as a screening test. The prevalence of eOA in subjects of ≥60 years old is *ca.* 20%. After a simple pre-screening questionnaire, our test increases PPV and NPV for population screening to 93% and 95%, respectively, decreasing false discovery rate by 9-fold which greatly improves the effectiveness of expert referral. We conclude diagnostic algorithms with trace level plasma glycated amino acids as features provides a simple blood test for detection of eOA of the hip.

Abstract 14: profiling of glycation products in an untargeted metabolomics approach

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Introduction:

The non-enzymatic reaction between amino acids (AAs) and reducing sugars, also known as the Maillard reaction, is the primary source of free glycation products (GPs) in vivo and in vitro. Liquid chromatography−mass spectrometry (LC−MS) is often used for GP analysis due to its high selectivity, sensitivity, and throughput. Advanced glycation end products (AGEs) derived from lysine and arginine are the most widely studied and targeted quantified GPs in food and biological samples. However, GPs have diverse structures, and biologically important ones are not always these well-studied AGEs. Thus, there is a need to establish a reliable method for comprehensively qualifying and quantifying free GPs. In current study, we aim to develop an approach for simultaneously characterizing a wide range of free GPs using model systems in an untargeted metabolomics way. This approach comprises a validated HILIC-MS method for GP analysis and a publicly available spectral library for GP annotation.

Materials and methods:

Twenty proteinogenic AAs were reacted with glucose, respectively, to obtain GPs with a wide range of physicochemical properties. Biological sample extracts were mixed with model systems to evaluate the optimized HILIC-MS method. Public repository mining was used to demonstrate the existence of GPs in metabolomics data sets as unidentified compounds.

Results:

We established a HILIC-MS method for untargeted GP profiling through steps of column selection, mobile phase optimization, and method validation. The ZIC-cHILIC column operating under acidic conditions offered the best potential to discover GPs by providing good peak shapes and maintaining comparable compound coverage. Finally, the optimized HILIC-MS method can detect 70% of free GP features despite interference from the complex endogenous metabolites. We further used the validated HILIC-MS method to analyze model systems for the establishment of a searchable spectral library. Based on the conceptional reaction processes and structural characteristics of products, we classified GPs into common GPs (CGPs) and modified AAs (MAAs). A workflow for annotating GPs was established based on the structural and fragmentation patterns of each GP type. The final spectral library contains 157 CGPs, 499 MAAs, and 2426 GP spectra with synthetic model system information, retention time, precursor m/z, MS/MS, and annotations. As a proof-of-concept, we demonstrated the use of the library for screening GPs in unidentified spectra of human plasma and urine. The AAs with the C6H10O5 modification were the most found GPs. With the help of the model system, we confirmed the existence of C6H10O5-modified Valine in human plasma by matching both retention time, MS1, and MS/MS without reference standards.

Discussion and conclusion:

The HILIC-MS method we developed enables reproducible free GP analysis without ion-pair reagents. Additionally, our GP library can serve as an online resource to quickly screen possible GPs in an untargeted metabolomics workflow, furthermore with the model system as a practical synthesis method to confirm their identity.

Abstract 15: Glycation biomarkers in diabetic patients' fingernails – LC-MS/MS quantification of early and advanced glycation products in an underexploited, non-invasive biological matrix

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Introduction:

Fingernails have been examined for signs of ill health since Antiquity. More recently they have been used in toxicology, pharmacology and forensic medicine, and in particular for "biomonitoring" of exposure to pollutants. Fingernail clippings can be sampled non-invasively by untrained personnel, appear not to need refrigerated transport or storage, and contain a time-integrated record of certain circulating metabolites from the preceding 3-6 months. Despite pioneering reports in the 1980s, and more recent spectroscopic studies, fingernails have not been widely studied in glycation research. We examined their use in the non-invasive assessment of glycation with modern, specific, isotope-dilution liquid-chromatography with tandem mass spectrometric detection (LC-MS/MS).

Materials and methods:

Extensive methodology was performed, and the method used to analyze fingernails from a hospitalized population of over 80 type 1 and 2 diabetic patients. Results for the amino acids lysine, arginine and phenylalanine, the early glycation surrogate furosine (an acid-hydrolysis derivative of fructose-lysine), and the advanced glycation end-products (AGEs) carboxymethyllysine (CML) and carboxyethyllysine (CEL), were assessed in the light of patients' characteristics, notably their %HbA1c values.

Results:

The LC-MS/MS method had limits of quantification of 100µg/g nail for the amino acids, and 0.1µg/g for the glycation biomarkers. Coefficients of variation for trueness and intra- and inter-day precision were all ≤16%. The average % recovery after 6- or 12-months' storage of nail clippings at +/-25°C ranged from 87-105%. Reduction with sodium borohydrate prior to acid hydrolysis proved essential to obtain accurate measurements of CML in nails. There was a moderate linear correlation between ungual (nail) concentrations of furosine and patients' %HbA1c at nail sampling (r_S = 0.339, p = 0.0011). There were weak correlations between ungual AGE concentrations and %HbA1c (r_S = 0.225, p = 0.0187 CML; r_S = 0.205, p = 0.0294 CEL).

Discussion and conclusion:

Building on underexploited research spanning 40+ years, we developed a sensitive, robust and specific LC-MS/MS method to quantify biomarkers of early and advanced glycation in fingernails. Ungual concentrations of the early glycation biomarker furosine were correlated with diabetic patients' %HbA1c at nail sampling among this relatively heterogeneous, hospitalized population. The ease of collecting, transporting and storing this non-invasive sample matrix will facilitate its use in glycation research in large cohorts across a wide range of investigative scenarios.

Abstract 16: A maternal diet enriched in advanced glycation end-products reduces impulsive and anxiety-like behavior and increases adiposity in female offspring

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Introduction:

The brain is the most energetically expensive organ and humans have a larger than would be expected brain relative to their body size. One possibility is that humans were able to maintain such a metabolically taxing brain due to the shift to a cooked diet. Advanced glycation end products (AGEs) are a heterogeneous group of molecules produced endogenously in the body and found in some food products, particularly following heat treatment. Consumption of AGE-enriched foods is associated with altered biological and metabolic outcomes, yet the influence of dietary AGEs on neurocognitive outcomes is poorly understood. In this study, we aimed to determine whether a heat-treated maternal diet rich in dietary AGEs affects neurocognitive and metabolic outcomes in offspring using a rodent model.

Materials and methods:

Female Sprague Dawley rats were placed on either a heat-treated (BKD; AIN-93G diet heated at 160C for 60min) or control (CTL; AIN-93G diet without heat treatment) diet 1 week prior to breeding and remained on this diet throughout breeding, gestation, and lactation. Offspring were weaned onto a standard chow diet and cognitive and metabolic outcomes were assessed in adulthood.

Results:

Behavioral experiments conducted in offspring starting at postnatal day 60 revealed that females from dams fed the BKD diet showed significant cognitive differences relative to their CTL diet counterparts, with BKD animals exhibiting significantly lowered levels of anxiety-like behavior and impulsive responding for palatable foods. Additionally, these animals showed a distinct metabolic phenotype, weighing significantly less than CTL animals despite having a significantly higher percentage of fat mass. These outcomes were not observed in male offspring of dams fed the BKD diet relative to CTLs.

Discussion and conclusion:

Our findings reveal a novel cognitive and metabolic phenotype for female offspring of dams fed a heated diet rich in AGEs. Female, but not male offspring of dams fed an AGE-enriched diet display decreased anxiety-like behavior, decreased impulsive responding, and decreased body weight coupled with increased fat mass. Ongoing experiments are exploring our hypothesis that these results may be partially explained by differences in the maternal oxytocin system, which is known the have anxiolytic and anorexigenic affects, and whose transport into the central nervous system relies on the receptor for AGEs.

Abstract 17: Glyoxalase 1 overexpression improves neurovascular coupling response and reduces cognitive decline in an animal model of type 1 diabetes

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Introduction:

Diabetes is associated with cognitive impairment, however, the underlying mechanism remains unclear. Methylglyoxal (MGO) is a small but highly reactive molecule, which arises from the breakdown of glucose and is increased in diabetes. MGO is associated with microvascular dysfunction in diabetes. We hypothesise that MGO accumulation in diabetes causes cognitive impairment which can be prevented by overexpression of *glyoxalase 1 (Glo1)*; the enzyme involved in the breakdown of MGO.

Materials and methods:

Diabetes was induced in 8-9 week old *Glo1* overexpressing C57Bl/6 mice and wild type littermates by streptozotocin injection (STZ, 50mg/kg IP) on 5 consecutive days, resulting in 3 groups (control, diabetes, *Glo1*/diabetes, n=10-12/group). Fasting blood glucose was measured at week 13. Mouse cognitive function was tested at 5-7 and 14-16 weeks using various cognitive tests. At 17 weeks, cerebral blood flow (CBF) was measured by laser speckle contrast imaging. Neurovascular coupling (NVC) was measured by assessing the change in CBF in the barrel cortex upon whisker stimulation (10Hz, 30s). Mice were sacrificed at 17 weeks and GLO1 activity was measured in the cortex.

Results:

Glucose levels in diabetes (25.6±3.0mM) and *Glo1*/diabetes (23.6±2.7mM) groups were increased vs control (7.0±1.0mM) ($p<0.0001$). GLO1 activity was increased 2.5-fold in *Glo1*/diabetes ($p<0.0001$) and decreased 10% in diabetes ($p<0.05$) compared to control. The average CBF increase in the barrel cortex upon whisker stimulation was unaffected. However, the NVC response time was increased in diabetes group, and this was normalised in *Glo1*/diabetes. A decreased visuospatial memory was observed in diabetes mice, compared to control, which was normalised in the *Glo1*/diabetes group.

Discussion and conclusion:

We found that diabetes impairs long-term memory in mice and reduced the NVC response speed, which was prevented by overexpression of *Glo1*. These data suggest that MGO formation in the brain in diabetes has an effect on cerebrovascular and cognitive functions.

Abstract 18: Glycation reduces albumin's protective effect against amyloid-β: Insights for Brain Health

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Introduction:

The process of glycation can significantly impact the structure and function of a protein. Human serum albumin (HSA) is a ubiquitous carrier protein in the body, prone to in vivo glycation. One of the roles of HSA is to scavenge and transport amyloid-β (Aβ), a protein known for its pro-inflammatory signalling effects in the central nervous system, while its accumulation in the brain is a condition observed in Alzheimer's disease patients. Aβ is also a known ligand for the Receptor for Advanced Glycation End-products (RAGE). Given Aβ's relevance to brain ageing and Alzheimer's disease, our in vitro study aimed to delve into the potential protective role of HSA against Aβ-induced inflammation. Additionally, we investigated how glycation of HSA might modify its protective effects against Aβ.

Materials and methods:

Briefly, HSA was glycated with glucose, methylglyoxal, or carboxylic acid, and the levels of carboxymethyllysine and carboxyethyllysine were measured with LC-MS/MS. Next, native or glycated HSA was incubated with Aβ for 3 days. Subsequently, HMC3 microglia, THP-1 macrophages and primary macrophages were exposed to Aβ only, Aβ with native HSA or Aβ with glycated HSA for 48 hours. To investigate the involvement of RAGE, RAGE inhibitor FPS-ZM1 was added to the cells. Supernatants were collected, and the secretion of cytokines was measured using ELISA.

Results:

Aβ caused an inflammatory response in HMC3, THP-1 and primary macrophages, while the response partially decreased in the presence of FPS-ZM1. Furthermore, when Aβ was pre-incubated with native HSA, the inflammatory response was reduced compared to Aβ only, whereas glucose-modified glycated HSA did not show this effect.

Discussion and conclusion:

Aβ-induced inflammation is partially RAGE-dependent, and only the glucose-modified HSA led to compromised scavenging properties for Aβ compared to native HSA. Our findings indicate that glycation of HSA alters the interaction between HSA and Aβ, hence affecting its physiological characteristics. This enhances our understanding of how in vivo protein glycation might contribute to the onset and progression of neurodegenerative disorders. Our future investigations will focus on comprehending the impact of glycation on the elimination and transportation of Aβ via the blood-brain barrier.

Abstract 19: Gut bacterium Intestinimonas butyriciproducens improves host metabolic health: evidence from cohort and animal intervention studies

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Introduction:

The human gut microbiome strongly influences host metabolism via fermentation of dietary components to metabolites that allow communication with peripheral tissues. Short chain fatty acids are among most known microbial metabolites that signals to the host. *Intestinimonas butyriciproducens* is a prevalent commensal bacterium that has a unique capability of converting dietary fructoselysine to butyrate and acetate and has a completed fructoselysine catabolic pathway. Dietary fructoselysine is an abundant Amadori product formed in foods during processing and is part of food products rich in dietary advanced glycation end products which can be potentially toxic. Therefore, understanding of the role of this bacterium and fructoselysine metabolism in metabolic health is highly relevant.

Materials and methods:

We accessed associations of *I. butyriciproducens* with metabolic risk biomarkers via both strain and functional levels using the Swedish IGT cohort (n=1011) characterized by fecal metagenomic analysis. Subsequently, we isolated and studied the capacity and genomic made-up of fructoselysine fermentation by four human *Intestinimonas* isolates. We later administered *Intestinimonas butyriciproducens* GL3 isolate to diet-induced obesity mice to explore the impact on obesity and host metabolism and to achieve a proof-of-concept study in human.

Results:

We observed that the level of the bacterial strain as well as fructoselysine fermentation genes were reversely associated with BMI, triglycerides, HbA1c and fasting insulin levels. We also investigated degradation capacity of fructoselysine within *Intestinimonas* genus using a culturing dependent approach and observed that *I. butyriciproducens* as a key player in the butyrogenic fructoselysine metabolism in the gut. To explore the function of *I. butyriciproducens* on host metabolism, we employed the diet-induced obesity mouse model to mimic the human metabolic syndrome. Oral supplementation of *I. butyriciproducens* counteracted body weight gain, hyperglycemia as well as adiposity. Moreover, within the inguinal white adipose tissue, bacterial administration reduced inflammation, and promotes pathways involved in browning and insulin signaling. The observed effects are attributable to the formation of the short-chain fatty acids butyrate and acetate from dietary fructoselysine, as their plasma levels were significantly augmented by the bacterial strain, thereby contributing to systemic effects of the bacterial treatment.

Discussion and conclusion:

Intestinimonas butyriciproducens ameliorates host metabolism in the context of obesity and may thus be a good candidate for a new microbiota-therapeutic approaches to prevent or treat metabolic diseases.

Abstract 20: Metabolization of the Amadori Product N-ε-Fructosyllysine by Probiotic Bacteria

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Introduction:

Thermally treated food contains large quantities of various Maillard reaction products (MRPs) which we ingest with our daily diet. The most important group in terms of quantity are the Amadori products (APs), of which we ingest up to 1 g per day. Preliminary work has shown that only a very small proportion of the ingested amount of APs can be recovered in urine and feces. Thus, Amadori products enter the colon largely unmodified, where they encounter a variety of different bacterial species. To date, there is very little information on how these substances are metabolized by the colonic microbiome. The aim of this study was to examine how dietary Amadori products can be metabolized by probiotic bacteria.

Materials and methods:

Nine commercially available probiotic preparations from pharmacies and online shops, as well as five single pure strains thereof, were incubated anaerobically for 72 h in a minimal medium containing different amounts of free N-ε-fructosyllysine (FruLys) as the only carbon source. Additional experiments with isotopically labeled FruLys allowed the clear identification of metabolites formed from the Amadori product. Analysis of FruLys-degradation and formation of metabolites was performed by LC-MS/MS and GC/MS.

Results:

Of the nine preparations tested, one was able to completely degrade a physiologically relevant amount of the Amadori product within 72 h. Model incubations with individual pure strains of the respective preparation identified two *Lactobacillus* strains and one *Pediococcus* strain as being able to deglycate FruLys albeit with different degradation capacities. Free lysine and lactic acid could be quantified as metabolites of the amino acid and sugar moiety, respectively. The strain *Intestinimonas butyriciproducens* – a FruLys degrading bacteria strain known from the literature and therefore the referene strain in our studies – metabolized both the amino acid and the sugar moiety to butyric acid.

Discussion and conclusion:

In conclusion, our experiments allowed the identification of three new deglycating bacteria species and showed for the first time that Amadori products can be utilized as substrates in lactic acid fermentation. These results may have a great significance with respect to an evolutionary adaptation of the human intestinal microbiota to a constant supply of MRPs via heated foods.

Abstract 21: How do early and lifelong exposure to dietary AGEs affect tissue, gut sensitivity, and microbiota in mice?

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Introduction:

Diet plays a crucial role in triggering deleterious physiological responses. Dietary Advanced glycation end-products (dAGEs) are suggested to lead to chronic low-grade inflammation (CLGI), oxidative stress, and alter gut bacteria. Studies investigate how glycation products like dietary carboxymethyl-lysine (dCML) and the Receptor for Advanced Glycation End-Products (RAGE) affect cellular responses, but it is unclear if early-life or lifelong exposure to dAGEs could contribute cited consequences of AGE intake, and whether these effects can be reversed.

Materials and methods:

Our study employed standard and an in-house prepared protein-dCML model to enrich mice food and explored how a high (13-times) dCML-enriched diet affects Wild-Type (WT) and RAGE KO mice from birth to 6, 35, and 70 weeks. We also investigated if switching to a standard diet at 6-weeks of age could reverse the potential dCML effects in the "Switch group". We assessed outcomes at systemic and local levels using quantitative (LC-MS/MS) and molecular biology methods (qRT-PCR, Metagenomics) to quantify dCML and physiological changes, respectively, in multiple organs.

Results:

We confirmed the accumulation of free dCML in kidneys (3x), ileum (17x), and colon (20x) from 6 to 70 weeks of diet, regardless of RAGE expression. Switching diets lowered dCML comparable control conditions. The dCML-enrich diet did not notably affect endogenous glycation, inflammation, or senescence. *TNFα, VCAM1, IL6*, and *P16* gene expression roughly doubled with age, especially in WT kidneys. There were notable increases in *TNFα* expression detected in the intestinal tract of the Switch group, suggesting an increased inflammatory reaction that could be related to timing of dietary shifts. Minor alterations in gut microbiota communities were observed.

Discussion and conclusion:

While persistent intake of dCML resulted in increased free dCML levels within tissues, there were no notable increases in parameters associated with inflammageing, oxidative stress, or significative microbial community shifts. However, early-life diet switching seemed to contribute with greater sensitivity to intestinal inflammation and increased *TNFα* expression. In addition, the lack of RAGE seemed to lower age-related *TNFα* increase in a healthy rodent model. It provides a potential avenue for future research to delve into understanding the influence of dAGEs on the onset of different diseases and health conditions.

Abstract 22: The impact of different glycation methods on the immunomodulatory effects of human serum albumin

C.C.C. Croes [1], M. Chrysanthou [1], H.J. Wichers [1], T. Hoppenbrouwers [1], J. Keijer [1], H.F.J. Savelkoul [1], M. Teodorowicz [1]

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Introduction:

Diabetes is a growing concern in the modern age, characterized by high blood glucose levels. Elevated glucose levels can lead to the generation of Advanced Glycation End Products (AGEs). AGEs form through a non-enzymatic reaction between amino acids and reducing sugars, known as the Maillard reaction. The accumulation of AGEs in the body has been linked to several diseases. It has been shown that in the onset and pathology of diabetes, glycated human serum albumin (HSA) increases as a consequence of elevated blood glucose and other glycating agents, such as methylglyoxal. However, limited information is available regarding how these different types of glycation impact the immunomodulatory properties of HSA. This study investigated the effects of three different glycating agents on HSA protein, their impact on binding to known AGE receptors, and their effects on macrophages.

Materials and methods:

HSA was glycated with D-glucose, methylglyoxal, or CML-directed glycation via glyoxylic acid and NaBH3CN. Glycation-related biochemical changes were characterized using dot blot, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Native-PAGE, o-phthalaldehyde (OPA)-assay, and Liquid Chromatography-Mass Spectrometry (LC-MS). The binding of differentially glycated HSA to AGE receptors, including RAGE, SR-A1, Galectin-3, and CD36 receptors, was determined with inhibition ELISAs. The impact of differentially glycated HSA on the expression of pro-inflammatory cytokines in macrophage cell line THP-1 and macrophages isolated from human peripheral blood mononuclear cells (PBMCs) was studied using quantitative polymerase chain reaction (qPCR) and a phosphorylation of NF-κB p65 via Western blot.

Results:

All glycation methods led to unique AGE profiles and had a distinct impact on protein structure. Glycation resulted in the binding of HSA to the AGE receptors RAGE, Galectin-3, CD36 and SR-A1, with MGO modification showing the highest binding, followed by glucose and lastly CML. Additionally, modification of HSA with MGO led to the increased expression of pro-inflammatory markers in THP-1 macrophages including: IL-1β, IL-8, TNF-α and RAGE as well as enhanced phosphorylation of NF-κB p65. The same pattern although less prominent was observed for HSA glycated with glucose and CML respectively. An increase in pro-inflammatory markers was also observed in PBMC-derived macrophages exposed to glycated forms of HSA. However, significant variations between donors were evident, suggesting individual differences in responsiveness to AGEs.

Discussion and conclusion:

Out of three different methods of glycation of HSA, MGO modification was identified as the most immunogenic in THP-1 macrophages, followed by glucose, and lastly, CML specific modification. These results suggest that various glycation methods, resulting in specific modifications of HSA structure, exert unique impacts on protein immunogenicity. This effect is likely due to selective receptor recognition by immune cells. Moreover, it is crucial to pay increased attention to regulating MGO levels in diabetics, in addition to glucose regulation, to prevent potential harmful effects from endogenous protein glycation.

Abstract 23: Glycation and aldosterone antagonistic interactions inhibit the progression of diabetic nephropathy and cardiomyopathy in streptozotocin-induced diabetic BALB/c mice

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Introduction:

In diabetic nephropathy (DN) and diabetic cardiomyopathy (DC), hyperglycemia elevates protein glycation forming advanced glycation end products (AGEs) and hyperaldosteronism. The receptor for AGEs (RAGE) and aldosterone-bound mineralocorticoid receptor (MR) are known to activate NF-κB pathway. The pharmacologic antagonist of MR, spironolactone (SPIR) and RAGE antagonist FPS-ZM1 inhibit MR and RAGE respectively. The present study focuses on the crosstalk between aldosterone and glycation in diabetic mice.

Materials and methods:

Streptozotocin-induced male BALB/c diabetic mice were treated with antagonists (SPIR- 50 mg/kg B.W/day, FPS-ZMI-1.5 mg/kg B.W/day) separately and also in combination of SPIR + FPS-ZM1 (n=6). During 21 day's treatment, biochemical parameters like blood glucose, triglycerides, glycated haemoglobin, microalbumin, sodium, potassium urea and complete blood count were measured using commercial kits. After the completion of treatment, plasma and tissue (kidney, heart) AGEs fluorescence and antioxidant markers (SOD, Catalase and GSH) were analysed. Tissue histopathology and immunohistochemistry (IHC) for renal NF-κB expression were performed.

Results:

Treatments with SPIR and FPS-ZM1 attenuated the biochemical, nephropathy markers and improved the sodium-potassium dysregulation in a time-dependent manner. Various AGEs (crossline, pentosidine and malondialdehyde) in plasma and tissues were significantly reduced with improved antioxidant markers. The activity of detoxification enzymes GLOI and GLOII in kidney and heart tissues was assessed to study the impact of hyperglycemia on the detoxification process. GLOI activity in kidney tissue of diabetic mice demonstrated 4-fold decrease as compared to control mice. Antagonist treatment reversed the increased glomerular basement membrane thickness, inflammatory cell infiltration, and expression of NF-κB.

Discussion and conclusion:

The action of SPIR counteracted the aldosterone-mediated influence by increasing the catalase, GSH, and SOD activity in the kidney tissue. It is well known that AGEs and oxidative stress contribute to NF-κB activation, antagonist SPIR and FPS-ZM1 showed a renoprotective effect from diabetes by improving renal hypertrophy and downregulating the expression of NF-κB. In the presence of SPIR and FPS-ZM1, the levels of antioxidant enzymes, IHC, and biochemical markers demonstrated a considerable reduction in the deleterious effect of aldosterone and glycation. As evident, interaction causes the progression of diabetic kidney and heart disease, the correlation between glycation and aldosterone offers new therapeutic approach or treatment modalities to condense future benefits for DN and DC.

Abstract 24: Inflammation drives increased chemical modification of proteins by fumarate and itaconate

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Introduction:

The metabolism of glucose results in the production of tricarboxylic acid cycle (TCA) intermediates including fumarate. Inflammatory stimuli, such as lipopolysaccharide (LPS), stimulate the production of an additional TCA-derived metabolite, itaconate, in activated macrophages. Both fumarate and itaconate react non-enzymatically with protein cysteine residues to yield S-2-succinocysteine (2SC) and 2,3-dicarboxypropylcysteine (2,3-DCP). Increases in 2SC and 2,3-DCP during immunometabolism have primarily been explored in macrophages cultured in vitro. We sought to determine if 2SC and 2,3-DCP increase in vivo in LPS-treated mice, modeling a bacterial inflammatory stimulus. We further examined the inflammatory response and levels of the protein chemical modifications in a mouse model of mitochondrial Complex I deficiency; the Ndufs4 knockout mouse.

Materials and methods:

Wildtype and Ndufs4 knockout mice were injected with 5mg/kg LPS or saline control with tissue collected at 6 or 18 hrs. Following tissue processing and ethyl esterification, the 2SC and 2,3-DCP levels were quantified by LC-MS/MS. Inflammatory cytokines were quantified by flow cytometry and ELISA. Mouse peritoneal macrophages were isolated and stimulated in vitro with 100ng/ml LPS. Gene expression, metabolite levels and cytokine production were quantified.

Results:

We employed LC-MS/MS following ethyl esterification to detect the immunometabolite derived protein modifications 2SC and 2,3-DCP. As expected, the levels of itaconate derived 2,3-DCP increased upon stimulation with LPS. Interestingly, fumarate derived 2SC levels were increased 2.5-fold in serum upon LPS stimulation and were further increased ~3.8-fold in the Ndufs4 KO mouse. Inflammatory cytokine data suggested a hyperinflammatory response in the Ndufs4 KO mouse, with elevation of pro-inflammatory cytokines such as interferon-gamma.

Discussion and conclusion:

We describe the dual quantification of two non-enzymatic protein modifications by LC-MS/MS, 2SC and 2,3-DCP, that have recently been associated with the immunometabolic response. We note that the Ndufs4 KO mouse has a hyperinflammatory response to LPS versus wild type controls, occurring in parallel with sustained elevation of fumarate-derived 2SC.

Abstract 25: Biodegradation of food melanoidins via solid state fermentation by Aspergillus awamori

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Introduction:

Melanoidins are high molecular weight compounds formed during the final stage of the Maillard reaction and contributing to the browning of thermally processed foods. Melanoidins can behave as antioxidant dietary fiber, having beneficial effects on gut microbiota. They also display effects like cation-binding activity, lowering the bioavailability of amino acids, flavor binding, and dark coloring, which are not desirable in many products. Microbial treatment could be a promising green strategy for melanoidins degradation during food production. This study aims to investigate the effectiveness of melanoidin degradation by *Aspergillus awamori*.

Materials and methods:

Several melanoidin-rich substrates having different molecular features, including distilled spent grain (DSG), bread crust, roasted cocoa, roasted coffee, and spent coffee grounds were employed. In the first part of the study, melanoidins from DSG were used as the only carbon and nitrogen source to grow *A. awamori* in a submerged fermentation set-up. In the second part, *A. awamori* was cultivated by solid-state fermentation (SSF) on melanoidin-containing materials.

Results:

The results showed that *A. awamori* exhibited a robust growth on DSG melanoidins, reaching a maximum biomass of 2.50 g/L. The browning index of broth was reduced by 24.4% after 3 days of fermentation. *A. awamori* increased the amount of soluble matter in extracts of coffee, bread crust, and spent coffee grounds via SSF. The browning index decreased by 17.4%, 24.3%, 61.0% and 89.8% in bread crust, coffee, spent coffee grounds and cocoa, respectively. SDS-PAGE and protease activity analysis revealed that melanoidin degradation did not correlate with the proteolytic activity of the *A. awamori*, suggesting a possible key role of ligninolytic peroxidase enzymes.

Discussion and conclusion:

The study shows that melanoidins are a very good substrate for the growth of *A. awamori*. Moreover, SSF by *A. awamori* can be used as a biotechnological approach to degrade undesired melanoidins in certain food and by-products, while its edible mycelium offers a valuable food resource, showing promise for upcycling melanoidin-rich waste into biomass.

Abstract 26: Inhibitory Potential of Vitamins on Heat-Induced Protein Modifications in Whey-Based Foods

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Introduction:

Whey-based foods, including infant formulas and enteral nutrition products, serve as the sole source of essential amino acids and vitamins in many diets. Thermal treatment of these products results in the formation of heat-induced non-enzymatic post-translational protein modifications (nePTMs), which impair the protein quality. The objective of this study was to examine the impact of seven fat-soluble and eight water-soluble vitamins and vitamers on nePTM formation. We aimed to identify promising inhibitors that could be used to develop effective strategies to mitigate nePTM formation. Therefore, a LC-ESI-MS/MS method was developed for the structure- and site-specific detection of 14 nePTMs at 30 binding sites in the major whey protein β-lactoglobulin. This approach allows for a comprehensive analysis of the specific effects of vitamins on distinct nePTMs or binding sites.

Materials and methods:

Raw cow's milk whey was spiked with phylloquinone, menaquinone 4, DL-α-tocopherol, retinol, retinoic acid, cholecalciferol, β-carotene, pyridoxamine, nicotinic acid, L-ascorbic acid, cyanocobalamin, biotin, thiamin pyrophosphate, riboflavin or folic acid. The samples were then heated at 60 °C for 3 d, which results in a similar amount of nePTMs as industrial processing. Whey proteins were enzymatically hydrolyzed using the specific endoproteinase Glu-C, and the resulting peptides were separated via micro-LC. Detailed analysis was performed with tandem mass spectrometry operating in scheduled multi-reaction monitoring mode.

Results:

Riboflavin, folic acid, and cyanocobalamin strongly inhibited all nePTMs. The differences in vitamers provided valuable information, with retinol exhibiting greater mitigation potential compared to retinoic acid and β-carotene. Ascorbic acid was found to promote the formation of some advanced Maillard products, including N-terminal carboxymethylleucine, Nε-carboxymethyllysine, and Nε-formyllysine. However, it exerted no effect on N-carboxymethylarginine and an inhibitory effect on 4-imidazolidinone. Pyridoxamine, thiamine, and biotin demonstrated a tendency to promote some modifications. In general, the primary Maillard reaction and oxidation can be inhibited by several vitamins, while the effects on advanced Maillard products are more complex.

Discussion and conclusion:

Despite potential nePTM inhibition, some vitamins may have conflicting impacts on protein quality. Therefore, the structure- and binding site-specific assessment is important for a molecular-level investigation of vitamin mechanisms in processed foods. Our study has identified promising candidates for tailored approaches to improve the quality and safety of whey products.

Abstract 27: Key Contributors to Color Formation? – Elucidating the Role of Short-Chained Maillard Reaction Products in Melanoidin Formation

C. Kanzler [1], T. Rueger [1], L. Bork [1], S. Rohn [1]

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Introduction:

The formation of colorants during thermal food processing is one of the most obvious changes in food caused by the Maillard reaction. Nevertheless, the formation mechanisms and the structure of melanoidins as heterogenous end-products in this complex reaction cascade are still not comprehensively understood. Approaches to isolate individual food melanoidins are important but gain limited insights into the overall reaction because it is to be expected that thousands of compounds with significant differences in chemical structure and molecular weight are formed. A useful tool to analyze complex Maillard reaction mixtures is high-resolution mass spectrometry (HRMS). With the help of Kendrick mass analysis reoccurring structural elements can be identified in HRMS data sets allowing to draw more overarching conclusions. In this study, model melanoidins formed from short-chained Maillard reaction intermediates were investigated to reveal the most relevant reaction steps and resulting structural pattern in these colorants.

Materials and methods:

Short-chained key intermediates of the Maillard reaction, such as methylglyoxal, glyceraldehyde, dihydroxyacetone, erythrose, and ribose, were heated with different amino acids in aqueous solution (100 mM, pH 5, 100 °C) and at low water content (60 wt% of water, 180 °C). The resulting reaction mixtures were analyzed in regards to color (420 nm, CIE-Lab), antioxidant activity (TEAC assay), conversion of the reactants (HPLC), molecular weight distribution (size exclusion chromatography), and molecular composition of the reaction mixtures (HRMS).

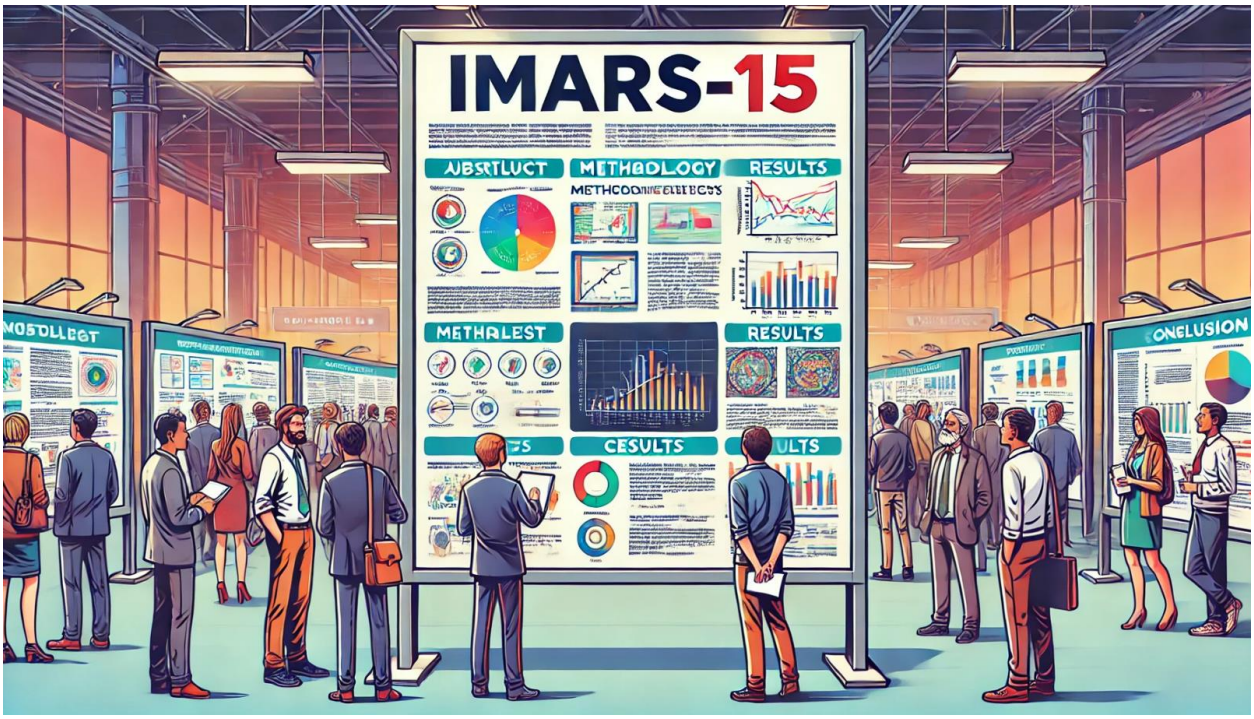
Results:

It was found that the backbones of the individual Maillard reaction intermediates and of the amino acids as well as all of their typical cleavage products were integrated into oligomeric colorants. The high-molecular weight (HMW) fractions of the Maillard reaction mixtures were formed with yields under 10 wt% of the dry mass. However, these fractions contributed in higher proportions to the overall color of the reaction mixtures. On the other hand, the antioxidant activity was more in line with the yield of the HMW colorants.

Discussion and conclusion:

Overall, the results underline the high complexity and structural variety of colorants formed in course of the Maillard reaction. Even when the reactants are limited to two components – a single Maillard reaction intermediate and an amino acid – hundreds of compounds are formed in aldol reactions and nucleophilic reactions of the native reactants as well as their cleavage and degradation products. Based on their dry mass the “classic” melanoidins in form of the HMW colorants are responsible for the majority of the absorbance at 420 nm in most reaction mixtures. However, it should be noted that even oligomeric products (molecular weight below 12 kDa) contribute strongly to color and antioxidant activity. The present results indicate that the analysis of food melanoidins should not solely rely on individual marker compounds, but also on structural patterns traceable by HRMS methods based on reoccurring mass differences.

Poster Presentations



Session 1

(Tuesday, 17th September, 12:05 - 14:00), 36 abstracts:

Abstracts: 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 74, 75, 76, 77, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96

Session 2

(Wednesday, 18th September, 12:05 - 13:30), 33 abstracts:

Abstracts: 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 72, 73, 79, 81, 83, 85, 87, 89, 91, 93, 95

Abstract 28: Inhibition vs. Promotion – The Role of Hydroxycinnamic Acids in the Maillard Reaction

L. V. Bork [1], N. Proksch [1, 2], L. S. Steinbauer [1], S. Rohn [1], C. Kanzler [1]

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Introduction:

The non-enzymatic conversion of food ingredients plays a vital role for the quality of processed food. Among these reactions, the Maillard reaction is of high relevance due to the ubiquitous presence of carbohydrates and amino compounds in food. On the one hand, it induces the formation of low-molecular weight, characteristic aroma compounds, whose structures and formation mechanisms have been mostly identified, to date. On the other hand, subsequent reactions leading to the formation of complex, heterogenous, high-molecular weight colorants, so called melanoidins, are not comprehensively described. As these polymers significantly contribute to the sensory quality of food and exhibit prebiotic and antioxidant properties, knowledge regarding their structural composition is essential to understand and eventually control these attributes.

Materials and methods:

This study aimed to build a general understanding of the reactivity of ubiquitous hydroxycinnamic acids, such as caffeic acid and ferulic acid with carboyhdrates, alanine, and reactive Maillard intermediates (pyrrole-2-carbaldehyde, 5-hydroxymethylfurfural, and furfural) by incubation under roasting conditions at 220 °C for up to 10 min. The obtained reaction mixtures were characterized regarding their browning (420 nm), the conversion of the reactants (HPLC-DAD, GC-MS), and their antioxidant properties (TEAC assay). The structural composition of colored reaction products was revealed by HRMS analysis which further enabled the identification of repetitive elements, potentially contributing to polymerization.

Results:

In summary, the present study showed that decarboxylation reactions play a crucial role in heat-induced browning reactions of hydroxycinnamic acid, which are catalyzed by nitrogen-containing compounds. Cross-linking with bifunctional carbonyls like pyrrole-2-carbaldehdye enables the formation of larger chromophores, which correlated with the highest browning intensity of the corresponding reaction products.

Discussion and conclusion:

The data obtained can serve to understand the fundamental properties of phenol-containing melanoidins formed in food like coffee, cocoa, or roasted nuts, by demonstrating the potential contribution of hydroxycinnamic acids to the color and antioxidant properties of melanoidins in plant-based food.

Abstract 29: Deciduous teeth are a novel biosample reflecting perinatal accumulation of AGEs and predictive of psychotic symptoms during adolescence: preliminary study

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Introduction:

The embryonic and early postnatal periods are critical for brain development, and stressors during these periods significantly impact brain development and increase the risk of psychiatric disorders across the lifespan. However, obtaining accurate patient information from the perinatal period is challenging in clinical settings, and there's currently no established objective method for quantifying perinatal stress. Deciduous teeth have gained attention as a novel biosample to address this issue. Deciduous teeth, which form during the embryonic and early postnatal periods when the brain is highly sensitive to stress, serve as "fossils" which permanently record stress exposures. We have previously found pentosidine accumulation in plasma of approximately 40% of individuals with schizophrenia and an association between glycation stress and the risk of developing schizophrenia in adolescence. These findings suggest a potential role for glycation stress in the pathophysiology of schizophrenia across the lifespan. However, the effects of perinatal exposure to glycation stress remain unclear. Therefore, we examined whether quantitative analysis of advanced glycation end products (AGEs) concentrations in deciduous teeth, indicating perinatal stress exposure, is linked to the onset of psychotic symptoms in adolescence among community children participating in a birth cohort study.

Materials and methods:

This study included 15-year-old children without a history of psychiatric disorders who were part of the Tokyo Teen Cohort. Psychotic symptoms were assessed by interviews conducted by three psychiatrists. Incisor teeth from 8 participants with psychotic symptoms and 12 age- and sex-matched participants without psychotic symptoms were analyzed for AGEs concentrations in deciduous teeth using liquid chromatography-mass spectrometry (LC-MS/MS) or high performance liquid chromatography (HPLC).

Results:

The concentration of certain AGEs in deciduous teeth was significantly higher in children with psychotic symptoms during adolescence than in those without psychotic symptoms. Logistic regression analysis showed that elevated AGEs concentration significantly predicted the onset of psychotic symptoms in adolescence. This finding was statistically significant even after adjusting for the effects of covariates.

Discussion and conclusion:

Elevated AGEs concentrations in deciduous teeth significantly predicted the emergence of psychotic symptoms in adolescence. This finding suggests that exposure to perinatal glycation stress may be one of the pathological bases for the appearance of psychotic symptoms in adolescence. As this was a preliminary study with limited data, we aim to expand the dataset to validate the robustness of our findings.

Abstract 30: Current and forward-looking experimental approaches in Maillard reaction product formation in gluten-free bread matrices

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Introduction:

Heat treatment of foods is a key operation in industry and results in the development of a wide range of flavors and tastes through the Maillard reaction. Apart from sensory modifications, the Maillard reaction impacts the nutritional and toxicological properties of food. The consumption of highly thermally treated foods rich in Maillard reaction products (MRPs) could increase the total i*n vivo* advanced glycation end product (AGEs) accumulation. Recent years have seen increased consumer interest in gluten-free products, a trend that reflects increasing awareness of celiac disease and the increase in the number of people with non-celiac gluten sensitivity. Although the attention of the scientific community in the past years has been focused on dietary fiber incorporation into bakery products, fiber deficiency in the gluten-free diet is still present globally. There is some evidence suggesting that a gluten-free diet might positively influence type 1 diabetes mellitus pathology, onset, and clinical course. However, instituting a gluten-free diet could be a significant hurdle because many gluten-free foods have a high glycemic index. This has led to rise in the demand for low-sucrose gluten-free products. For the moment, scarce information has been reported regarding the formation of MRPs in gluten-free bread. Considering the reformulation of traditional recipes of gluten-free products, the evaluation of heat-induced chemical markers in this food category is a priority. Therefore, the present study aimed to determine the effects of the various approaches used to improve gluten-free bread recipes by evaluating the effects of food ingredients (types of sugar) and additives (different fibers) on Nε-(carboxymethyl) lysine (CML) formation in the crust and crumb of model gluten-free bread.

Materials and methods:

The studies involved the preparation of gluten-free bread with the addition of various fibers. The proximate composition of the fiber was specified according to the AOAC method. Additionally, a kinetics experiment was conducted during bread baking (0 to 30 min at 230°C), where sucrose was substituted with erythritol. The control sample contained 100% sucrose, while the experimental samples contained a 50%/50% mixture of sucrose and erythritol, as well as 100% erythritol. The concentration of CML in the gluten-free bread was determined using LC/MS-MS.

Results:

After a baking time (5 min), a significant level of CML was observed and maintained at a consistent level throughout the remainder of the thermal treatment. Replacing sucrose with erythritol does not exclude the formation of CML in the analyzed samples. The presence of fiber tended to increase CML in gluten-free breads. A significant correlation was obtained between the SDF dietary fiber content and CML generation in the bread crust and crumb.

Discussion and conclusion:

The comprehensive character of these studies could contribute to a better understanding of the mechanisms of the formation of CML in gluten-free bread matrices. In addition, these findings underline the importance of conducting risk-benefit consideration when introducing reformulated recipes to reach not only an improved nutritional profile but also control toxicological aspects.

Abstract 31: All-aqueous emulsion droplet reactors for accomplishment of the Maillard reaction

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Introduction:

Besides accomplishing the Maillard reaction by dry heating of the reactants and wet state-heating of single-phase solutions, liquid-liquid biphasic systems on the basis of oil-water emulsions have been assessed as reaction platforms. Nonetheless, the use of organic solvents or edible oils, also synthetic low-molecular weight surfactants, as well as requirement for utilising high-energy emulsification approaches in making oil-water emulsions are disfavoured. All-aqueous (water-in-water) emulsions which are produced by size reduction of one of the phases of an aqueous two-phase system (ATPS) within the pairing immiscible phase, have a ocparable ability to oil-water emulsions for partitioning guest molecules between two phases. The objective of the present study was to assess whether all-aqueous emulsions can be used as rather sustainable and consumer-friendly liquid-liquid biphasic systems for the development of the Maillard reaction.

Materials and methods:

All-aqueous emulsions were prepared using polymer-salt and polymer-polymer pairs either supplemented with deep eutectic solvents (DESs) or not. Then reducing sugar and amino acids were added as reactants into emulsions and the emulsions were heated up to accomplish the Maillard reactions. The reactants were either segregated between the interior (droplet) and exterior (continuous) phases of emulsions or co-encapsulated within the droplet phase. Concentration of the Maillard reactants, and reaction products through the process was measured, and their partition coefficients were calculated.

Results:

When the reactants were segregated, they could interfacially react and most of the Amadori products, also the advanced reaction products partitioned into the rather hydrophobic phase of the emulsions. When co-encapsulated, certain reaction derivates showed affinity towards the rather hydrophilic phase of the emulsions. The incorporation of DES in emulsion formulation affected the reactants partitioning and the reaction kinetics.

Discussion and conclusion:

All-aqueous emulsions enable the Maillard reaction accomplishment and the reaction products downstream fractionation in one-pot. We could modulate the reactants partitioning and the reaction rate by tailoring emulsion composition.

Abstract 32: (Glyc)oxidation during barbecuing and in vitro gastrointestinal digestion of extruded pea and pork balls

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Introduction:

Plant-based meat alternatives have become increasingly popular for various reasons, however, their nutritional quality and possible health implications are poorly investigated. Oxidative reactions occur during thermal processing and gastrointestinal digestion of foods, leading to the formation of various biologically active metabolites derived from lipids and proteins. In this study, we investigated the (glyc)oxidative stability of model extruded pea and pork balls with different added carbohydrates (glucose, sucrose, or starch) and herbs during heating and simulated gastrointestinal digestion.

Materials and methods:

High-moisture extruded (HME) pea and pork balls with equal protein and fat content were manufactured by the addition of 5% coconut oil, 10% rapeseed oil and 9% starch to HME-pea, and the addition of 13% lard and 2% starch to minced pork shoulder. Thereafter, 3% glucose, sucrose, or starch and 0% or 0.5% herbs mixture were added. Equal-sized balls were barbecued at 260°C for 16 min with flipping every 4 min. The heated samples were subjected (in quadruplicate) to an *in vitro* gastrointestinal digestion model, mimicking the conditions in the human mouth, stomach and small intestine. Raw, heated and digested samples were assessed for α-oxoaldehydes (glyoxal (GO), methylglyoxal (MGO), 3-deoxyglucosone (3-DG) by UPLC-MS/MS), advanced glycation endproducts (AGEs: protein-bound and free N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(carboxyethyl)-lysine (CEL) and N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) by UPLC-MS/MS and protein-bound pentosidine (PEN) by HPLC-FLD), lipid oxidation (4-hydroxy-2-nonenal (4-HNE) and hexanal (HEX) by HPLC-FLD), protein oxidation (protein carbonyl content (PCC) by spectrophotometry).

Results:

The HME-pea as an ingredient was found to contain higher levels of α-oxoaldehydes, protein-bound AGEs, and lipid- and protein oxidation products, compared to raw pork. The heated pea balls contained lower amounts of free AGEs, whereas higher levels of GO and protein-bound pentosidine were found, next to a higher extent of lipid- and protein oxidation compared to the heated pork balls. Gastrointestinal digestion increased the levels of α-oxoaldehydes, free AGEs, and stimulated lipid oxidation, whereas levels of protein-bound AGEs decreased. The formation of α-oxoaldehydes and free AGEs was especially promoted during heating and digestion of the pea balls with added glucose. Furthermore, compared to pork ball digests, pea ball digests contained higher levels of protein-bound PEN and PCC, while being comparable in levels of other protein-bound AGEs and lipid oxidation products. Only relatively minor effects of the other carbohydrates on these reactions were observed during digestion. The addition of herbs resulted in only a minor reduction of lipid oxidation in heated and digested pork balls, but not in HME-pea samples.

Discussion and conclusion:

The present study shows that (glyc)oxidation of HME-pea balls during gastrointestinal digestion was generally higher than pork balls subjected to the same heating conditions. This work has also been presented at the 8th International Conference on Food Digestion in April 2024, Porto.

Abstract 33: Assessing the influence of advanced glycation end products due to biosocial synergy on tumor biology

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Introduction:

It is estimated that over 30 million people are living with a cancer diagnosis worldwide and due to successes in screening and treatment, that number may double by 2050. There is a need to unite research across the cancer continuum to address the needs of cancer survivors to ensure optimal quality of life and improve mortality outcomes. However, health constructs that influence cancer survivorship are multifactorial, highly dynamic, and vary across the lifespan. As a pathophysiological process that lies at the intersection between societal, environmental, and biological constructs, the irreversible accumulation of advanced glycation end products (AGEs) can inform on quality of life and mortality outcomes for cancer survivors.

Materials and methods:

Epidemiological, basic and clinical evidence is provided to support that as a “biosocial” driver of health, non-enzymatic glycoxidation leading to the formation of AGEs influences malignant progression. Epidemiological, basic and clinical evidence is provided to support that as a “biosocial” driver of health, non-enzymatic glycoxidation leading to the formation of AGEs influences malignant progression.

Results:

The relationships between social and biological drivers and AGE exposure are epitomized by nutritional behavior. The consumption of cheaper unhealthier foods such as those high in sugar and fat and/or ultra-processed that promote obesity and are associated with increased cancer risk now represent a significant source of AGE exposure. The investigators first of kind transdisciplinary studies have defined the oncogenic potential of nutritional AGE exposure:

1. Epidemiological studies assign high levels of AGE consumption with increased cancer risk, aggressiveness, and mortality.

2. Translational studies indicate that circulating AGE levels in breast cancer patients correlate with worse disease-free survival and other poor prognosis indicators.

3. Basic studies also show that chronic AGE consumption by mice increases tumor growth ($p<0.001$), and causes rapid progression towards a malignant phenotype by causing a regulatory program of stromal activation.

4. Clinical studies support that lifestyle and pharmacological intervention is a viable option to reduce either AGE exposure or their pathogenic effects

Discussion and conclusion:

As a biosocial driver of health AGE accumulation and its pathogenic effects serve as informative and/or functional markers indicative of the current health status and well-being of cancer survivors and may inform of potential future outcomes. Therefore, prevention and control interventions aimed at reducing AGE exposure represent viable strategies for improving quality of life and disease recurrence for cancer survivors.

Abstract 34: Glucoselysine, a fructose-derived advanced glycation end-product: Exploring its potential as a novel indicator of polyol pathway activity

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Introduction:

Glucoselysine (GL) is the unique advanced glycation end-product (AGE) derived from fructose. The main source of fructose *in vivo* is the polyol pathway, which metabolizes excess glucose independently from the insulin-dependent pathway. Enhanced polyol pathway activity leads to the pathogenesis of diabetic complications. Here, we aimed to demonstrate that GL could serve as an indicator of the polyol pathway activity.

Materials and methods:

Spontaneously immortalized Schwann cells of wild-type and aldose reductase (AR) knockout were starved for 4 hours after 20 hours of pre-culture, and then exposed to high glucose conditions for 3 days. The protein-bound GL content in cells and the free GL content in the medium were measured by liquid chromatography-tandem mass spectrometry after acid hydrolysis without reduction.

Results:

GL levels in both the cells and culture medium of AR knockout cells exposed to high glucose were significantly lower compared to the wild-type. However, under normal glucose conditions, no significant changes in GL levels were observed in AR knockout cells compared to the wild-type. Additionally, quantifiable levels of GL were still detected in AR knockout cells under both normal and high glucose conditions.

Discussion and conclusion:

This study demonstrated that GL reflects the downstream activity of the polyol pathway, suggesting its potential as a new indicator. Additionally, this study has reinforced the importance of evaluating GL by demonstrating the insufficient effect of AR inhibition in blocking the polyol pathway. It is also noteworthy that GL is produced exclusively through the polyol pathway, in contrast to many AGEs that have multiple production pathways. In the future, conducting research focusing on drugs capable of inhibiting GL accumulation may lead to the development of new medications capable of inhibiting the progression of vascular diseases caused by the enhancement of the polyol pathway.

Abstract 35: Establishment of culture method for industrial use of Drosera sp. targeting anti-glycation activity

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Introduction:

Advanced glycation end-products (AGEs) can worsen diseases by degenerating various proteins in the body, potentially reducing their function. Therefore, searching for food components that block AGEs formation could help prevent lifestyle-related diseases. Additionally, to consistently supply food components that inhibit AGEs formation, it's important to find more affordable cultivation methods. In this study, we screened natural compounds that inhibit AGEs formation and explored cost-effective cultivation conditions by focusing on light.

Materials and methods:

Various food extracts were examined whether they prevent the N^{ε}-(carboxymethyl)lysine (CML) and N^{ω}-(carboxymethyl)arginine (CMA), which are produced by oxidative stress. The inhibitory effect of the natural compound on AGEs formation was evaluated by ELISA, then active compounds were analyzed by instrumental analyses such as HPLC, mass spectrometry, and NMR. Furthermore, we investigated whether changes in LED wavelength during cultivation affect the inhibitory effect of AGEs formation.

Results:

Some food extracts, especially from *D. rotundfolia*, *D. spatulata*, and *D. tokaiensis*, showed a high inhibitory effect on AGEs formation. Especially, *D. tokaiensis*, a native Japanese species, contained ellagic acid and other phenolic compounds. Although *D. rotundforia* and *D. spatulata* also had high anti-glycation activity, they lacked certain components compared to *D. tokaiensis*. Furthermore, using LEDs for cultivation provided similar results to traditional methods, whereas compound production varied under different light conditions.

Discussion and conclusion:

D. rotundfolia contains ellagic acid and other compounds that could inhibit AGEs formation, suggesting its potential in preventing glycation-induced diseases. However, *D. spatulata* lacks ellagic acid, indicating that other unidentified compounds may contribute to its anti-glycation effects. *D. tokaiensis*, an amphidiploid between *D. rotundiforia* and *D. spatulata*, may produce compounds from both species, hinting at additive benefits. Despite requiring careful light control, these plants can be grown without losing their anti-glycation activity, promising future drug development possibilities.

Abstract 36: Evaluation of AGEs levels in sera using new pretreatment system for detection of diabetic vascular damages

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Introduction:

Currently, it is known that advanced glycated end-products (AGEs) would be linked to the pathogenesis or progression of diseases such as diabetic complications. Therefore, measurement of AGEs *in vivo* is desired to evaluate the involvement of diseases. Although mass spectrometry allows precise AGEs measurement in tissues such as serum or skin, the complex preprocessing makes clinical measurements difficult. The cation exchange column in AGEs pretreatment has repeated steps such as washing and eluting, resulting in errors. Thus, we focused on optimizing cation exchange and attempted to establish an efficient method for handling both free and total AGEs. Then sera from diabetic patients were evaluated through pretreatment, and we hypothesized that AGEs levels could distinguish macrovascular complications.

Materials and methods:

First, standard and internal standard of amino acids (Arg, Lys) and AGEs (N^{ε}-(carboxymethyl)lysine, CML; N^{ω}-(carboxymethyl)arginine, CMA; N^{ω}-(carboxyethyl)lysine, CEL; and N^{δ}-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine, MG-H1) were prepared, and the those samples were treated with cation exchange column using full-automated solid phase extraction system (FSPES). Next, Sera in patients with diabetes were processed after adding internal standard of amino acids (Arg, Lys) and AGEs (CML, CEL, MG-H1, CMA), isolating them with an MW3000 cutoff filter or hydrolyzing with HCl. Next, each serum was treated with a cation exchange column using FSPES, and free AGEs (CML, CEL, MG-H, CMA) and total AGEs (CML, CEL, MG-H1, CMA) were measured using triple quadrupole mass spectrometer.

Results:

Our new FSPES method showed precision similar to the manual method in processing multiple AGEs simultaneously. In diabetic patients with macrovascular complications (MacC), increases in free CML, CEL, MG-H, total CML, CEL, free AGEs z score, and total AGEs z score were observed. Moreover, logistic regression analysis for identifying MacC in diabetic patients showed significant differences in AGEs z score (free AGEs z score; p= 0.003, total AGEs z score; p= 0.004). In assessing the diagnostic model with the AGEs z score, we found that the AUC for the total AGEs z score was 0.71 based on ROC analysis.

Discussion and conclusion:

Efficient and stable measurements of both free and total AGEs using FSPES were established for clinical application. Increased each AGE in macrovascular complications would occur by concurrent metabolic abnormalities such as ROC generation and lipid peroxidation. And z score with multiple AGEs could be more useful than a single AGE when metabolic diseases are evaluated with AGEs. In conclusion, the AGEs z score would be a unique marker to detect macrovascular complications in progressive diabetes.

Abstract 37: The association between schizophrenia and methylglyoxal modification and glyceraldehyde 3 phosphate dehydrogenase (GAPDH)

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Introduction:

Schizophrenia (SCZ) is diagnosed based on the patient's subjective opinion. The onset mechanism and pathology of SCZ remain unclear. In clinical practice, there is a strong demand for diagnostic methods with high reliability and validity for the disorders. In this study, we measured the glyceraldehyde 3 phosphate dehydrogenase (GAPDH) protein expression level, its activity, and methylglyoxal (MG) modification levels in lymphoblastoid cell lines (LCLs) of SCZ patients and healthy control subjects (CON), and analyzed their associations.

Materials and methods:

The SCZ diagnosis was based on unstructured patient interviews and reviews of their medical records in accordance with the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) criteria. All CON were selected from the general population and psychiatrically screened by interviews. This study was approved by the Ethics Review Committee of Shubun University (#2023SR011) and was conducted in accordance with the Helsinki Declaration. LCLs derived from SCZ and CON were established with Epstein-Barr virus (EBV) transformation. This process eliminates the effects of drug administration, meals, diurnal fluctuations. Dehydrogenase activity was measured using the GAPDH Activity Assay Kit. GAPDH protein expression level and MG-modified proteins were measured by ELISA and Western blotting, respectively.

Results:

Compared to CON, increases in both GAPDH expression levels and MG-modified protein levels were observed in LCLs of SCZ. The dehydrogenase activity of GAPDH in LCLs of SCZ was shown to be decreased.

Discussion and conclusion:

The lower dehydrogenase activity in LCLs of SCZ than in CON may be due to the possibility that GAPDH loses its function as a glycolytic enzyme and exerts other functions. Based on the analysis of copy number variations in genomes derived from SCZ, we identified deletions and duplications in gene regions involved in oxidative stress response (Mol Psychiatry. 2016). Deletion or duplication of a gene region results in loss of its function. While GAPDH has dehydrogenase activity, another function is to metabolize carbonyl compounds derived from oxidative stress. MG selectively modifies Cys-149, which is the active center of GAPDH, causing it to lose its function as a dehydrogenase activity. We also demonstrated that GAPDH modified with carbonyl compounds translocated to the nucleus and played a role in regulating apoptosis in SH-SY5Y cells (Neurosci Lett. 2002). GAPDH also translocates to mitochondria under stress conditions, binds to voltage-gated anion channels, and induces apoptosis by promoting the release of cytochrome c. On the other hand, when oxidative stress induces apoptosis via cytochrome c release, MG-modified proteins, such as MG-heat shock protein 27 (HSP27), exert a chaperone function and regulate the induction (BBA - Molecular Basis of Disease. 2011). We have reached a stage where the association of GAPDH expression levels, its dehydrogenase enzyme activity, and MG modification can evaluate in LCLs of SCZ. In the future, we plan to analyze the MG modification of GAPDH and examine its correlation with the activity and apoptosis in LCLs.

Abstract 38: Nε-(carboxymethyl)lysine-modified albumin exacerbates doxorubicin-induced NOX5-dependent oxidative stress in endothelial cells

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Introduction:

Doxorubicin (Dox) therapy, a potent chemotherapeutic agent for multiple malignancies, is associated with cardiovascular complications. Dox-induced cardiotoxicity is accompanied by N^{ε}-(carboxymethyl)lysine (CML) accumulation in the cardiac microvasculature, which is potentially mediated by NADPH oxidase (NOX)-induced oxidative stress. However, whether NOX5, as well as extracellular CML play a role in Dox-induced vascular dysfunction is unclear. This study investigated the impact of Dox and CML-albumin on endothelial oxidative stress, focusing on the involvement of NOX5.

Materials and methods:

Human umbilical vein endothelial cells (HUVECs) were treated with Dox and/or CML-albumin for 6h, either or not in the presence of NOX5 inhibitor ML090. Cell viability was assessed using a MTT assay. Reactive oxygen species (ROS), Nitrotyrosine (NT), NOX5 and CML were visualized and quantified using confocal immunofluorescence imaging and intensity analysis. Subcellular localization of NOX5 was confirmed via immunofluorescence and electron microscopy.

Results:

Dox treatment induced endogenous CML generation and exacerbated accumulation of CML-albumin in HUVECs. Dox significantly reduced cell viability, which was not affected further by CML-albumin. Combined Dox and CML-albumin treatment induced cellular oxidative stress, evidenced by increased ROS/NT accumulation and NOX5 expression, which was inhibited by ML090. NOX5 was found predominantly in the Golgi apparatus, which was fragmented as a result of Dox treatment.

Discussion and conclusion:

This study showed that extracellular CML increased Dox-induced accumulation of CML in endothelial cells, which exacerbated NOX5-related oxidative stress. These findings suggest that high plasma CML may worsen Dox-induced cardiovascular toxicity, which is in line with the observed aggravated Dox-induced cardiotoxicity in diabetes patients.

Abstract 39: Effect of brewer's spent grain melanoidins on Maillard reaction products during simulated UHT treatment and storage of lactose-hydrolysed skimmed milk.

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Introduction:

Lactose-hydrolysed (LH) milk is a commercial dairy product gaining an increasing popularity amongst consumers especially those suffering from lactose intolerance [1] Nevertheless, prior research has demonstrated that processing and storage of LH milk at high temperatures can enhance the rate of Maillard reaction (MR) in comparison to regular milk. This is mainly due to the presence of free glucose and galactose which are more reactive towards free amino acids and amino groups of peptides and proteins than lactose [2]. Additionally, UHT thermal milk processing and subsequent storage induce the occurrence of undesirable MR products (MRPs) including C5 and C6 α-dicarbonyl compounds (α-DCs), hydroxymethylfurfural (HMF) and advanced glycation end products (AGEs) [3] . In this study, high-molecular weight melanoidins generated by roasting of brewer's spent grains (BSGM) were tested for their effect on the formation of MRPs during UHT treatment of LH milk and its subsequent storage.

Materials and methods:

Brewer's spent grains were roasted according to 3 roasting regimes increasing in temperature: low roasting (LR): 160 °C – 60 min; intermediate roasting (IR): 185°C – 60 min and high roasting (HR): 210 °C – 60 min. After their extraction [4], melanoidin fractions were added to fresh LH skimmed milk at 3 concentrations (2, 4 and 10 mg/mL) prior to UHT treatment [5]. Storage of UHT samples at 50 °C for up to 8 weeks followed. Freshly heated and stored heated samples were subjected to HPLC analysis for the quantification of methylglyoxal, glyoxal, diacetyl, 3-deoxyglucosone (3-DG) [6] and HMF [7] and to LC-ESI-linear ion trap Orbitrap for carboxymethyllysine (CML), carboxyethyllysine (CEL), methylglyoxal-hydroimidazolone 1 (MG-H1) and furosine determinations [8].

Results:

The short-chained α-DCs were not detected in the UHT control and melanoidin fortified milk samples after heating and upon storage, highlighting their high reactivity with amino groups present in milk. For all other target analytes, the reduction was dose-dependent in the melanoidin groups. IR BSGM was the most effective treatment in lowering 3-DG and HMF in the freshly heated samples with reductions of 20.43 ± 0.20 and 7.72 ± 0.57 μM, respectively, and in the 8-week stored samples with decreases of 32.59 ± 3.70 and 16.79 ± 6.21%, respectively, compared to the control. The most significant inhibitions in CML, MG-H1 and furosine contents were also recorded after the addition of 10 mg/mL IR BSGM with inhibition rates of 9.42 ± 0.41, 4.78 ± 1.23 and 147.10 ± 12.53 μM, respectively, before storage and of 32.42 ± 2.48, 25.57 ± 5.11 and 28.40 ± 3.13%, respectively, after the storage span elapsed. No significant reduction in CEL content was observed in any of the tested freshly treated or stored UHT LH milks.

Discussion and conclusion:

BSGM can be considered as a valuable source of bioactive compounds that can contribute to the mitigation of MRPs and AGEs occurrence in thermally processed LH milk. Further research is needed to elucidate the mechanistic pathways underlying these mitigations.

Abstract 40: Dietary Maillard Reaction Products and Inflammatory Bowel Disease: Effects on Cytotoxicity and Intestinal Permeability

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Introduction:

The Western diet plays a crucial role in the aetiology of Inflammatory Bowel Disease (IBD). Maillard reaction products (MRPs), formed in protein- and sugar-rich foods, may contribute to the onset or progression of IBD as they have been linked to other inflammatory diseases. The course of the Maillard reaction (MR) can vary depending on sugar and protein source and other external factors, resulting in different MRPs. This study aims to investigate the effect of different sugar and protein sources on the course of the MR and to determine toxicological effects of resulting MRPs on endpoints related to IBD.

Materials and methods:

A combination of 100 mM sugar (glucose, fructose, sucrose or lactose) and 5 g/L protein (casein, whey or soy protein) was heated to 100°C. Samples were taken before (unheated control) and after 15, 30, 60, 90 and 120 minutes of heating. MRP formation was assessed by measuring fluorescence of vesperlysine (λexc=370nm, λem=440nm). Cytotoxic effects of the different heated sugar-protein combinations were assessed after a 24h-exposure in CaCo2-cells (passage 20-40) and effects on cell viability were determined after a 6h-exposure in Thp1-macrophages. Multiple dilutions of the heated sugar-protein combinations (10-100%) were used. Furthermore, the effects of 33% and 100% fructose-whey MRPs on intestinal permeability were tested after a two-hour exposure on differentiated CaCo2-monolayers (passage 30-40) in transwell inserts.

Results:

Lactose yielded the highest MRP-formation, followed by glucose and fructose when casein was used as protein source. For both soy and whey derived MRPs, fructose gave the highest MRP-formation, followed by lactose and glucose. For all proteins, sucrose gave the lowest vesperlysine-formation. For fructose and sucrose whey-MRPs, there were no significant effects on cell viability of CaCo2-cells. For glucose and lactose, whey MRPs seemed to have a protective effect on cytotoxicity as all heated exposures were significantly less cytotoxic than their unheated controls. In Thp1-macrophages, MRPs showed no significant effects on cell viability. Exposure to fructose-whey MRPs led to a reduction in intestinal integrity when compared to the unheated control.

Discussion and conclusion:

In conclusion, sugar and protein sources clearly affect the course of the MR. Higher MRP-formation however, does not translate to more cytotoxicity in CaCo2-cells or Thp1-macrophages, but does seem to increase intestinal permeability, implying a barrier-modulating role for MRPs in IBD.

Abstract 41: Multi-center clinical validation of plasma protein glycation and oxidation-based blood test for autism

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Introduction:

Autism Spectrum Disorder (ASD) is a common neurodevelopmental disorder in children. It is currently diagnosed by behavior-based assessments made by observation and interview. In 2018 we reported a discovery study of a blood test for ASD based on an algorithm with features of four plasma protein glycation and oxidation adducts: N_{ε}-carboxymethyl-lysine (CML), N_{ω}-carboxymethylarginine (CMA), 3-deoxyglucosone-derived hydroimidazolone (3DG-H), and o,o'-dityrosine (DT). The test had 88% accuracy in children 5 – 12 years old. Herein, we sought to validate the blood test in a large, independent cohort with ASD and of typical development (TD), including evaluation of applicability to children of younger age.

Materials and methods:

Children of 1.5 – 12 years of age were recruited for this study: 311 children with ASD (age 5.2 ± 3.0 years) and 167 children with TD (age 4.9 ± 2.4 years) at Sidra Medicine and Hamad Medical Corporation hospitals, Qatar, and Hospital Regional Universitario de Málaga, Spain. ASD was diagnosed by Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) and ASD severity assessed by Autism Diagnostic Observation Schedule-Second Edition (*ADOS-2*) score by child development experts. Blood samples were collected with EDTA as anticoagulant. Glycation, oxidation and nitration adduct content of plasma protein was quantified by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry. Classification algorithms were developed by the Ensemble method to validate the discovery study algorithm in children of 5 – 12 years old. Thereafter, we assessed its applicability to children 1.5 – 12 years old. Algorithms were trained and tested using 5-fold cross-validation, repeated 5 times.

Results:

For subjects 5 – 12 years old, an algorithm with features CML, CMA, 3DG-H, and DT, age and gender had accuracy 83% (CI 79 – 89%), sensitivity 94% (CI 90 – 98%), specificity 67% (CI 57 – 76%) and area-under-the-curve of receiver operating characteristic plot AUROC 0.87 (CI 0.84 – 0.90). Inclusion of additional plasma protein glycation and oxidation adducts increased the specificity and applicability to younger children. ASD severity correlated positively with plasma protein methylglyoxal-derived glycation adducts, hydroimidazolone MG-H1 and N_{ε}(1-carboxyethyl)lysine (CEL).

Discussion and conclusion:

We envisage this test being applied after questionnaire-based pre-screening. For ASD prevalence of 1 in 36 children, our test will decrease false discovery and false omission rates *ca*. 4-fold, thereby greatly increasing effectiveness of expert referral and ASD detection. We conclude that ASD diagnosis may be supported using an algorithm with features of plasma protein CML, CMA, 3DG-H and DT. This may indicate a mechanistic link of ASD to increased lipid peroxidation, neuronal plasticity and proteotoxic stress.

Abstract 42: Increased cellular protein modification by methylglyoxal activates endoplasmic reticulum-based sensors of the unfolded protein response

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Introduction:

The unfolded protein response (UPR) detects increased misfolded proteins and activates protein refolding, protein degradation and inflammatory responses. In the endoplasmic reticulum (ER), UPR sensors are IRE1α, PERK and ATF6. Misfolded proteins with unexpected surface hydrophobicity bind and activate IRE1α and PERK whereas ATF6 is activated by proteolytic cleavage when released from complexation with protein disulfide isomerases (PDIs). Metabolic dysfunction leading to the activation of UPR sensors remains unclear. The cellular concentration of reactive dicarbonyl metabolite, methylglyoxal (MG), is increased in impaired metabolic health, producing increased MG-modified cellular proteins. The aim of this study was to assess the effect of high glucose concentration and related increased cellular MG concentration on the activation status of UPR sensors in endothelial cells *in vitro*.

Materials and methods:

Human aortal endothelial cells and HMEC-1 microvascular endothelial cells were incubated in low and high glucose concentration to model impaired blood glucose control, with further increase or decrease of MG by silencing or overexpression of glyoxalase 1 (Glo1). UPR pathways were analyzed by immunoblotting and mRNA analysis.

Results:

High glucose concentration activated IRE1α, leading to downstream increased splice variant XBP1s, thioredoxin-interacting protein (TXNIP) and inflammatory signalling by TXNIP linkage to the NLRP3 inflammasome. PERK was activated with downstream activation of eukaryotic translation initiation factor-2α (eIF2α) and increased expression of apoptotic factor, C/EBP homologous protein (CHOP). ATF6 was activated, as judged by increased formation of ATF6-N terminal fragment, with downstream increased expression of chaperone GRP78. UPR activation was exacerbated by MG increase by Glo1 silencing and prevented by normalizing MG levels by increasing Glo1 expression with vector transfection or Glo1 inducer, trans-resveratrol and hesperetin, treatment.

Discussion and conclusion:

MG modification of proteins produces unexpected surface hydrophobicity through arginine-derived hydroimidazolone MG-H1 formation, with related protein unfolding. MG-modified proteins are thereby competent to bind and activate IRE1α and PERK. MG preferentially targets PDIs for modification leading to activation of ATF6 which also prolongs activation of IRE1α and PERK. MG also targets chaperone pathways for modification. Increased MG thereby poses a major challenge to proteostasis and activates UPR sensors. We conclude that increased MG is a major activator of the UPR in endothelial cells in model hyperglycemia and Glo1 inducer offers a novel treatment to counter UPR activation, particularly in hyperglycemia associated with diabetes.

Abstract 43: Maillard reaction flavor formation in extruded model system: a case on coffee

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Introduction:

Traditional model systems often oversimplify the complex dynamics of the Maillard reaction in solid food processing.

Materials and methods:

A solid food matrix model system was developed to address this gap by using high moisture extrusion to simulate real food processing conditions and explore the impact of Maillard reaction processing conditions and different reactants on coffee flavour generation. Several formulations based on legumes, oil and different amino acids and sugars were tested to set up the model which underwent fluidised bed roasting to stimulate the flavour formation. Bulk density and expansion ratio were employed to characterize the impact of oil addition on physical properties of the model. GC/MS was used to measure the generation of volatile compounds under different treatments.

Results:

Results showed that the addition of oil significantly altered the expansion ratio of the extrudates, but had negligible effects on bulk density. Findings highlight the critical role of initial pH and reactants in generating key Maillard compounds, such as furanic and pyrazine compounds. The presence of chlorogenic acid in the model system selectively inhibited pyrazine production while enhancing the formation of furfural and 2-furanmethanol. The initial concentrations of sugars and amino acids were found to differentially influence the generation of Maillard-type compounds. Specifically, increased sugar levels facilitated the production of furanic compounds, whereas higher amino acid concentrations boosted the generation of pyrazine compounds.

Discussion and conclusion:

The study shows that solid model systems are useful tools to explore Maillard reaction flavour formation.

Abstract 44: Maillard reaction of creatine in vivo – A cross over human intervention study

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Introduction:

Methylglyoxal is a by-product of glycolysis and highly reactive towards proteins and DNA. Increased formation and/or decreased metabolization of methylglyoxal *in vivo* contributes to “dicarbonyl stress" and is related to pathophysiological pathways of diseases such as diabetes or uremia. In model incubations it was shown that creatine rapidly reacts with methylglyoxal under physiological conditions forming N-(4-methyl-5-oxo-1-imidazolin-2-yl)sarcosine (MG-HCr). The purpose of this study is to investigate, whether creatine reacts with methylglyoxal *in vivo* as well.

Materials and methods:

A human intervention study with 12 healthy participants was conducted. Subjects supplemented 5 g creatine monohydrate (equals 4,4 g creatine) each day or not for two weeks in a cross-over design. Fasting blood samples were collected before and after the intervention and analyzed for creatine, creatinine, MG-HCr and methylglyoxal via liquid chromatography coupled to mass spectrometry.

Results:

MG-HCr increased from (0.35 ± 0.26) µmol/L to (1.1 ± 0.68) µmol/L in erythrocytes and from (0.05 ± 0.03) µmol/L to (0.10 ± 0.04) µmol/L in plasma following creatine supplementation. MG-HCr concentrations in erythrocytes correlate with creatine concentrations. Creatine supplementation also increased creatine concentrations in erythrocytes and in plasma from (335.3 ± 63.2) µmol/L to (565.1 ± 116.8) µmol/L and from (25.2 ± 11.3) µmol/L to (72.5 ± 27.5) µmol/L.

Discussion and conclusion:

Creatine reacts with methylglyoxal in erythrocytes to form MG-HCr. This could be an additional function of creatine to prevent protein and DNA from being damaged by methylglyoxal.

Abstract 45: Phytate, a Natural Chelator, Mitigates Glycation and Short-Term Memory Impairment in an Aging Iron-Overloaded Rat Model

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Introduction:

The complex interaction among iron overload, glycation, and cognitive impairment represents substantial challenges in the context of aging and neurodegenerative diseases. Iron accumulation and glycation are commonly observed in affected brain regions, contributing significantly to neurodegeneration. Phytate (IP6), a natural chelator abundant in various plant-based foods, emerges as a candidate for fulfilling a therapeutic role. Its capacity to bind with iron cations, suggests potential utility in inhibiting glycation reactions and combating the pathological mechanisms underlying neurodegeneration. The aim of this study was to investigate the efficacy of IP6 in ameliorating glycation in in vitro, and cognitive dysfunction in an aging rat model of iron overload.

Materials and methods:

In vitro studies monitored the formation of Fe^{3+}-catalyzed advanced glycation end products (AGEs) using fluorescence spectroscopy in a solution containing Ac-Lys, Ac-Arg, ribose, and Fe^{3+} with different concentrations of IP6 (0 to 2 µM). In the animal model, 40 male Wistar rats, aged 2 months, were divided into 4 groups receiving different diets until 18 months: i) *Control Group* (n=10): AIN93G diet; ii) *Fe Group* (n=10): AIN-93G diet enriched with Fe^{3+}; iii) *IP6 Group* (n=10): AIN-93G diet enriched with IP6; iv) *Fe+IP6 Group*: AIN-93G diet enriched with Fe^{3+} and IP6. Throughout the study, animals were continuously monitored and subjected to a novel object recognition task at 18 months of age. At 19 months, they were euthanized, and samples of organs, fluids, and tissues were extracted and preserved for subsequent analysis.

Results:

In vitro studies indicated that IP6 effectively reduced the formation of Fe^{3+}-catalyzed AGEs. Animals fed a high-iron diet exhibited notably diminished short-term memory (STM) retention compared to those in the control, IP6, and Fe+IP6 groups. Fe group presented a lower recognition index than the other groups ($p < 0.05$). However, there were no differences in long-term memory (LTM) recognition across the various groups.

Discussion and conclusion:

These findings suggest that IP6 may prevent glycation and age-related memory dysfunction. Dietary supplementation with IP6 could be considered a novel strategy for inhibiting glycation and preserving cognitive function in aging populations. Further research into IP6's pharmacological properties and clinical efficacy may offer new insights into glycation and its role in neurodegeneration.

Abstract 46: Lactobacillus delbrueckii is associated with methylglyoxal levels in type 2 diabetes

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Introduction:

Methylglyoxal (MGO) is a highly reactive dicarbonyl compound that is formed as a byproduct of glucose metabolism and is known to be cytotoxic and can cause damage to various cellular components including proteins , DNA , and lipids. Its formation and metabolism in prokaryotic cells has remained relatively neglected in the setting of human diseases. This study investigates the relationship between the human gut microbiome and MGO to eventually find new therapeutic targets to lower MGO.

Materials and methods:

In this study, we focused on a subset of the BARIA cohort, which included 292 patients (76 % female , mean age 47 ± 10 years) with morbid obesity. Fecal metagenomics alongside targeted metabolomics (UPLC-MS/MS , MGO analysis) was used to explore the association between the gut microbiome and plasma MGO levels. For bioinformatic analyses the cohort was divided into two groups: 220 individuals without diabetes and 72 individuals with type 2 diabetes (mean 7.4 % HbA1c ± 1.3). Spearman correlation was used to identify associations, while hierarchical clustering was used to identify microbes that co-occur and visualise trophic networks. Linear models were used to adjust for age and sex , while P-values were adjusted for multiple testing with $P < 0.05$ considered significant.

Results:

Plasma MGO was significantly higher in individuals with diabetes (295 ± 60 nmol/L) compared to individuals without diabetes (252 ± 71 nmol/L) ($P = 0.001$). No correlation was found between plasma MGO and the microbiome alpha diversity in both groups. However, upon correlation analysis on the microbial family *Lactobacillaceae*, a correlation was observed between *Lactobacillus delbrueckii* abundance and plasma MGO in the individuals with diabetes (Spearman rho = 0.37, $P = 0.02$). which persisted after adjusting for age and sex. In the individuals without diabetes no correlation was found between *Lactobacillus delbrueckii* abundance and plasma MGO (Spearman rho = -0.01, $P = 0.9$). *Lactobacillus delbrueckii* formed the closest clusters with *Streptococcus thermophilus* in both groups of individuals with and without diabetes.

Discussion and conclusion:

We found that plasma MGO was higher in diabetes. Higher abundance of *Lactobacillus delbrueckii* was associated with higher plasma MGO in individuals with diabetes. We will further investigate the relationship between *Lactobacillus delbrueckii* and MGO to validate the potential causal direction of these findings.

Abstract 47: The Role of Electrophilic Aromatic Substitution Reactions for Color Formation of Hydroxycinnamic Acid Derivatives and Heterocyclic MAILLARD Reaction Intermediates

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Introduction:

Commonly, electrophilic aromatic substitution reactions of phenolic compounds, most prominently flavonoids, are understood to quench reactive Maillard intermediates by formation of phenol-carbonyl adducts. However, hydroxycinnamic acids are postulated to play a vital role in the formation of coffee melanoidins, whose phenol content was found to exceed 20 %. Overall, there is a lack of data regarding the mechanisms preceding the incorporation of these phenolic acids into food melanoidins. This knowledge is essential to better understand the structural composition of this heterogenous group of complex Maillard reaction end-products which would allow to derive structure-activity relationships. Therefore, the main objective of this study was to build an understanding of the key reaction pathways of hydroxycinnamic acids derivatives in combination with heterocyclic carbonyl compounds deriving as key intermediates from the Maillard reaction and their implication on the color and the antioxidant properties of the corresponding reaction products.

Materials and methods:

A wide range of structurally related phenolic compounds, among them caffeic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, and vinylguaiacol were incubated in binary mixtures with prominent Maillard intermediates, such as hydroxymethylfurfural, furfural, pyrrole-2-carbaldeyhde, and 2-acetyl pyrrole under roasting conditions at 220 °C for up to 30 min. The reactivity of the corresponding mixtures was characterized by their brown color (420 nm) and the conversion of the reactants (HPLC-DAD, GC-FID). The structural composition and the structure of novel reaction products were identified using HRMS and NMR, respectively.

Results:

The present study revealed that the substitution of the aromatic ring is decisive for color formation involving phenolic compounds. Consequently, the highest yield of brown colorants was observed for the reaction systems of caffeic acid. Further, the electron-rich nitrogen-containing heterocyclic compounds, pyrrole-2-carbaldehyde, and 2-acetyl pyrrole exhibited significant browning in combination even with less reactive phenolic compounds. The identification of several different reaction products indicated that the vinyl moiety of hydroxycinnamic acids is a vital reactivity center acting as a donor and acceptor in nucleophilic reactions.

Discussion and conclusion:

The findings of this study show that the reactivity of hydroxycinnamic acids in the Maillard reaction might significantly differ compared to those reactions characterized for flavonoids. Consequently, nucleophilic and electrophilic addition reactions of their vinyl moiety might be key to understand the incorporation of hydroxycinnamic acid derivatives into melanoidins in plant-based food.

Abstract 48: Metabolic and behavioral effects of intranasal oxytocin administration in cafeteria-diet induced obesity model in wild-type and RAGE-/- female mice.

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Introduction:

Obesity-associated metabolic syndrome is a global health issue that increases the risk of cardiovascular morbidity and mortality. Advanced glycation end-products (AGEs) represent a diverse group of molecules formed via different metabolic pathways and play a pathogenetic role in the development of obesity and its complications. Neuropeptide oxytocin (OXT) controls, among others, energy expenditure and behavior. The uptake of the OXT into the brain is mediated by the receptor for advanced glycation end products (RAGE). We studied the metabolic and behavioral effects of intranasally administrated OXT in cafeteria-diet-induced obesity models in wild-type (WT) and RAGE deficient ($^{-/-}$) mice.

Materials and methods:

At the age of 10 months, wild-type C57/BL6 female mice were divided into the control group (CTRL; n=7) fed a standard chow and the experimental group receiving a cafeteria diet (WT/CAF; n=7). CAF diet was administered to RAGE$^{-/-}$ (n=7) mice. After 10 weeks of dietary intervention, OXT was administered intranasally to all animals in a dose of 1.25 IU/kg daily for 5 weeks. Thereafter, animals underwent behavioral testing, morphometry was performed, and blood was collected for biochemical analyses.

Results:

Before initiation of OXT administration, both groups of mice fed an obesogenic CAF diet displayed higher body weight ($p<0.001$) than the WT/CTRL animals. After 5 week-long administration of OXT, WT/CAF animals showed higher body weight than the CTRL group ($p<0.01$), while RAGE$^{-/-}$CAF did not differ significantly from WT/CTRL mice. In the open-field test, WT/CAF and RAGE$^{-/-}$/CAF groups displayed lower locomotor activity compared with the CTRL mice (by 31%, $p<0.05$ and 28%, $p=0.07$, respectively), and showed higher anxiety-like behavior evaluated as less time spent in the central zone (WT/CAF: 59%; $p<0.001$; RAGE$^{-/-}$/CAF: 39%; $p<0.01$). Both groups of mice consuming the CAF diet performed significantly worse in the olfactory test on the ability to find a buried pellet. At sacrifice, WT/CAF but not the RAGE$^{-/-}$/CAF mice had higher BMI, fasting glycemia, cholesterol, and inflammatory cytokine levels (all: $p<0.01$) than the CTRL group; while both CAF groups were less insulin sensitive than CTRL animals. The concentration of ecDNA did not differ.

Discussion and conclusion:

In old female RAGE$^{-/-}$ mice, but not their WT counterparts, intranasal OXT administration partially ameliorated the CAF diet-induced weight gain and obesity-associated metabolic alterations. The importance of crosstalk between OXT and RAGE needs to be further investigated. This study was supported by grant *No. 21-0355* from the Slovak Research and Development Agency.

Abstract 49: Renewed efforts to determine the structure of LW-1(Glucuronidine), a marker of cardiovascular disease

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Introduction:

Protein-bound long wavelength fluorescence (LWF) with excitation/emission (ex/em) 370/440 nm was introduced by our laboratory as a marker for AGE formation. LW-1 is an acid-labile fluorescent molecule (ex/em 348/463) of unknown structure found in human insoluble skin collagen and has a molecular weight of 623 Da. Commercial devices that measure skin autofluorescence (SAF) at the same wavelength as LW-1 showed that SAF is highly associated with severity of micro-and macrovascular disease, in T1DM and T2DM. LW-1 levels increase with age and reach 1–3 nmol/mg collagen in skin digests from nondiabetic and diabetic-end stage renal disease patients, respectively. It is also associated with established CHD (coronary heart disease) and CAD (coronary artery disease). The Dialong study of long-term survivors of T1DM revealed that LW-1 and SAF are significantly higher in the group with established CHD compared to the participants without CHD. Mean±SD glucuronidine/LW-1 levels in the patients with established CHD was 1495 pmol/mg±650 versus 965 pmol/mg±614, p=0.003 in participants without established CHD. Partial structure elucidation of LW-1 revealed presence of a glucuronide, suggesting presence of a circulating glycating precursor undergoing glucuronidation in the liver or kidney . Hence, elucidation of the structure of LW-1 is crucial to understand the source of the glycating molecule. Therefore, our aim is to isolate and purify~10 mg of LW-1 from human skin collagen and elucidate its structure.

Materials and methods:

Human skin specimens were obtained at autopsy and all samples were stored frozen at 80 °C. To release LW-1 highly insoluble collagen skin fractions (~270 g) is exhaustively digested with collagenase followed by 5% pronase, 1% achromopeptidase, 1% enterokinase and 1% prolidase. This digest is passed over a preparative column packed with reverse-phase tC18 resin and the highly fluorescent peak was eluted with 60% acetonitrile. The fluorescent fractions are further purified over HPLC using semi-prep tC18 column. The LW-1 peak is collected, freeze-dried, and analysed by fluorescence, LC/MS and NMR for peak purity.

Results:

Currently only preliminary results are available. Using, HFBA as pairing ion LW-1 eluted at 95.00 min based on standard LW-1 available from prior purification, and a second peak at 100 min with LW-1 like fluorescence (LD). Both peaks have ex/em of 348/463. Mass spectrometry confirmed the expected parent ion at m/z 624 and MS/MS analysis showed expected fragment ions at m/z 448>403>318>274. NMR shows the presence of an impurity suggesting need for further purification of the isolated LW-1 peak.

Discussion and conclusion:

Unequivocal determination of the structure of LW-1 requires large quantities of pure LW-1 to determine its structure using X-ray diffraction since existing NMR and MS data have not provided sufficient data. Changes in the purity and composition of commercial enzymes to release intact LW-1 have added unexpected challenges to the purification of LW-1. At present LW-1 and LW-1 like peptide-linked material can be isolated from human skin collagen and we remain hopeful that enough highly pure LW-1 will be obtained for structure analysis. NMR and MS data will be presented.

Abstract 50: Bioinformatics prediction of hydrophobicity of methylglyoxal-derived hydroimidazolone and its effect on protein folding and functional activity

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Introduction:

Reactive dicarbonyl glycating agent, methylglyoxal, is precursor of the major physiological advanced glycation endproduct, hydroimidazolone MG-H1. The cellular concentration of MG and MG-H1 content of cellular protein increases in hyperglycemia leading to activation of the unfolded protein response (UPR). Unexpected surface hydrophobicity on proteins is a recognition factor for UPR activation. The aim herein was to employ a bioinformatics approach to: 1. deduce the Eisenberg hydrophobicity *H* of MG-H1, $H_{MG\text{-}H1}$; and 2. use this to replace H_R to predict the effects on protein misfold and activity – taking as example, proteins modified by MG-H1 in human proximal tubular epithelial cells (PTECs) cultured in high glucose concentration.

Materials and methods:

$H_{MG\text{-}H1}$ was deduced from the molecule transfer energy Etr. For amino acids, *H* is inversely correlated to Etr (r = -0.87); *H* = -0.143 Etr + 1.043. Etr values for modified amino acids are deduced empirically by increments for change of side chain charge and atomic composition. MG-H1, compared to arginine, has gain of 1 x C_{sp2}, 2 x C_{sp3}, 2 x uncharged H and 1 x O, and loss of 1 x charged H. $H_{MG\text{-}H1}$ was deduced from the calculated $Etr_{MG\text{-}H1}$. Thereafter, Receptor Binding Domain (RBD) plots of target proteins were prepared: a plot of mean hydrophobic dipole moment < $H\mu$ > against mean hydrophobicity < *H* > for a windows of 5 amino acids moving residues-by-residue through the primary sequence. This identifies residues in 4 domains: G, globular interior; M, membrane seeking; S, surface; and RBD, sites of protein-protein or protein ligand/substrate interaction required for functional activity. To deduce the effect of MG-H1 formation on protein folding and activity, we replaced H_R with $H_{MG\text{-}H1}$ and applied the following interpretation for change in residue location in the RBD plot: S → RBD, activation; RBD → S, M or G, inactivation; S or RBD → G or M (or vice versa), protein misfolding.

Results:

Calculated Etr and H values for MG-H1 are 11.65 and -0.62; *cf*. 17.31 and -2.5 for R. This indicates MG-H1 is markedly more hydrophobic than R, similar to that of asparagine, *H* = - 0.78. Sites in proteins found with MG-H1 modification in PTECs in high glucose concentration by proteomics analysis were: actin (ACTG) - R177, R206, R254 and R312; glyceraldehyde-3-phosphate dehydrogenase (GA3PD) – R248 and R323; calmodulin-1 (CALM1) - R127; and triosephosphate isomerase (TPI) - R206. RBD analysis indicated in ACTG, $R_{MG\text{-}H1}177$ moved 2 residues from S to G (misfolding), $R_{MG\text{-}H1}206$ and $R_{MG\text{-}H1}254$ displaced two residues from RBD to G and $R_{MG\text{-}H1}312$ moved one residue from RBD to G (inactivation and misfolding). In GAPDH, $R_{MG\text{-}H1}248$ and $R_{MG\text{-}H1}323$ moved two residues from RBD to G (inactivation and misfolding).

Discussion and conclusion:

We provide a novel bioinformatics procedure by which the effects of MG modification on protein folding and functional activity may be predicted.

Abstract 51: Early life “processed” diet-induced memory impairments are driven by advanced glycation end-products and the gut microbiome in rats

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Introduction:

“Ultra-processed” foods constitute ~65% of total energy intake among children in the US, UK, and Canada. The impacts of excessive processed food consumption during development are poorly understood. Heat treatment is a common food processing step, which can induce the Maillard reaction and lead to foods high in advanced glycation end-products (AGEs). The neurocognitive implications of an AGE-rich diet consumption, particularly during early life, are unknown. Given that memory processes that rely on the hippocampus (HPC) are particularly vulnerable to early life dietary influences, and that changes in the gut microbiome are causally related to these outcomes, here we evaluated the effects of consuming a heat-treated AGE-rich diet during early life on HPC-dependent memory function and gut microbial taxa.

Materials and methods:

Rats received an otherwise healthy AGE-rich diet (AGE rats; AIN-93G diet heated at 160ºC for 60min) or a non-AGE-rich diet (CTL rats; AIN-93G diet without heat treatment) during adolescence (postnatal days [PN] 26-60). Half of the rats per diet group received a drug that disrupts AGEs (alagebrium, 1mg/kg/day) in drinking water (CTL+ALA, AGE+ALA) and the other half received drinking water alone (CTL+H2O, AGE+H2O). Behavioral assessments were conducted during adulthood (PN 60+).

Results:

AGE+H2O rats exhibited HPC-dependent contextual episodic memory impairments (novel object in context procedure) relative to AGE+ALA, CTL+H2O, and CTL+ALA rats, indicating a diet-induced memory deficit that is driven by dietary AGEs. Analyses of gut microbial taxa revealed that *Lactococcus* abundance was decreased in rats consuming the AGE-rich diet and that lower abundance of *Lactococcus* was linked with impaired memory function. These changes appear to have a causal role in the memory impairments, as replenishing *Lactococcus lactis* (10^9 colony forming units/day) via oral gavage administration during exposure to the heat-treated diet (from PN 26-40) prevented AGE-induced memory impairments.

Discussion and conclusion:

Collective findings reveal a functional connection between early life dietary AGEs, the microbial taxon *Lactococcus*, and impaired memory function.

Abstract 52: Exploring the impact of dietary advanced glycation end products (AGEs) on the production of gut microbial metabolites by infant microbiome

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Introduction:

The Maillard reaction, a non-enzymatic reaction between reducing sugars and free amino groups of proteins, leads to the formation of compounds, such as dietary advanced glycation end-products (dAGEs). This process is particularly significant in infant formulas that are highly susceptible to the Maillard reaction due to their high content of lactose and proteins and their long shelf life. A growing body of evidence suggests that dAGEs could alter host gut microbiota, potentially increasing the risk of inflammatory diseases and allergies, particularly when exposed early in life during the microbiota's developmental stage. The objective of the research was to investigate how glycated and non-glycated dietary proteins can modulate microbial metabolites.

Materials and methods:

Four infant donors were used to inoculate 48-h fecal batch cultures using native whey protein concentrate (WPC) and glycated WPC with glucose. In addition, a dynamic *in vitro* simulator model of the human digestion system (SHIME®) was employed to mimic the proximal and distal colonic regions of infants. Two infant donors were employed to simulate protein-gut microbiota interactions. Maillard reaction products and tryptophan metabolites were analyzed using LC-MS/MS and short-chain fatty acids (SCFAs) using GC-FID. LC-QTOF-MS was used to explore unreported metabolites linked to dAGEs present in the fermented samples.

Results:

Firstly, as expected, AGEs were present in higher amounts at the start of the fermentation in the presence of glycated WPC. Glycated and native WPC fermentations showed no significant differences in the production of SCFAs. Infant microbial communities had the capacity to catabolize dAGEs, decreasing concentration levels over time. Correlation analysis of native WPC and glycated WPC with gut-related metabolites shows that glycated proteins might affect the pattern of microbial metabolites *in vitro*.

Discussion and conclusion:

Overall, these findings shed light on the complex interactions between dietary components, microbial communities, and metabolite profiles in the infant gut.

Abstract 53: Rapid, efficient, and one-step synthesis of succinated thiol compounds

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Introduction:

In vivo, when excess glucose increases fumaric acid in the TCA cycle, it reacts with cysteine residues of proteins to form *S*-(2-succinyl) cysteine (2SC). Several succinated proteins (2SC-proteins) have been reported and are thought to cause dysfunction, but the details of the molecular mechanism are unclear. In this study, we report rapid and efficient synthesis method of succinated thiol compounds as a tool for analyzing molecular mechanisms *in vivo* by succination.

Materials and methods:

The succination reaction of the thiol group was performed by adding an excessive amount of maleic anhydride dissolved in ether to the thiol compounds (cysteine, acetylcysteine, and cysteine-containing peptides). The reaction efficiency was confirmed by quantification of free thiol groups with Ellman reagent and HPLC. The molecular weight of the target succinated thiol compounds were confirmed by mass spectrometry.

Results:

Maleic anhydride was selected as a reagent that reacts specifically and irreversibly with a thiol compound (SH group) and can also be easily removed from the reaction system. When an excessive amount of maleic anhydride was added to various thiol compounds, the thiol group was added to the double bond of maleic anhydride, and the succination reaction occurred quantitatively in a short time at room temperature. On the other hand, the excess maleic anhydride that was not involved in the reaction with the thiol group was rapidly hydrolyzed in an aqueous solution and could be easily removed.

Discussion and conclusion:

The conventional synthesis of succinyl compounds, such as 2SC requires complicated operations, long reaction times, and/or hydrochloric acid hydrolysis treatment. In this study, we have developed a simple and efficient synthesis method of succinated thiol compounds. Present results are expected to lead to the synthesis of various succinated thiol compounds and be used as a tool to deepen understanding of the molecular mechanism of succination *in vivo*.

Abstract 54: Development of methods for measuring AGEs and 2SC in mice sperm

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Introduction:

The World Health Organization report in 2023 demonstrates that around 17.5% of the adult population experienced infertility, which is a major health issue worldwide. Male infertility makes up approximately 50% of the total human infertility. It is considered that acquired factors such as aging, obesity, and stress are involved in reduced fertility in addition to pathological factors. However, its onset mechanisms are not clear. The glycation and succination modify proteins in a non-enzymatic way, causing a decrease in the enzymatic function and degeneration, eventually leading to disease onset and development. Therefore, advanced glycation end-products (AGEs) and *S*-(2-succinyl)cysteine (2SC), which is a cysteine modification product by fumarate of tricarboxylic acid cycle intermediates, have been actively studied concerning aging and lifestyle-related diseases. Although aging, obesity, and hyperglycemic disorders may also affect fertility *via* nonenzymatic modification of proteins, few studies have reported the accumulation of AGEs and 2SC in germ cells. Thus, to clarify the effects of AGEs and 2SC modification of sperm proteins in fertilization, methods for measuring AGEs and 2SC in mice sperm by liquid chromatography-tandem mass spectrometry (LC-MS/MS) were developed.

Materials and methods:

Male C57BL/6J mice of 18 weeks of age were dissected, and sperm were collected from the cauda epididymis. Pretreatment such as homogenization, protein precipitation, and hydrolysis were conducted, thereafter, N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(carboxyethyl)lysine (CEL), N^{δ}-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1), N^{ω}-(carboxymethyl)arginine (CMA) and 2SC were measured by LC-MS/MS.

Results:

Clear peaks of each AGE and 2SC were detected, allowing their quantification. CMA in mice sperm was markedly higher than other AGEs and 2SC.

Discussion and conclusion:

Sperm proteins were shown to be non-enzymatically modified even during the short period of sperm production and maturation. Since CMA is the highest, it is suggested that the sperm collagen protein may be modified.

Abstract 55: Immunoassay for the quantitation of glycation site lysine 414 of serum albumin in human plasma samples

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Introduction:

Diabetes mellitus is characterized by elevated blood glucose levels due to deficiency and/or reduced insulin action. The disease is widespread throughout the world and its prevalence is steadily increasing. Life-threatening complications can be prevented by early treatment, and there is a great interest in easy-to-use and reliable diagnostic methods. Different glycation sites in human serum albumin (HSA) are considered to be promising biomarkers of systemic glycemic status. Mass spectrometric methods developed for the quantification of glycated peptides are not yet suitable for high-throughput screening and routine diagnostics. Immunoassays, such as the enzyme-linked immunosorbent assay (ELISA), are a good alternative. Therefore, we focused on the development and validation of a sensitive and clinically applicable indirect ELISA for the quantification of the glycation site Lys414 in HSA (HSA_{K414}).

Materials and methods:

Monoclonal antibodies (mAbs) were generated by immunizing mice with a glycated peptide synthesized on solid phase and coupled to bovine serum albumin. Mice with the highest titers and best specificity for the glycated sequence were selected for cell fusion. MAbs from the resulting cell clones were tested by indirect ELISA, either at the peptide level using glycated and unglycated peptides or at the protein level using HSA glycated with glucose *in vitro*. The mAbs were purified by protein affinity chromatography. The best anti-HSA_{K414} antibodies, in particular mAb 50D8, were used to develop an indirect ELISA to detect this glycation site in human plasma.

Results:

An indirect ELISA was established with a limit of detection of 0.39 nmol/g glycated HSA and a lower limit of quantification of ~0.5 nmol/g glycated HSA. The linear range of the assay was between 0.5 and 6.25 nmol/g glycated HSA. Intra- and interassay variability was good with coefficients of variation less than 20%. To validate and demonstrate the accuracy of the assay, the *in vitro* glycated HSA samples and plasma samples from diabetic patients and matched controls were measured and compared to the LC-MS/MS reference method. Both data sets correlated very well. Finally, further plasma samples from men and women were measured. Values between 0.70 and 4.14 nmol/g glycated HSA were determined, with the glycation level of HSA_{K414} in the diabetic samples being significantly higher than in the control samples.

Discussion and conclusion:

The obtained values for plasma samples were comparable to previous results[1] regarding quantity, the separation between diabetic and control samples, as well as sensitivity, specificity and area under the curve of the receiver operating characteristic (ROC) analysis. Overall, the developed indirect ELISA could be a valuable tool for the determination of glycated HSA_{K414} in different cohorts to evaluate its clinical relevance.

[1] S. Spiller, Y. Li, M. Blüher, L. Welch, R. Hoffmann, Clin. Proteomics. 2017, 14, 10.

Abstract 56: Methylglyoxal as Precursor for Colorants in the Malillard Reaction – Formation of Novel Intermediates and Characterization of the Resulting Melanoidins

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Introduction:

In the complex cascade of the Maillard reaction, methylglyoxal is formed by cleavage reactions of Amadori products or 1,2-dicarbonyl compounds. Given its two electrophilic carbonyl groups and the C-H-acidic methyl group, methylglyoxal is highly reactive and an essential precursor for the formation of both, colorants and flavor compounds such as pyrazines and pyrroles. It is postulated to participate in the formation of melanoidins, the high-molecular-weight, nitrogen-containing, heterogenous end products of the Maillard reaction, *via* aldol reactions. Although melanoidins are consumed in significant amounts on a daily basis as part of our diet, their chemical structure and the underlying mechanisms of their formation are mostly unknown.

Materials and methods:

The present study aimed to characterize model melanoidins formed from methylglyoxal and different amino acids. For this purpose, methylglyoxal was incubated in binary mixtures with alanine, proline, glutamic acid, or phenylalanine at 100 °C and pH 5 for up to 300 min. The resulting melanoidins were isolated from the colored reaction mixtures by dialysis (cutoff >12 kDa). Novel Maillard reaction intermediates were isolated using preparative HPLC and structurally elucidated by HRMS and NMR. The obtained fractions were characterized regarding their browning (absorbance at 420 nm), and antioxidant properties (TEAC assay).

Results:

The yield of melanoidins was comparable for the studied reaction systems, being 2–4 wt% of the dry mass. In contrast, their contribution to browning in aqueous solution depended significantly on the model system, ranging from 5 % (methylglyoxal/proline) to 44 % (methylglyoxal/alanine). The water solubility of these melanoidins was strongly influenced by the amino acid used with the lowest solubility observed in combination with the apolar amino acid phenylalanine. TEAC assay showed that the melanoidin fraction of methylglyoxal/alanine contributes to approximately 5 % of the antioxidant activity of the entire reaction mixture. HRMS data of the reaction mixtures revealed that condensation products with pyrrole or pyridine structure serve as building blocks in these colorants. Some of them were quantitatively the most relevant intermediates in the reaction mixtures and could be isolated. This allowed the elucidation of structure and formation mechanism of novel intermediates.

Discussion and conclusion:

Depending on the amino acid in the model system, melanoidins show different properties: The color contribution of the melanoidin fraction can greatly exceed its low weight contribution in the mixture, particularly in the system methylglyoxal/alanine. The contribution to the antioxidant activity of the whole reaction mixture, in contrast, largely corresponds to the weight proportion. During the formation of colorants, the novel pyrrole and pyridine derivatives are incorporated into oligomeric structures. Their identification expands our knowledge of the product spectrum that is formed as part of non-enzymatic browning reactions.

Abstract 57: Hyperglycemia and vascular aging nexus: From phenotypic interactions towards treatment strategies

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Introduction:

In the aging population, hyperglycemia is frequently observed and is known to exacerbate cardiovascular and renal impairments, along with further metabolic disturbances and organ dysfunction characteristic of aging. Our hypothesis is that hyperglycemia expedites non-atherosclerotic vascular aging (NAVA) by compromising cardiovascular performance and renal function through glycation and unfavorable bioenergetic conditions.

Materials and methods:

In vascular age-accelerated mouse models with endothelium-selective Ercc1 DNA repair gene excision (EC-KO) and the corresponding wild type littermates (WT), we administered either streptozotocin (STZ) (50mg/kg/over 5 consecutive days, intraperitoneal) or Citrate buffer (vehicle) at 8 weeks of age to induce hyperglycemia. Urine and blood samples were collected in 24-hour metabolic cage sessions at 10 and 18 weeks old to measure plasma glucose and renal function. One week before sacrifice, echocardiography was performed to assess cardiac function. After sacrifice, thoracic aorta was used for myography, and kidneys were used for mitochondrial high-resolution respirometry.

Results:

Mice were considered diabetic with blood glucose >14 mmol/l (diabetic WT (WTd): 22.9±1.35 and EC-KO diabetic (EC-KOd):18.2±3.6 mmol/l). Endothelium-dependent vasorelaxation was impaired in the aorta of EC-KOd mice compared to EC-KO ($p < 0.02$). Cardiac output is decreased in both LMd and EC-KOd mice compared to the non-diabetic controls ($p = 0.01$). With respect to renal function, both WTd and EC-KOd mice showed polyuria at both 10 and 18 weeks of age compared to their non-diabetic controls ($p < 0.01$). However, water and food intake were significantly increased only in WTd mice. At 18 weeks of age, there were no discernible differences in body weight among the groups, except for the lower body weight observed in the WTd group compared to the WT group. Renal function declined in EC-KOd mice as indicated by increased plasma creatinine compared to EC-KO controls at 18 weeks of age. Albumin-to-creatinine ratio was augmented in LMd mice compared to their non-diabetic controls. We observed increased in mitochondrial oxygen consumption rate (OCR) in the presence of: (1) ADP, (2)glutamate, complex 1 substrate, (3)succinate, a complex 2 substrate in EC-KOd compared to EC-KO, and (4) oligomycin in LMd compared to LM.

Discussion and conclusion:

In conclusion, the introduction of hyperglycemia on top of accelerated vascular aging exacerbates renal dysfunction , which is associated with an increase of mitochondrial respiration as a result of a cumulative increase of complex V activity, which is diabetes-induced, and of proton flux capacity, which is dually diabetes/vascular aging-induced.

Abstract 58: Tandem Amadori-Heyns Rearrangement: A possible route to non-enzymatic de-glycation

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Introduction:

Deglycation can occur through the action of various enzymes, however, non-enzymatic deglycation has not been proposed yet. Amadori rearrangement products, being keto sugars, can undergo a subsequent Heyns rearrangement in the presence of free amino acids; the resulting ene-diamine intermediate can exist in equilibrium with the Schiff base of Amadori compound, that can be hydrolyzed. Sulfur or oxygen atoms of the side chains of amino acids can also initiate de-glycation through nucleophilic substitution reaction at the imine carbon of the Amadori Schiff bases.

Materials and methods:

Glycation of lysozyme with D-glucose was performed according to published procedures at 45ºC for 2 and 5 days. The amino acids were mixed with glucose or fructose at a 1:1 ratio, in stainless steel grinding jars and milled at a frequency of 30 Hz for 30 min. The de-glycating amino acid was added to the grinding jar and the milling was repeated under the same conditions. Alternatively, around 5 mg of glycated lysozyme was transferred to a glass vial and the de-glycating amino acid (5% by weight of protein) was added and the mixture was dissolved in water and incubated in closed vials for 24h at 60oC. All experiments were performed in two replicates and analyzed by ESI/qTOF/MS or by ATR/FTIR. Samples were diluted in water/methanol mixture, and solutions were applied to the detector via a syringe pump. The analysis was performed on a quadrupole time-of-flight mass spectrometer operated in negative mode. The FTIR spectra were acquired on a FTIR spectrometer, equipped with DTGS detector and a single-bounce ATR crystal. One drop of the solution was added onto the ATR crystal and the solvent was evaporated.

Results:

Analysis of the data by q-TOF-MS/MS, have indicated that Amador products were de-glycated to a significant extent and were converted into the Heyns products of the added amino acids as identified by their characteristics MS/MS fragmentation patterns. On the other hand, when glycated lysozyme was incubated in an aqueous solution at 60ºC for 24h at RT in the presence of cysteine or serine, a significant reduction in the intensity of the C-O stretching band of the sugar moiety of the glycated lysozyme at 1079 cm-1 was observed after deglycation when analyzed by ATR/FTIR.

Discussion and conclusion:

In this study, the principle of non-enzymatic deglycation was demonstrated first in individual Amadori products and later applied to proteins. The amino acids cysteine and serine were found to be the most efficient amino acids in de-glycating the tested Amadori products due to the nucleophilicity of the sulfur and oxygen atoms on their side chians and their ability to attack the imine carbon of the Amadori Schiff bases.

Abstract 59: Protective effect of unsaturated fatty acids against albumin glycation modifications and diabetic nephropathy: Approaching RAGE-NF-κB pathway in renal cells

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Introduction:

The interaction between amino groups of protein with reducing sugars through the Maillard Reaction (MR) modifies the structure and function of Human Serum Albumin (HSA). The accumulation of advanced glycation end products (AGEs) upregulates the Receptor for Advanced Glycation End products (RAGE)-NF-κB pathway leading to elevated oxidative stress and inflammation contributing diabetic nephropathy (DN). Unsaturated Fatty Acids (UFAs) exhibit significant antioxidant activity. This study aims to explore UFA-mediated glycation prevention by investigating structural changes in HSA and activation of RAGE-NF-κB pathway, inflammation and fibrosis in HEK-293 cells which is not fully understood so far.

Materials and methods:

HSA (10 mg/mL) and methylglyoxal (55 mM) were incubated for 8 days at 37°C in phosphate buffered saline, with aminoguanidine and USFs such as Oleic acid (OA), Linoleic acid (LA), Docosahexaenoic acid (DHA) (300 µM). Fructosamine, thiol group and fluorescence were examined. Structural (free amino group, CD, FTIR, ^{1}HNMR), functional (ABTS, FRAP, TGA) and aggregation markers (FE-SEM, β -amyloid content, zeta potential) were studied. HEK-293 cells were treated with HSA, glycated HSA (GHSA, 400 µg/mL), OA, LA, DHA, RAGE antagonist (FPS-ZMI,150nM) and NF-κB antagonist (Bay11, 15 µM) separately. Subsequently, western blotting and RT-qPCR were done to study gene and protein expressions of RAGE, NF-κB, VEGF, IL-6, TNF-α, collagen IV (Col IV) and fibronectin. Immunofluorescence and luciferase assays were employed for NF-κB nuclear translocation and transcriptional activity in treated cells. All experiments were conducted in biological repeats.

Results:

Glycation and functional markers were elevated in GHSA group and OA and DHA groups restored these markers significantly. DHA protected the secondary and tertiary structure modifications of HSA against glycation confirmed by CD, FTIR and 1HNMR. A comprehensive understanding of the interaction based on aggregation and charge associated with particles was offered by FE-SEM and zeta potential respectively. Glycation-induced increase in RAGE, NF-κB expression was reduced by DHA and OA groups. GHSA treatment elevated gene expression of IL-6 (fold change, 1.8), VEGF (fold change, 1.5), TNF-α and was reduced by OA and DHA treatment. UFAs attenuated NF-κB nuclear translocation, transcriptional activity and expression of fibrosis markers.

Discussion and conclusion:

Structural and aggregation analysis suggests preventive role of UFAs against glycation due to UFAs intrinsic structures, degree of unsaturation and interaction with HSA. The study concluded the potential of DHA and OA for treating DN, validating their protective role as an effective therapeutic strategy.

Abstract 60: Maillard reaction products from carboxylic acid modified sugars: The role of glucuronic acid in generating volatile and non-volatile compounds

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Introduction:

The Maillard reaction (MR) is responsible for generating the distinctive color and flavor during food processing. While many studies on the MR have been focused on neutral sugars, it is noteworthy that acidic sugars, such as glucuronic acid, have not been studied yet. This study investigates the impact of glucuronic acid on the Maillard reaction using mechanochemistry.

Materials and methods:

Sugars were ball milled at 30 Hz for 30 min with amino acids at a 1:1 molar ratio to generate stable Maillard precursors, mainly Amadori rearrangement products. All samples were analyzed at least in two replicates. For ESI-qToF-MS thermal treatments were carried out by dissolving milled Maillard intermediates in water and heating in tightly closed reactors at 120 ℃ for 2 h. The solutions were then heated in open vials at 120 °C for 1 h until dry. For HS-GC-MS, milled precursors were heated directly in closed headspace vials for 30 min at 120 °C. Milled precursors were dissolved in methanol and heated in open vials at 90 °C. The dry residues were re-dissolve in methanol for UV-Vis analysis at a single wavelength of 420 nm.

Results:

Milling glucuronic acid and alanine together produced a yellowish color, intensifying notably upon heating. In contrast, the glucose-alanine sample milled under the same conditions showed a less intense yellowish color and undergo a slower browning process upon heating. High-resolution-ESI-qToF-MS detected GlcA disaccharides in milled, heated, and milled-heated GlcA samples, confirmed by MS/MS. GlcA's carboxylic acid group, acting as an intrinsic buffer, may promote self-protonation, facilitating condensation without catalysts or enzymes. Reaction with amino acids yielded GlcA disaccharide ARP. Ribose ARP found in GlcA-containing model systems may enhance MR rate and yield desirable end-products. Ribose's high reactivity may increase mutagenic activity, limiting its use as a food additive. Using GlcA instead could offer a solution to enhance Maillard flavors. The glucuronic acid-derived 3-hydroxypridiniums were proposed within GlcA-containing model systems. Each of these showed significant relative intensity, suggesting potential antioxidant properties. Ongoing process: compare the aroma-generating potential of milled-heated GlcA+Ala and milled-heated Glc+Ala, based on the quantity and diversity of volatiles generated.

Discussion and conclusion:

When using GlcA in the MR model system, a faster browning rate is observed with the detection of previously unreported compounds. The carboxylic acid group has the potential to modify the acidity of the reaction environment, promoting acid catalyzed disaccharide formation. Decarboxylation of GlcA generates ribose ARPs, which further contribute to the cascading reaction. The specific family of betaines derived from glucuronic acid ARPs are characterized as hydroxy-pyridinium compounds whose specific role in the MR has not been studied yet. These effects collectively have the potential to enhance the diversity of MR products. This study opens new research perspectives on the role of carboxylic acid modified sugars in the MR, suggesting their potential application as color and flavor precursors.

Abstract 61: In favor of the first-born - the specificity of glycation assays for glucose and fructose induced Maillard protein modifications

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Introduction:

Since the discovery of the Amadori rearrangement product (ARP) and the importance of its protein-bound derivatives for the detection of diabetes, several clinical assays for the detection of HbA_{1c}, glycated albumin or serum fructosamines have been developed. Due to their non-enzymatic nature and relatively high blood glucose levels, proteins are continuously "glucated", with the degree of modification increasing sharply at the elevated sugar levels typical of diabetes. Although fructose glycation ("fructation"), yielding Heyns rearrangement products (HRP) is well established, promising *in vitro* data have rarely been translated into meaningful *in vivo* data. Low peripheral fructose concentrations could be addressed by a comparatively higher reactivity, but the lack of HRP-specific analytics and possible discrimination by established assays may explain the limited research. Thus, clinical glycation assays were evaluated for their ability to detect defined levels of ARP and HRP.

Materials and methods:

Synthetic standards were used to study the enrichment of Amadori and Heyns peptides in boronate affinity chromatography (BAC), including phenylboronic acid (PBA) and boronic acid derivatives bound to agarose, silica particles or PEG-based resins. Glycated peptides and their reduced hexitol derivatives were enriched using different buffer systems. Recoveries were estimated using RP-HPLC combined with targeted mass spectrometry (MS). Reactivity of glucose and fructose derived isomers were determined by various chemical assays.

Results:

We reported the separation and quantitation of Amadori and Heyns peptides resembling protein glycation sites using targeted MS and specific fragment ion ratios.[1] When proteins were incubated with fructose *in vitro*, the formation of significant amounts of ARP were detected in addition to the expected HRP, whereas only ARP were present in a BAC enriched plasma sample. Therefore, the enrichment protocol originally optimized for ARP was reviewed and found to be inappropriate for the analysis of HRP, since the peptide-bound amino sugars exhibited significantly different affinities for boronic acids. Under aqueous conditions, neither buffer composition nor pH altered retention, but reduction with borohydride to yield the non-cyclic sugar alcohols significantly improved binding. Although reduction provides much needed enrichment of low abundant HRP in complex samples, reduced glycation isomers have the same masses, retention times in RP-HPLC, and tandem mass spectra without characteristic neutral losses. Due to the lack of analytical techniques, we have developed a robust strategy to selectively quantify HRP in mixtures with highly abundant ARP.

[1]Schmutzler et. al. (2022) *Anal Chem* 94, 7909.

Discussion and conclusion:

Analysis of fructation sites requires HRP-validated techniques, because fructose and glucose derived glycation products, despite structural similarities, demand different analytical strategies. This includes their different affinities for boronic acids in the commonly used PBA-assay, which we have confirmed under numerous conditions. The novel peptide-based strategy presented here allows the specific analysis of HRP and ARP at levels commonly found in blood proteins.

Abstract 62: Assessing the addition of lupin for the production of dough-based potato crisps and reduction of acrylamide

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Introduction:

Potato crisps are widely consumed worldwide, with a market size of 35 billion U.S. dollars, and are currently regarded as the largest product by volume on the savoury market. Supermarkets are likely to sell either crisp made from cut-potato or from commercial potato flakes, categorising them as dough-based crisps. Because of the presence of reducing sugars and amino acids, the formation of potentially toxic compounds including carbonyls and acrylamide represents a major concern. Indeed, the addition of vegetable protein and their extracts can have mitigating influences. Lupin (*Lupinus angustifolius* L.) has gained interest in the recent years for its nutritional properties including a high protein content, dietary fibre and low-fat content. This study aimed to investigate the addition of lupin protein isolate in dough-based potato crisps (0%, 5%, 10%, 20%) and its effect on acrylamide levels, oil uptake, and firmness.

Materials and methods:

Coarse lupin powder was obtained through dehulling and grinding lupin beans. Isoelectric precipitation of defatted powder was used to isolate lupin proteins. Protein content was quantified using Kjeldahl analysis and the resulting protein extract was used as key ingredient into a modified dough-based crisp model system. Lupin protein isolate (LPI) was tested at 5%, 10%, and 20% in place of potato powder. The dough was extruded, cut, and fried. To evaluate the quality of the final product firmness, acrylamide levels and oil absorption during frying were assessed.

Results:

The results showed that by partially substituting potato powder with 20% lupin protein isolate, the acrylamide content decreased from 503.41ppm to 312.92ppm. The results suggest that the acrylamide reduction was promoted by the Michael addition reaction supervised by nucleophiles and amino acids present in lupin, which are more stable compounds that can interact with acrylamide formed, thus promoting its elimination. The results also showed an increase in oil uptake between the control sample, 0% lupin protein isolate (15.45%) and 20% lupin protein isolate (19.69%). The chemical reactions supervised by oil uptake were investigated through metabolomics and molecular networking revealing that oxidation reactions of conjugated double bonds can be the key pathway.

Discussion and conclusion:

The addition of lupin protein isolates reduced acrylamide levels in potato crisps but impacted firmness and increased oil uptake. Lupin protein isolates show potential as a coating agent to reduce acrylamide, warranting further investigation. Future studies should focus on protein characterization, expanding to other food categories, and examining the interplay between lipid oxidation and Maillard reaction during frying for a comprehensive understanding.

Abstract 63: Impact of first-line antidiabetic treatment by metformin and/or empagliflozin on the glyoxalase system in Proximal Tubular Epithelial Cells exposed to diabetic milieu: An in vitro study

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Introduction:

Diabetes mellitus is characterized by chronic hyperglycemia leading to increased oxidative stress and formation of advanced glycation end-products (AGEs) and modified profucts, which are pivotal in the initiation of hyperglycemia-mediated cell injury and progression of clinical diabetic complications. The glyoxalase system, primarily glyoxalase I and II (GLO I and II), plays a crucial role in detoxifying methylglyoxal, a reactive glycolytic by-product and precursor of AGEs. Understanding how therapeutic agents such as metformin and empagliflozin (renal SGLT-2 inhibitor) influence this system in diabetic environments can provide insights into the mechanisms responsible for observed reno- and cardio-protection. This study investigates the effects of metformin, empagliflozin and their combination in normo- vs. hyperglycemic conditions on the efficiency of the glyoxalase system in proximal tubular cells (PTEC), a cell type exclusively expressing SGLT-2.

Materials and methods:

Human PTEC (HK-2) were cultured under normo- (6.6 mM glucose) and hyperglycemic (25 mM glucose) conditions to simulate the diabetic environment. Cells were treated with metformin (500 µM), empagliflozin (690 nM) or their combination. The glyoxalase system efficiency was assessed by measuring the protein expression of GLO I using Western blot analysis to compare relative expression between the conditions. Additionally, the concentration of D-lactate in the culture medium was quantitatively measured using a commercial D-lactate assay kit.

Results:

Concentrations of D-lactate were 628±184 nM in normoglycemic (N) vs. 611±247 nM in hyperglycaemic (H) conditions. Furthermore, D-lactate assay showed 633±253 nM for N + metformin, 596±214 nM for N + empagliflozin, 638±211 nM for H + metformin, 534±116 nM for H + empagliflozin (HG) and 561±268 nM for H + metformin + empagliflozin. None of the comparisons ascertained a statistically significant difference. Similarly, GLO I protein expression comparison between conditions described above did not reveal statistically significant differences.

Discussion and conclusion:

The study investigated the impact of metformin, empagliflozin and their combinations on the glyoxalase system activity in vitro in human PTEC. Renal tubules exclusively mediate the beneficial clinical effects of SGLT-2 inhibition in diabetics as shown with many trials so far. The findings indicate that the concentration of D-lactate remains relatively constant across all experimental conditions despite the addition of metformin and/or empagliflozin. Similarly, the protein expression levels of GLO I did not show significant variations across different experimental setups. Therefore, based on these pilot data, we can conclude that the glyoxalase system does not appear as one of the candidates mediating the beneficial effects of those pharmacological treatments. However, data are pilot and additional experiments are warranted to draw more specific conclusions.

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Abstract 64: Intake of dietary dicarbonyl compounds and colorectal cancer risk in European adults: findings from a large prospective cohort study

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Introduction:

Incidence of colorectal cancer (CRC), for which diet is an important modifiable risk factor, is high in many world regions. But epidemiological evidence on CRC risks of dietary dicarbonyl compounds is limited. These molecules, with both potentially deleterious and protective metabolic effects, can be found in a wide variety of foods. We evaluated CRC risk associated with the intake of three main food-derived dicarbonyls: methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG), in European adults.

Materials and methods:

Dietary intakes of MGO, GO, and 3-DG were estimated using a detailed food composition database from 450,111 EPIC participants (median follow-up=14.9 years; 5,899 accrued CRC cases). Hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations between dietary dicarbonyls and CRC were computed using Cox proportional hazard models adjusted for sociodemographic, lifestyle, and clinical variables. Sub-group analyses by anatomical subsite (colon, rectum) and by biological sex were also conducted.

Results:

Inverse CRC risk associations were suggested for dietary GO, (HR comparing extreme quintiles=0.86, 95% CI=0.79-0.96, P-trend=0.002). Dietary 3-DG was associated with higher CRC risk (HR=1.15 (1.06-1.26), P-trend=0.002). No associations were observed for dietary MGO (HR=0.98 (0.90-1.07), P-trend=0.478). Analyses by anatomical sub-sites showed significant heterogeneity between colon and rectal cancers for dietary MGO (P-value-for-heterogeneity = 0.006). Associations differed by sex for dietary GO, suggesting inverse associations among men only (P-value-for-heterogeneity=0.007).

Discussion and conclusion:

Our findings suggest differential associations with CRC risk depending on the specific dietary dicarbonyl consumed. Further research is needed on the potentially divergent roles of these common food components in CRC development.

Abstract 65: Blocking glycation stress in chemoresistant colon cancer: effects on cancer stem cells

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Introduction:

Our team recently demonstrated that KRAS-mutated colorectal cancer (CRC) exhibits high methylglyoxal (MG) glycation stress. This stress is linked to the reprogramming of glucose metabolism, a crucial event observed in many cancers during tumor progression and therapy resistance. Increasing evidence suggests that cancer stem cells (CSCs) are primarily responsible for cancer aggressiveness, drug resistance, and tumor relapse. Based on these findings, we propose to study MG stress in CRC CSCs.

Materials and methods:

Human CRC cell lines HT-29, SW480, and SW620 (the metastatic derivative of SW480) were used in this study. Selective cell sorting (FACS) was employed to isolate ALDH-high and ALDH-low cells using the Aldefluor kit. Intracellular MG levels were quantified using a specific fluorescent probe. The fluorescence was analyzed by FACS. Spheroids enriched in CSCs were generated using a serum-free medium supplemented with B27, EGF, and bFGF and low-attachment plates. Extracellular acidification rates (ECAR) and oxygen consumption rates (OCR) were measured using a Seahorse XF Analyzer. L-lactate production was measured by lactate dehydrogenase A (LDHA) activity, and glucose uptake was evaluated using the Glucose Uptake Glo assay (Promega). Organoids were derived from genetically engineered mouse CRC models, specifically Apc^min/+ and Lgr5-EGFP-IRES-creERT2 mice. Matched patient-derived organoids were cultured from normal or tumoral colon mucosa, providing precious human material for the study.

Results:

Interestingly, CSCs characterized by high ALDH activity demonstrated a significant increase in intracellular MG and MG protein adducts, suggesting a correlation between MG and this marker of stemness. We compared cells cultured in 2D conditions to 3D spheroids to assess their enrichment in CSCs. Elevated levels of stem cell markers such as ALDH, OCT-4, and CD133 were successfully detected in 3D cultures. Preliminary experiments showed a significant increase in ALDH1A1 in HT29 spheroids and correlated with elevated levels of CD44 and cyclin D1, suggesting activation of the Wnt pathway. RNA sequencing analysis performed on LIM1215 CRC cells stably depleted of GLO1 using specific shRNAs revealed enhanced expression of several Wnt activators, including CTNNB1 (coding for β-catenin), WNT6, WNT7B, WNT9A, LEF1, and TCF4. This suggests that MG stress could, at least in part, contribute to Wnt activation in CRC. Encouraging preliminary results indicate that following exogenous MG stress, there is a dose-dependent accumulation of MG adducts paralleled by increased TCF1 expression in HT29 cells.

Discussion and conclusion:

The recent demonstration of 5FU-induced promotion of stemness in CRC suggests that our FOLFOX-resistant models may recapitulate the sequence of stemness enrichment leading to glycolysis, MG stress, and Wnt activation. We propose that neutralizing MG in CSCs could significantly enhance the efficacy of FOLFOX therapy in CRC. Understanding the energetic modalities of CSCs and their ability to adapt their metabolism in response to therapy promises numerous applications that can help improve cancer patient care.

Abstract 66: Glycation of albumin: The missing link in the association between Diabetes and Alzheimer's disease?

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Introduction:

The global rise in type 2 diabetes mellitus (T2DM) is associated with an increased risk of Alzheimer's disease (AD) among affected individuals. One of a pathological feature of AD is formation of a plaques in the brain, that are associated with peptides called amyloid beta (AMB). In healthy individuals, AMB in circulation binds to human serum albumin, forming HSA-AMB complexes. Glycation of HSA in T2DM patients may reduce binding of AMB adding to plaque formation and AD symptoms. Additionally, glycation of HSA also results in the formation of Advanced Glycation End Products (AGEs) recognized by the Receptor for Advanced Glycation end Products (RAGE). While glycation of HSA in people with diabetes is well-known, its consequences in relation to AD have not been explored. The objective of this study is to investigate whether glycation of HSA impacts the formation of complexes with AMB, and subsequently, the binding of these complexes to the RAGE receptor.

Materials and methods:

Human serum albumin (HSA) was glycated using 3 different glycations methods; with glucose, with methylglyoxal (MGO), and with glyoxylic acid leading to formation of carboxymethyl-lysine (CML). The level of glycation of HSA was characterized by UPLC-MS/MS and immunoblot. HSA and glycated HSA were incubated with AMB peptides to form HSA-AMB complexes. The binding potential of glycated vs non glycated HSA to AMB, was analysed by Western blot and dot blot. Competition ELISA was used to measure the binding of glycated vs non glycated HSA-AMB complexes to the RAGE receptor and their potential to elicit an immune response in THP-1 macrophage was measured by qPCR and a phosphorylation of NF-κB p65 via Western blot.

Results:

All three HSA glycation methods led to an unique MRP/AGEs profile. The highest level of CML was observed in glyoxylic acid modification, an intermediate level of CML was found in glucose-modified HSA, and MGO modification resulted in selective MGO presence. Both non-glycated and glycated HSA were shown to form complexes with AMB. Interestingly, HSA-MGO modification exhibited enhanced binding to AMB compared to its non-glycated control, followed by HSA-glucose, which also showed higher binding to AMB than its control. HSA-AMB complexes formed with glycated HSA showed significantly higher affinity to RAGE compared to the complexes formed from non-glycated variant. Blocking with anti-AMB antibody prior to performing an inhibition ELISA significantly, but not completely, lowered the binding of the glycated complexes to RAGE. When looking at the inflammatory response in THP-1 macrophages, all HSA-AMB complexes led to a slight increase in inflammatory markers.

Discussion and conclusion:

Overall, our results show that non-glycated HSA indeed adopts its protective role in AD symptoms, as the non-glycated HSA-AMB complexes showed low affinity for RAGE. Glycation of HSA with glucose and MGO appears to compromise this protective role by generating ligands for RAGE. While we cannot definitively conclude that the enhanced binding to RAGE directly leads to increased inflammatory response, it may be possible that binding to RAGE facilitates AMB transport across the blood brain barrier, possibly increasing risk of AMB plaque formation.

Abstract 67: Intradermal advanced glycation end-products relate to reduced sciatic nerve structural integrity in type 2 diabetes

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Introduction:

Cardiovascular risk management is beneficial, but stringent glycemic control does not prevent the progression of distal symmetric polyneuropathy (DSPN). Persistent hyperglycemia-induced alterations and cardiovascular factors may contribute to diabetes-associated neuronal damage. This study aimed to evaluate the correlation between skin autofluorescence (sAF), an indicator of dermal advanced glycation end-product (AGE) accumulations, cardiovascular risk, and changes in peripheral nerve integrity.

Materials and methods:

Sixty-two individuals with type 2 diabetes (T2D) (20 women and 42 men), including 29 diagnosed with DSPN (7 women and 22 men), and 10 healthy controls (HC) underwent diffusion tensor imaging of the sciatic nerve to assess fractional anisotropy (FA), an indicator of nerve structural integrity. sAF measurements were combined with clinical, serological, and electrophysiological evaluations. Arterial stiffness was assessed via pulse wave velocity (PWV).

Results:

sAF (HC 2.1±0.25 AU, nDSPN 2.3±0.47, DSPN 2.6±0.43; p=0.005) was higher in individuals with DSPN compared to HC (p=0.010) and individuals without DSPN (p=0.035). Within the group of T2D FA correlated negatively with sAF (r=-0.49, p<0.001), PWV (r=-0.40, p=0.009) and hsTNT, a marker of microvascular damage (r=-0.39, p<0.001). In DSPN, sAF correlated positively with hsTNT (r=0.58, p=0.005) and with PWV (r=0.52, p=0.007), the sciatic nerve´s FA correlated negatively with PWV (r=-0.47, p=0.010).

Discussion and conclusion:

This study is the first to show a close correlation between reduced peripheral nerve integrity and both intradermal AGE deposition and arterial stiffness in individuals with T2D. Advanced glycation and vascular damage are linked to neuronal damage, emphasizing the importance of cardiovascular risk management in preventing DSPN.

Abstract 68: Formation of dicarbonyl, furanic compounds and Maillard reaction derivatives during in vitro digestion of a pea-based sponge cake

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Introduction:

In the context of more sustainable lifestyles, the use of vegetable instead of animal proteins has raised the question of replacing traditional ingredients, such as milk, meat, or even cereals, with legumes. These ingredients contain higher concentrations of precursors for the Maillard reaction, caramelization, and oxidation which, after high-temperature processing, could generate reactive dicarbonyl compounds and advanced glycation end-products (AGEs), potentially hazardous for human health. Questions on the formation, reactivity and bioaccessibility of these newly formed compounds (NFCs) during cooking and digestion therefore arises. The goal of this study was to investigate these questions for selected NFCs during the in vitro digestion of a pea-based in comparison with a wheat-based cake product.

Materials and methods:

A cake model based on a reconstituted flour containing pea protein isolate and maize starch was developed and compared with a wheat-based cake reference. Cakes were baked in an instrumented oven (200°C) and submitted to the in vitro INFOGEST static digestion. Relevant analytes including dicarbonyl compounds (glyoxal, methylglyoxal, dimethylglyoxal, glucosone, 1-deoxyosone, 3-deoxyosone), furanic compounds (5-HMF, furfural), and AGEs (carboxymethyllysine (CML), carboxymethyllysine (CEL)) were extracted and quantified in the cakes and after the gastric and intestinal phases of the digestion using UHPLC-DAD, UHPLC-MS-QToF and UPLC-MS/MS.

Results:

Among the NFCs tested, 3-deoxyosone and 5-HMF were most abundant. The pea-based formulation yielded higher concentrations of dicarbonyl and furanic compounds than the wheat-based flour; while CML was not different between the cakes, CEL was more concentrated in the pea-based cake. During the in vitro digestion, dicarbonyl compounds increased and furanic compounds decreased. 1-deoxyosone and furfural evolved differently in pea-based and wheat-based formulations. Glyoxal was detected during the gastric phase and significantly increased during the intestinal phase. These results and the compounds' behaviour during in vitro digestion will be discussed in light of the existing literature.

Discussion and conclusion:

At the end of the digestion, although NFCs behaved differently, their final amounts remained high. At each digestion step, NFCs amounts resulted from an equilibrium between formation and consumption reactions determined by the different gastric and intestinal environments, cake composition, digestibility and structure. Further investigations are necessary to shed light on the link between processing intensity/product structure and bioaccessibility of the compounds of interest. This line of research could, in future, contribute to optimisation of industrial processes to limit the generation of NFCs during the production of legume-based processed food.

Abstract 69: Ultrasound and additives treatment to mitigate acrylamide formation in potato crisps on large scale

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Introduction:

The growing concern about acrylamide presence in foods and the increasing constraints in its regulation underline the urge for mitigation strategies applicable on industrial scale. The aim of this work was to examine the applicability on large scale of additives and ultrasound treatment during washing of potatoes before frying, during the production of crisps, to reduce precursors levels and acrylamide formation at both laboratory and pilot plant scale.

Materials and methods:

$CaCl_2$ and citric acid were applied at laboratory scale to establish optimum conditions, then scaled up to pilot plant. The additives were tested alone or in combination, in various concentrations, orders and washing regimes. A 2 min ultrasound treatment was applied in a first trial to test optimal powers and amplitudes. Based on the findings from trial one, a second trial was designed to investigate different washing combinations.

Results:

At laboratory scale, the highest reduction (91.0%) in acrylamide was obtained applying 0.1 M $CaCl_2$ in hot wash + 0.1 M citric acid in cold wash. Both concentration and order of additives influenced the mitigation observed, with higher concentration of additive in the 2nd wash being beneficial. When upscaled to factory pilot plant, the reduction observed was not consistent across 3 trials, with a 33.4% reduction in the first trial but no significant reduction in following studies. Up to 67.1% of acrylamide reduction was recorded after 2 min ultrasound in cold wash followed by hot wash; ultrasound was not effective in reducing acrylamide or its precursors when solely applied or when followed by cold wash under the tested conditions.

Discussion and conclusion:

This study considered scaling up two mitigation strategies that have previously proven effective in reducing acrylamide formation in fried potato products and could potentially be easily implemented within a crisps production line. From our results, it is hypothesized that the studied additives are more effective in reducing acrylamide in crisps with a higher starting reducing sugars content in the tubers. The mitigation of acrylamide achieved in the pilot plant trials was less than anticipated from results obtained within laboratory trials. It is observed that higher concentrations of additives, which might be effective in reducing acrylamide also in crisps from fresh potato tubers and/or at pilot plant scale, resulted not feasible due to their impact on the taste of the end consumer product. The findings from the ultrasound trials suggest that, in the studied conditions, the hot wash appears to be the main contributor in reducing precursors levels, consequently leading to a lower acrylamide content in the crisps, while the only use of ultrasound or the combination ultrasound – cold wash had little to no effect. The precision and accuracy which characterize laboratory trials are difficult to control during scaling up and this might affect the reproducibility and repeatability of the observed results. This is valid for both the pilot plant additives trials and the processing plant ultrasound trials, where reductions in acrylamide formation observed in a first study were not always confirmed in the following ones.

Abstract 70: Methylglyoxal has no direct effect on immune cell recruitment or activation but is involved in trained immunity

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Introduction:

Immune cell activatImmune cell activation leads to increased glycolysis. During glycolysis, methylglyoxal (MGO), a reactive dicarbonyl, is formed. MGO is thought to activate immune cells. We investigated whether MGO directly affects immune cell recruitment and inflammation. Moreover, since MGO can modify DNA, inducing epigenetic changes, we investigated its effects on trained immunity.

Materials and methods:

C57BL/6J miceC57BL/6J mice received a single iv injection of 25 µg or 1 mg MGO, and were euthanized within 24h, or received 50 mM MGO in drinking water for 12 weeks. Leukocytes and inflammatory markers were assessed in blood and in liver with flow cytometry and qPCR. Bone marrow was isolated and cultured for 7 days into bone marrow derived macrophages (BMDMs), and stimulated with LPS. Inflammation was assessed using qPCR and nitric oxide (NO) assays. Primary human monocytes were trained with β-glucan with or without aminoguanidine or MGO. After 6 days, cells were restimulated with LPS, and assessed with ELISA. MGO was quantified with LC-MS/MS.

Results:

Neither short-term MGO spike nor long-term exposure to MGO affected immune cell recruitment, plasma cytokines or hepatic inflammation. Interestingly, a single high-dose MGO injection enhanced LPS-induced NO production (from 7.0±1.2 to 10.3±0.84 µM; $p<0.05$) and inflammatory gene expression (fold changes for IL1b: 4668 ± 252 to 13062 ± 4943, TNF: 20 ± 5.8 to 51.7 ± 9.1, iNOS: 1140 ± 118 to 8612 ± 1598, for all $p<0.05$) in BMDMs from these mice, suggesting trained immunity induction. In primary human monocytes, β-glucan-induced innate training enhanced MGO formation (313.1 ± 71.8 to 549.1 ± 16.9 nmol/gram protein). Moreover, β-glucan-induced trained immunity response for TNF (2.4± 0.8 to 1.0±0.3 ng/mL, $p<0.05$) was blunted by the MGO scavenger aminoguanidine, while it was enhanced by adding additional MGO (0.8±0.06 to 1.2± 0.02ng/mL, $p<0.05$).

Discussion and conclusion:

MGO is involved in the induction of trained immunity, but not in direct immune cell activation or recruitment.

Abstract 71: PDZD8, a tethering protein of ER-lysosome interaction, alters tubular inflammation in acute kidney injury mice

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Introduction:

Protein homeostasis is orchestrated through synthesis, post-translational modifications (such as glycation and phosphorylation), and degradation. This process is well organized by various organelles, including endoplasmic reticulum (ER), mitochondrial, and lysosome. Recently, the pathophysiological importance of organelle crosstalk in maintaining cellular homeostasis has emerged as a prominent research trend. In this study, we focus on PDZD8, a tethering protein located in the lumen that facilitates interactions with other organelles and promotes homeostasis at organelle contact sites. We aimed to elucidate the role of organelle crosstalk, specifically through PDZD8, in the context of kidney injury, especially acute kidney injury (AKI).

Materials and methods:

In vivo studies: 8-10 week-old male PDZD8 knockout (KO) and wild-type (WT) mice were administered 20mg/kg cisplatin to induce AKI and euthanized 48hr later. Kidney injury after AKI was assessed by kidney function measurement (BUN, Cre), pathological analysis (PAS), immunohistochemistry for inflammatory molecules, and real-time PCR. In vitro studies: human proximal tubular cell line (HK-2) was treated with 20mM cisplatin and assessed changes in organelle homeostasis and their effect on inflammatory response (TLR9-NFkB axis) using mass spectrometry-based immuno-precipitation proteomics and immunofluorescence microscopy for organelle function.

Results:

PDZD8 KO mice improved cisplatin-induced kidney injury compared to WT mice, as indicated by lower BUN and Cre levels and less severe tubular damage. This was accompanied by a diminished activation of the NFkB inflammatory pathway and decreased NFkB-downstream inflammatory gene expression (IL6, CXCL10). In vitro studies revealed that cisplatin-induced inflammation was also reduced in PDZD8-knockdown (KD) HK-2 cells, while cisplatin damage, estimated by mitochondrial DNA leakage, was identical between WT and KD cells. Interestingly, such PDZD8-mediated tubular inflammatory signal was closely correlated with the TLR9 activation level: 1) cisplatin-exposed HK-2 cells induced the intracellular translocation of TLR9 (to the lysosome), which activates NFkB inflammatory signaling, 2) PDZD8-KD significantly impaired the TLR9 translocation/activation, leading to the reduction of NFkB-mediated tubular inflammation. Further, PDZD8-KD significantly affected the lysosomal functions, characterized by insufficient lysosomal pH, which hindered TLR9 activation. These findings suggested the critical role of PDZD8-TLR9-NFkB axis as a novel pathway for regulating tubular inflammation.

Discussion and conclusion:

Tubular PDZD8 regulates TLR9-NFkB inflammatory pathway through its regulation of lysosomal function via ER-lysosome interaction, demonstrating the novel pathophysiological role of PDZD8 in tubules and the crucial importance of organelle contact site in the progress of tubular damage in acute kidney injury.

Abstract 72: Dietary fructose does not alter serum levels of methylglyoxal: analyses of human intervention studies

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Introduction:

Plasma methylglyoxal (MGO) levels are increased in hyperglycemic states and are associated with microvascular disease and cardiovascular mortality. Although hepatic fructose metabolism yields precursors for MGO, i.e. glyceraldehyde and dihydroxyacetone phosphate, it is unknown whether fructose consumption effectively augments systemic MGO levels in humans. In this study, we aim to investigate the effect of physiologically relevant doses of dietary fructose on serum MGO levels.

Materials and methods:

We investigated the effect of fructose on serum levels of MGO in an acute 20 grams fructose challenge in ten healthy individuals (NCT05826717). Furthermore, the effect of 45 grams daily fructose or glucose supplementation, at the background of a fructose-restricted diet, on serum MGO levels was evaluated in thirty-five adults with fatty liver index ≥60, i.e. a score for metabolic dysfunction-associated steatotic liver disease (NCT03067428). MGO in serum was quantified with ultra-performance liquid chromatography tandem mass spectrometry.

Results:

In the acute 20 grams fructose challenge in healthy adults, the serum MGO concentration did not significantly differ from baseline (mean concentration 298.5 ±42.0 nmol/L) compared to 2.5-hour post-fructose load (mean difference from baseline 9.4 nmol/L, 95% confidence interval -9.8; 25.1 nmol/L, p=0.294). Furthermore, no differences in serum MGO levels were observed after six weeks of glucose or fructose supplementation (difference between change from baseline -15.5 nmol/L, 95% confidence interval -111.0; 90.0 nmol/L, p=0.756).

Discussion and conclusion:

Acute consumption of a physiologically relevant fructose dose did not increase serum MGO in healthy adults. Moreover, six weeks of glucose or fructose supplementation on the background of a fructose-restricted diet did not result in differences in serum MGO. Although these findings suggest that dietary fructose does not increase systemic MGO levels, it cannot be excluded that dietary fructose induces MGO accumulation in tissue. This is of particular interest since fructose metabolism occurs primarily in the liver, intestine, and kidney. Further studies are warranted to quantify MGO and MGO-derived advanced glycation end products in human tissue.

Abstract 73: Dietary intake of acrylamide from acrylamide-rich Slovak foods associates with that of advanced glycation end products and α-dicarbonyls but not with autofluorescence of saliva, plasma or skin and plasma markers of oxidative status

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Introduction:

Acrylamide, advanced glycation end products (AGEs), and α-dicarbonyls are formed during the thermal processing of foods. Their dietary intake raises potential health concerns, yet the extent of exposure to AGEs and α-dicarbonyls from acrylamide-rich food remains unknown.

Materials and methods:

In 106 students aged 19-to-30 years (74% females), dietary acrylamide intake was estimated using food frequency questionnaires on 26 acrylamide-rich Slovak foods. To align acrylamide intake with that of AGEs and α-dicarbonyls, eight items not present in Dutch databases on AGEs or α-dicarbonyls in foods were excluded from the recalculated acrylamide intake. In 97 out of 106 students, the relationship between estimated total daily acrylamide intake and AGE-associated fluorescence of saliva, plasma (λex370/λem440 nm), skin (the AGE reader, Diagnoptics BV, Groningen, The Netherlands), or markers of oxidative status in plasma (ferric reducing ability of plasma - FRAP, thiobarbituric acid reactive substances – TBARS) was determined.

Results:

Total acrylamide intake was estimated to be 40.0±20.5 µg/d. Bread, breakfast cereals, pastries, snacks, crackers, and crisps accounted for approximately 49% of acrylamide intake. After excluding 8 food items, the recalculated (adjusted) acrylamide intake dropped to 33.0±17.3 µg/d. Concurrently, the CML, CEL, and MG-H1 intake reached 2.3±1.2 mg/d; 1.6 (0.9; 2.6) mg/d, and 19.5±12.0 mg/d, respectively. The intake of α-dicarbonyls, i.e., MGO, GO, and 3-DG, reached 1.7±0.9 mg/d, 1.6±0.8 mg/d, and 6.0 (3.3; 8.2) mg/d, respectively. The main sources of dietary AGEs and α-dicarbonyls were bread, breakfast cereals, pastries, cakes, and biscuits, representing 73%-to-82% and 75%-to-79% of their intake, respectively. Correlation coefficients between adjusted acrylamide and CML, CEL, or MG-H1 intakes reached 0.69, 0.71, and 0.63 ($p<0.001$, all); while those with MGO, GO, or 3-DG intakes equaled 0.74, 0.68, and 0.63, respectively ($p<0.001$, all). Estimated total daily acrylamide intake showed no significant relationship with salivary, plasma, and skin autofluorescence or markers of oxidative status. We did not detect sex differences in any of the studied parameters.

Discussion and conclusion:

As dietary intakes of acrylamide, AGEs, and α-dicarbonyls are strongly intercorrelated, nutritional research should explore their consumption's potential cumulative or synergistic adverse health effects. Our data underline that the restriction of dietary AGEs recommended to patients with diabetes and chronic degenerative diseases concurrently mitigates acrylamide intake.

Abstract 74: Tissue specific suppression of AGE levels by low glycemic diet and glyoxalase-1 (Glo1) overexpression in mice

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Introduction:

Consumption of high glycemic diets or diabetes leads to a dramatic systemic accumulation of AGEs which correlates directly with a number of adverse medical conditions and diseases. Here, we studied the effects of high (HG) and low (LG) glycemic diets on a transgenic (Glo1-Tg) C57BL/6J mouse model with overexpression of Glo1, a detoxification enzyme which previously has been shown to reduce AGEs by inhibiting methylglyoxal (MG) formation in vivo. In this study, we measured MG-derived hydroimidazolone (MG-H1) as a marker for MG modifications in relationship to other markers for glycation and oxidation as well as autofluorescence (AF) in Glo1-Tg vs. non-Tg (control) mice fed HG and LG diets.

Materials and methods:

Male C57BL/6J wild-type mice, age 12 months, and Glo1-Tg littermate-matched mice were fed identical amounts of HG and LG diets for 12 months. AGEs were determined in mouse tissues by liquid chromatography-mass spectrometry (LC-MS/MS) which included plasma filtrates (10 KD MW cut-off) and proteins extracted from plasma, kidney, lens, and tails prior to either acid hydrolysis (plasma, kidney, lens: 6 M HCL, 110°C, 16 hrs) or enzymatic digestion (tails: sequentially, collagenase, pronase, enterokinase, prolidase, each 24 hours). AF was determined in tail collagen-enriched protein digests of mice at Ex 370/Em 440 nm. Statistical analyses were performed by ANOVA, Spearman s correlation and Mann-Whitney U test.

Results:

Repeated measurements of AGEs showed reproducible patterns among tissues due to both diet and Glo1 overexpression. Levels of MG-H1 were significantly ($P<0.05$) higher in mice fed HG vs. LG diets and significantly ($P<0.05$) lowered by Glo1 overexpression in HG diets. In tail digests, diet was significantly correlated with fructose-lysine ($r=0.49$, $P<0.0001$), G-H1 ($r=0.57$, $P<0.0001$), CML ($r=0.36$, $r=P=0.009$), CEL ($r=0.29$, $P=0.04$), methionine sulfoxide ($r=0.32$, $P=0.02$) and o-tyrosine ($r=0.28$, $P=0.046$) while Glo1 overexpression significantly correlated inversely with glucosepane ($r= -0.39$, $P=0.004$), G-H1 ($r= -0.34$, $P=0.015$) and AF ($r= -0.30$, $P=0.029$). Glo1 overexpression in plasma proteins was inversely correlated with levels of MG-H1 ($r= -0.46$, $P=0.002$) and G-H1 ($r= -0.35$, $P=0.025$).

Discussion and conclusion:

Levels of AGEs were increased in HG-fed mice and decreased in Glo1-Tg mice. These findings were observed for collagen-enriched proteins of the tail as well as proteins of the plasma and lens. There was no difference between diets and Glo1-Tg groups for plasma filtrates and kidney proteins which could possibly be due to tissue turnover in these mice.

Abstract 75: Genetic reduction of glycative stress in high glycemic diet fed mice reduces age-related macular degeneration (AMD)-like features

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Introduction:

Western dietary patterns are associated with increased risk for AMD of the eye. High glycemic diets and high fat diets cause hyperglycemia and metabolic stress, which increase oxidative damage. Chronic hyperglycemia increases levels of glycoxidative products known as advanced glycation end-products (AGEs), which are associated with and potentially causative for AMD. Here, we test the importance of AGE formation in a mouse model of AMD by transgenic (Tg) overexpression of glyoxalase 1 (Glo1), a detoxification enzyme that reduces AGE formation.

Materials and methods:

WT or Glo1-Tg littermate male mice were aged to 12-months on regular diets and then fed macronutrient- and calorie-matched low glycemic (LG) or high glycemic (HG) diets for an additional 12-months. Glycemia was evaluated by intraperitoneal glucose tolerance test. Eyes were evaluated in vivo by fundus photography or via histological examination of the retina and RPE. Metabolomics and analysis of AGEs was performed on fasting tissue and plasma samples by LC-MS/MS. Glyoxalase activity was tested by enzymatic assay. Statistical analysis was performed using 2-way ANOVA with Tukey's post hoc.

Results:

Mice fed HG diets became obese and glucose intolerant, irrespective of genotype. Glyoxalase activity was not affected by diet but decreased with aging and increased by overexpression of Glo1. Aging of WT HG-fed mice led to increased numbers of white-yellow fundus lesions relative to WT LG-fed mice. HG-fed Glo1-Tg mice had reduced numbers of fundus lesions. Levels of several AGEs were increased in HG-fed mice and decreased in Glo1-Tg mice or LG-fed mice (see poster Sell et al). Mice fed HG diets had loss of photoreceptors, increased vacuolation of the RPE, and large lipid-containing basal deposits. These AMD features were reduced by Glo1 overexpression and were similar to those in mice fed LG diets. Analysis of the plasma metabolome revealed distinct metabolomes for HG-fed compared to LG-fed mice. We were also able to identify metabolites that were altered in Glo1-Tg mice compared to WT mice.

Discussion and conclusion:

Development of AMD features caused by consumption of an HG diet is a combined effect of altered metabolism and glycative stress downstream of chronic hyperglycemia. Reduction of glycative stress by overexpression of Glo1 ameliorated the effect of poor diet on retinal health. Therefore, interventions that decrease levels of AGEs may have potential therapeutic benefits.

Abstract 76: Glucose variability measured by continuous glucose monitoring is associated with SAF - The Maastricht Study

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Introduction:

Globally, the incidence of type 2 diabetes is increasing due to obesogenic lifestyle changes and aging of the population, leading to an increased risk of cardiovascular disease (CVD). Skin autofluorescence (SAF), a reflection of Advanced Glycation Endproducts (AGEs), is a strong predictor of CVD. SAF has been found to be increased in people with type 2 diabetes, and is thought to result directly from interstitial glucose. It is unknown whether 'spontaneous' hyperglycemia or glucose variability is the driver of the formation of AGEs, as measured by SAF. We therefore aimed to assess the association of SAF with glucose variability in people with prediabetes, type 2 diabetes and without diabetes using data from continuous glucose monitoring (CGM) downloads.

Materials and methods:

Data were derived from The Maastricht Study, a population-based cohort study. The population was stratified by diabetes type (normal glucose metabolism, prediabetes and type 2 diabetes individuals). Coefficient of variance (CV) and standard deviation (SD) assessed by CGM were used to investigate the correlation of SAF with glucose variability in the three groups, respectively. Spearman rank correlation was used for the non-normally distributed data. We further conducted multiple linear regression analyses to adjust for potential confounders including sex, age, BMI, HbA1c, serum creatinine, hypertension, total cholesterol-to-HDL ratio, smoking, alcohol intake, and nutritional factors (energy intake and adherence to Dutch Healthy Diet summary score).

Results:

We included 459 people with normal glucose metabolism, 174 people with prediabetes and 162 people with type 2 diabetes. SAF was increased in type 2 diabetes (2.26 ± 0.59, $p<0.001$ between the three groups). Furthermore, SAF was significantly related to SD ($R=0.24$, $p<0.001$) and CV ($R=0.2$, $p<0.001$) in the total population. These effects were driven by the effect in normal glucose metabolism and prediabetes subgroups ($R=0.13$, $p=0.004$ and $R=0.15$, $p=0.046$, respectively) and by normal glucose metabolism subgroup in CV ($R=0.11$, $p=0.016$), whereas the correlations were non-significant in the type 2 diabetes subgroup ($R=0.12$, $p=0.12$ and $R=0.11$, $p=0.16$, respectively). Finally, no further associations were found when we adjusted for the potential confounders in the total population.

Discussion and conclusion:

SAF is independently associated with glucose variability, in people without diabetes and prediabetes. The association was not significant to people with type 2 diabetes, possibly due to small sample size. Further research to explain this difference is needed.

Abstract 77: Development and validation of HPLC-Fluorescence method for pentosidine analysis in fingernails as a biomarker in diabetic patients

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Introduction:

Diabetes is a chronic metabolic disease characterized by elevated blood glucose level, leading to destructive complications that increase morbidity and mortality. Poor glycemic control in diabetes facilitates glycation, producing early and advanced-glycation end products (AGEs). Traditionally, the glycated hemoglobin (HbA1c) test is used to examine a glycemic control in the preceding 90-120 days, and it has some power to access risk of diabetic complications. However, the invasive nature of this test is believed to deter regular patient compliance, potentially compromising the effectiveness of clinical management. Alternatively, nail clippings are gaining attention as a surrogate matrix for reflecting medium-term of glycation status, and offer advantages compared to blood samples. We have developed a new method for quantification of the AGE pentosidine in fingernails, using high-performance liquid chromatography with fluorescence detection (HPLC-Fluo) in order to investigate its use as a biomarker for prognosis of diabetic complications.

Materials and methods:

Nail samples, were hydrolyzed with 6M hydrochloric acid. Pentosidine was quantified using a C18, reversed-phase column with a binary, gradient mode mobile phase (A: 25 nM aqueous citric acid; B: 25 nM aqueous citric acid/acetonitrile (50/50, v.v)) using fluorescence detector (excitation: 325 nm, emission: 385 nm). This HPLC-Fluo method complements our existing LC-MS/MS to determine amino acids and other fingernail AGEs.

Results:

We tested the effect of reduction with sodium borohydrate prior to acid hydrolysis, the use nonafluoropentanoic to resolubilize the sample in order to facilitated the peak separation, and the effect of different storage times and temperatures. Linearity, precision, intra- and inter-day repeatability tests on several standard calibrations of pentosidine in the range 0-1000 ng/mL showed excellent results, while recoveries of spiked samples were all >94% with low variability. Other methodological results will be presented, and pentosidine and amino acid levels in nails from diabetic patients will be showed in the light of parameters such as HbA1c, age, duration of diabete, *etc*.

Discussion and conclusion:

This study highlights the potential of quantifying fingernail pentosidine by HPLC-Fluo as a non-invasive biomarker, complementary to the conventional HbA1c test, for the assessment of long-term glycemic control and which may provide insights into diabetic complications.

Abstract 78: Reactions of α-dicarbonyl intermediates with Maillard reductones

A. Průšová [1], A. Mečířová [1], Z. Procházková [1], K. Cejpek [1]

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Introduction:

Dietary α-dicarbonyl compounds (α-DCs) have often been discussed with regard to both harmful endogenous effects after metabolic transfer and possible protective effects in gastrointestinal tract etc. During the Maillard reaction, highly reactive α-DCs are formed as key precursors of advanced MRPs. Thus, the Maillard reaction development in food systems may be significantly govern *via* the amount of α-DCs. Many ways to affect α-DCs amounts have been described using scavengers such as phenolic, guanidyl and other nucleophiles. This work aims to describe scavenging properties of methylene-active reducing MRPs such as norfuraneol (NF). Similarly, melanoidins possessing reductone moieties may also scavenge these reactive carbonyls. Another goal is to assess the effect of α-DCs addition reactions to reductones on antioxidant capacity given by the reductones.

Materials and methods:

The reaction of NF with MGO was studied at slightly acidic and neutral pH at 95 °C using kinetic models. Reactants and products were monitored (MGO in the form of 2-methylquinoxaline) by HLPC-PDA methods. The change in reducing power was determined by HPLC-ELD (amperometric detector, working voltage +0.8 V). LC-MS and other spectroscopic analysis were used to identify the products.

Results:

The reactions of NF and MGO have proceeded almost quantitatively within two hours under the conditions used. The transformation of MGO has proceeded significantly faster than equimolar NF, indicating the involvement of more MGO molecules in the products. The primary condensation products are isomers of (*Z,E*)-4-hydroxy-5-methyl-2-(3-oxopropan-2-ylidene)furan-3(*2H*)-one. The subsequent addition product forms more slowly when an additional MGO molecule attaches to the primary product. Results from the amperometric method indicate that at least the primary addition products of NF and MGO retain most of NF's reducing power.

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Abstract 79: Alpha-Dicarbonyl Compounds in Model Reaction Systems and Food with Maltooligosaccharides

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Introduction:

α-Dicarbonyl compounds (α-DCs) arising from sugars during the Maillard reaction are precursors of both positively perceived food components e.g., with sensory and/or reducing properties, and potentially harmful ones such as process contaminants and modified proteins. Comparing to monosaccharides, less attention has been paid so far to reducing oligosaccharides which can produce also specific α-DCs *via* different reaction mechanisms. The aim is to compare the formation of α-DCs from maltooligosaccharides under different reaction conditions with those from other oligosaccharides and monosaccharides. Moreover, determination of a set of α-DCs in selected maltose-rich foods were carried out.

Materials and methods:

Reaction mixtures of sugars with an amino acid at different concentrations, water activity and mode of α-DCs conversion to quinoxaline derivatives (*in statu nascendi* and after reaction) were heated at 95 °C up to 8 hr. As food samples, syrups and other cereal products rich in maltose, beers and beer-related systems (worts, malt extracts) were analysed. Both models and food samples were ultrafiltrated (1kDa) and α-DCs were determined as quinoxaline derivatives (α-DCs-qx) using an HPLC-PDA method with separation on phenylhexyl stationary phase and identified using LC-MS methods.

Results:

The development of α-DCs arising from maltose and maltulose was studied under different reaction conditions to find out the extent and pathways of their formation. The results were compared with analogous kinetic models of galactooligosaccharides and monosaccharides. In low-a_w systems, main α-DCs from maltose as well as maltulose were 1- and 3-deoxymaltosulose (1(3)-DMal) at the both derivatization modes (up to 58 %). In low-a_w maltooligosaccharide systems, the formation of oligo(deoxy)glycosuloses is thus faster than hydrolysis of 1,4-glycosidic bond. In water, maltulose is preferentially transformed *via* 4-deoxyglucosulose (4-DG, up to 76 %). In maltose systems, 4-DG is also fomed, but later and more slowly probably after maltose isomerization to maltulose. The α-DCs content in maltose syrups was up to 780 mg/l with 1(3)-DMal possessing up to 50 %. Dark beers and (hopped) worts were richer in α-DCs then pale ones (up to 55 mg/l). Even in these samples with high a_w, α-DC typical for maltooligosaccharides – 1(3)-DMal, 4-DG, 1,4-dideoxyglucosulose and 3-deoxypentosulose – represent relatively high α-DCs portion, up to 36 %.

Discussion and conclusion:

The results show that analyses focused only to common α-DCs - e.g., 3-deoxyglucosulose,3-DG, and methylglyoxal, MGO - may lead to a significant underestimation of α-DCs level especially in foods rich in oligosaccharides with 1,4-glycosidic bonds.

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Abstract 80: Evaluating the sensitivity of sRAGE, hs-CRP, Fructosamine and Extracellular Vesicles in the Detection of Cognitive Impairment Status

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Introduction:

Alzheimer's disease (AD) is a neurodegenerative disease classically associated with protein aggregation and progressive neuronal death. Mild cognitive impairment (MCI) is a transitional stage between healthy aging and AD onset. Blood-based biomarkers have become a promising area of study in AD prognostic and diagnostics. The receptor for advanced glycation end products (RAGE), Fructosamine and high-sensitive C-reactive protein (hs-CRP) are suggested to be mechanistically linked to AD pathogenesis and reflect whole-body inflammatory status. Further, extracellular vesicles (EV) characteristics (size and quaintly) may be sensitive to cognitive disease status. RAGE protein expression within EVs, however, have not been well characterized. Thus, the purpose of this study was to characterize soluble RAGE (sRAGE), hs-CRP, EV characteristics and EV RAGE protein expression across the cognitive impairment continuum.

Materials and methods:

Samples were acquired from the University of Michigan Alzheimer's Disease Research Center (n=156 CON, n=79 MCI, n=39 AD). All participants underwent cognitive assessments and Consensus Diagnosis, blood-sampling, anthropometric measurements, and structural MRI. Plasma sRAGE was quantified via ELISA. Hs-CRP and Fructosamine was quantified by a colorimetric assay. EVs were isolated utilizing size exclusion chromatography, and characterized via nanoparticle tracking analysis. EV protein was concentrated using protein precipitation and RAGE protein expression analyzed via Western blot.

Results:

Differences in age ($p=0.02$), education ($p<0.01$), and BMI ($p<0.01$) were detected between groups. As expected, there was a stepwise reduction in MoCA score across the groups ($p<0.01$). A difference in hs-CRP concentration was found between groups ($p<0.05$). Notably, we identified a +26% elevation in hs-CRP CON vs MCI and a -90% ($p<0.01$) difference in hs-CRP levels when comparing CON and AD. No differences in sRAGE or Fructosamine concentration were identified across groups. We found a significant 4.6% elevation in EV size ($p<0.01$) in AD patients compared to CON. No differences in plasma EV concentration were found. Further, no differences in EV RAGE protein expression were identified. EV size displayed moderate sensitivity by ROC curve in detecting CON vs MCI (AUC=67.5%) and MCI vs AD (AUC = 61.5%).

Discussion and conclusion:

These results indicate that sRAGE is not a sensitive biomarker in identification of cognitive impairment status. However, elevated hs-CRP may be an early indicator of cognitive impairment. EV size, but not EV RAGE protein expression, appears to be elevated with AD, and relatively sensitive in detecting MCI and AD. Further research is warranted to determine alternative inflammatory biomarkers sensitive to disease status.

Abstract 81: Effects of Acute Aerobic Exercise on Circulating sRAGE and sTLR in Type 2 Diabetes

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Introduction:

The receptor for advanced glycation end products (RAGE) is a multiligand receptor that instigates a cascading inflammatory pathway. Similarly, toll like receptor 4 (TLR4) is a transmembrane protein that plays a role in inflammatory signaling. Both RAGE and TLR4 have soluble counterparts (sRAGE; sTLR4) that act as decoys and disrupt receptor signaling. RAGE and TLR4 are expressed at higher levels in diseased populations where inflammation is elevated, such as type 2 diabetes (T2D). On the contrary, circulating solubilized receptor quantities (sRAGE and sTLR4) have been shown to be inversely correlated with inflammatory burden present in the diseased state. Lifestyle interventions such as aerobic exercise have the physiological effect of mitigating inflammation and other symptoms of metabolic impairment. We have previously found that sRAGE is increased after habitual periods of aerobic exercise training and intermittent fasting, though the effect of acute exercise remains inconclusive. This study aimed to determine the effect of acute aerobic exercise on sRAGE and sTLR4 levels in T2D. We hypothesize that acute aerobic exercise will increase the circulating concentration of both solubilized receptors.

Materials and methods:

T2D participants (n=55) underwent a baseline treadmill VO_{2max} testing (23±5 mL/kg/min) and comprehensive metabolic characterization including body composition (DXA; 41±8%) and oral glucose tolerance determination of insulin sensitivity (Matsuda Index; 4±3). On a separate day, the participants underwent an acute bout of aerobic exercise (treadmill) at 70-75% VO_{2max} for 60 minutes. Blood samples were taken before and 30 minutes after the exercise bout. Plasma sRAGE and sTLR4 were quantified using commercially available ELISA kits.

Results:

With acute aerobic exercise, circulating plasma sRAGE levels decreased by ~11±6% ($p=2.3x10^{-9}$), where 50 of 55 subjects decreased. However, circulating plasma sTLR4 was unchanged (0.7±1.1, p=0.27). Correlation analyses revealed an association between sRAGE and sTLR4 at baseline (r=-0.30; p=0.027) but not following acute aerobic exercise. Correlation analyses between baseline sRAGE and sTLR4 against baseline VO_{2max}, body fat %, and Matsuda index demonstrated no associations.

Discussion and conclusion:

Contrary to our hypothesis, acute aerobic exercise decreased circulating sRAGE concentrations. These observations are in opposition to chronic aerobic exercise training demonstrating increases in sRAGE by 10-20%. Thus, evaluation of the post-exercise temporal dynamics is warranted to better understand the mechanisms and physiological consequences of our observations.

Abstract 82: Galactooligosaccharides as Precursors of α-Dicarbonyl Compounds in Dairy Products

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Introduction:

α-Dicarbonyl compounds (α-DCs) play a key role as intermediates in caramelization and the Maillard reaction. Most research to date has focused on a limited range of α-DCs, usually 3-deoxyglucosulose (3-DG), glyoxal (GO), methylglyoxal (MGO), and/or biacetyl (BA), potentially underestimating the overall α-DC content. This study aims to identify as many α-DCs as possible, particularly those derived from galactooligosaccharides, to measure their concentrations in dairy products and to verify the impact of processing on their amounts. Another aim is to try to decrease α-DC content via reactions with selected phenolic compounds.

Materials and methods:

Model solutions of sugars at a concentration of 0.25 mol/l were prepared and then heated at 95 °C for 1-24 hr. In case of adding phenolic compounds, their concentration in the sample was 1 mmol/l. Separation of the target low molecular weight fraction from both dairy samples and model reaction mixures was performed using 1kDa ultrafiltration. Quinoxaline derivatives of α-DCs (α-DCs-qx) were obtained by reaction with o-phenylenediamine (OPD) for one hour at laboratory temperature. Samples were analysed by HPLC coupled to a photodiode array detector (HPLC-PDA) and individual α-DCs-qx were tentatively identified using LC-HRMS-ESI-TOF.

Results:

Each α-DC-qx was described with MS spectral data, molecular formula, other spectral and retention characteristics and by comparison with standards The elution order of four isomers: 1-deoxyglucosulose (1-DG), 3-deoxyglucosulose (3-DG), 3-deoxygalactosulose (3-DGal), and 4-deoxyglucosulose (4-DG) was established through a series of other experiments. A complex reaction scheme focused on particular α-DC formations was created. Chlorogenic, caffeic and rosmarinic acids proved to be effective during experiments to reduce α-DC content using phenolic substances. Of selected milk and dairy product samples, the highest α-DC content (up to 14 ± 1 mg/kg of dry matter) was found in dried, condensed, and lactose-free products and infant formulas.

Discussion and conclusion:

A series of experiments with reaction mixtures of lactose and lactulose led to the development of a comprehensive reaction scheme for a wide range of α-DCs. In total, more than 20 α-DCs were described. Further experiments showed that they could be scavenged by some phenolic acids. Analysis of a set of dairy samples revealed the highest α-DC levels in thermally and otherwise processed products.

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Abstract 83: The effect of glycated dietary protein on the in vitro intestinal epithelium and intestinal immune system; absorption and AGE-RAGE interaction

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Introduction:

Endogenous Advanced Glycation Endproducts (AGEs) are well-described to be involved in multiple disease pathologies, as their formation changes the tertiary and quaternary structure of body proteins, altering their functionality. Next to these endogenous AGEs, we are also exposed to dietary AGEs daily as they are formed when processing food. Related to health, much research has been performed on the digestibility and therefore bioavailability of these glycated dietary proteins. As many in vitro studies showed a decrease in protein digestibility, subsequently, different studies focused on the effect of AGEs-rich undigested protein fractions on the microbiome. However it is not yet clear what direct effect these glycated proteins might have on the intestinal epithelium and intestinal immune system. In this respect, we investigated their absorption by the intestinal epithelium and AGE-RAGE interaction on immune cells.

Materials and methods:

Transport of glycated beta lactoglobulin (BLG, dry-heated with glucose) over a Caco-2 monolayer grown on transwells was monitored by BLG ELISA. Inflammatory response of these cells was assessed by IL-8 ELISA. To further assess the inflammatory characteristics of glycated BLG, primary monocytes and M0 macrophages (differentiated using either M-CSF or GM-CSF) were used in this study and characterized for surface and total RAGE expression using flow cytometry. Cells expressing surface RAGE were exposed to glycated BLG for 24 hours. Their inflammatory response was assessed by measuring IL-8, IL-6, and TNF-α. Subsequently, the RAGE-specific blocker FPS-ZM1 and LPS-specific neutralizer polymyxin B (PMB) were used to explore the inflammatory mode of action.

Results:

Glycated BLG is partially transported over a Caco-2 monolayer without inducing inflammation in Caco-2 cells. By default, all cell models expressed RAGE but not on the cell surface. Only M-CSF differentiated M0 macrophages showed surface RAGE expression. When these cells were exposed to glycated BLG, an inflammatory response was observed that could not be inhibited by the RAGE-specific blocker FPS-ZM1. With PMB, a LPS-specific neutralizer, this response was clearly attenuated.

Discussion and conclusion:

Currently, literature show that the fate of undigested glycated proteins is being metabolized by the microbiome; however, our results indicate that glycated proteins could also pass the intestinal epithelium. Our data showed that that not all cells express RAGE on the surface. Accordingly, AGEs by themselves do not induce inflammation and we showed that LPS contamination of the glycated protein sample was the main driver of inflammation. Therefore, the hypothesis of AGEs directly inducing inflammation through interaction with RAGE needs to be reformulated. Possibly a second hit or a cascade of events is required to trigger the pro-inflammatory action of dietary AGEs.

Abstract 84: The effects of aerobic exercise training on circulating sRAGE in Type 2 Diabetes

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Introduction:

The receptor for advanced glycation end products (RAGE) is a membrane-bound receptor linked to inflammation. Soluble RAGE (sRAGE) acts as a decoy receptor for AGEs and other ligands as it lacks an intracellular domain to initiate signaling cascades. Similar to RAGE, TLR4 is a pattern recognition receptor that initiates proinflammatory responses. sTLR4 is TLR4's ligand decoy. High sensitive C-reactive protein (hsCRP) responds to inflammatory cytokines and is a principal marker of inflammation. Fructosamine is a measure of non-enzymatic glycation of circulating proteins that is present in high concentrations amongst individuals with poor blood glucose control. Individuals with Type 2 Diabetes (T2D) experience systemic inflammation linked to low levels of sRAGE and sTLR4 and high levels of hsCRP and fructosamine. T2D is hallmarked by insulin resistance which can result from excess body fat or lack of physical activity. We have demonstrated that altered body composition through weight or fat loss decreased RAGE protein expression while increasing circulating sRAGE. Further, we have established that acute bouts of aerobic exercise minimally modulate sTLR4 and sRAGE levels in healthy and obese adults. Aerobic exercise training (AET) is known to attenuate systemic inflammation, but its ability to reduce cardiometabolic risk through its relationship with sRAGE remains unknown. This study aimed to investigate the influence of AET on sRAGE and cardiometabolic risk factors within T2D.

Materials and methods:

Individuals with T2D (n=33, age: 57±9.5, BMI: 33.8±5.8) were randomized into control (CON: n=13) and AET groups (n=20). Those randomized to AET completed 12 weeks of supervised aerobic exercise (treadmill) at ~70% VO_{2max}, 5 days/week for 60 min/day. Fasting blood samples were taken before and after the 12-week intervention period under controlled metabolic conditions. Plasma sRAGE and sTLR4 levels were determined via ELISA. Plasma fructosamine and serum hsCRP were measured via colorimetric assay. Glucose and insulin were measured during an OGTT and insulin sensitivity was calculated via Matsuda index. Intervention deltas for CON and AET were determined for sRAGE, sTLR4, hsCRP, fructosamine, and Matsuda index.

Results:

Within AET, sRAGE increased by 25±31% (p=0.02), sTLR4 was unchanged (p=0.36), hsCRP decreased by 7±76% (p=0.13), fructosamine decreased by 16±24% (p=0.05), and Matsuda index increased by 78.7±186% (p=0.09). Correlational analyses demonstrated a negative correlation between sRAGE and sTLR4 amongst all cohorts (r=-0.45, p=0.02).

Discussion and conclusion:

AET augmented sRAGE levels comparable to that of healthy individuals while improving glycemic control within T2D. Overall, AET improved inflammatory conditions within T2D individuals. The mechanisms by which aerobic exercise training influences these factors within T2D are under further investigation.

Abstract 85: Longitudinal assessment of advanced glycation end products (ages) and bone mineral density: the Rotterdam study

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Introduction:

Advanced glycation end products (AGEs) are heterogeneous molecules produced irreversibly through non-enzymatic glycation of proteins, lipids, or nucleic acids. Skin AGEs can be measured as skin autofluorescence (SAF) and are used as a marker for long-term AGE accumulation. AGEs bind to collagen type 1 in bone and form cross-linking (e.g., pentosidine) and non-crosslinking collagen modifications (e.g., carboxymethyllysine (CML). We previously showed that SAF was associated with prevalent fractures independent of bone mineral density (BMD) but the exact role of AGEs on BMD remains unclear. This study aims to elucidate the relation between AGEs and BMD by assessing the longitudinal association of SAF with BMD and the cross-sectional association of serum CML with BMD in a population-based cohort study.

Materials and methods:

In the Rotterdam Study (RS), SAF was measured using the AGE Reader®, and CML in serum. Total body and site-specific BMD were assessed using dual-energy X-ray absorptiometry (DXA). SAF was analyzed in relation to baseline and follow-up total body (TB), femoral neck (FN), lumbar spine (LS), total hip (TH) BMD and trabecular bone score (TBS), employing a linear mixed-effects model with interaction analysis examined for sex, prevalent type 2 diabetes mellitus (T2D) and chronic kidney disease (CKD). The cross-sectional relation between CML and TB, FN, LS, TH BMD and TBS was analyzed using linear regression analysis. These associations were adjusted for age, sex, body mass index (BMI), smoking status, kidney function, physical activity and T2D.

Results:

For the analyses between SAF and TB BMD, 2553 participants were included with (mean (SD) age 68.3 (9.5) years, 1436 (56.2%) women) with follow-up time 4.9 (0.7) years. For 851 participants with available SAF and LS, FN, TH BMD and TBS, mean (SD) age was 58.0 (6.4) years, and 469 (55.1%) were women with follow-up time 5.6 (0.6) years. In the fully adjusted model, SAF was not associated with changes in the trajectory of TB, FN, LS, TH BMD and TBS. Interactions were observed of SAF with sex and T2D for the associations with TB and FN BMD, but not with TBS. Subsequent subgroup analysis by sex did not show significant associations between SAF and repeated measurements of TB BMD: in women -0.010 SD, 95% CI -0.022, 0.001, in men: 0.005 SD, 95% CI -0.008, 0.018. In individuals with T2D (n=57) an inverse association was observed for FN BMD, and a nonsignificant inverse association for TH BMD; no association was observed in individuals without T2D. In 300 participants with serum CML measurements and mean (SD) age 71.4 (5.3) years, 149 (49.7%) women) no significant associations were observed between CML and all BMD outcomes.

Discussion and conclusion:

We observed no longitudinal association between SAF and BMD at multiple sites or TBS, nor any cross-sectional association between serum CML and BMD or TBS. These findings suggest that AGEs may affect fractures by influencing bone quality rather than bone quantity. However, a larger sample size and longer follow-up time are needed to further elucidate these associations. Future incident fracture data could provide a clearer understanding of this relationship.

Abstract 86: Fiber-Type Specific Effects of GLO1 Expression in Type 2 Diabetes Following an Aerobic Exercise Training Intervention

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Introduction:

Glyoxalase-1 (GLO1) is a highly conserved myocellular protein involved in the detoxification of methylglyoxal, a toxic dicarbonyl byproduct that is spontaneously produced in glycolysis. We have previously shown elevated levels of dicarbonyl stress and reduced GLO1 protein expression in skeletal muscle tissue of individuals with obesity and Type 2 diabetes (T2D) that correlate with the degree of insulin resistance. However, there is little evidence concerning the role of aerobic exercise training (AET) on inducing skeletal muscle GLO1 protein expression. Our study aimed to identify whether aerobic exercise training upregulates GLO1 expression, specific to fiber-type, in human skeletal muscle.

Materials and methods:

Individuals with T2D (n=33, age: 57±9.5, BMI: 33.8±5.8) were randomized to control (CON; n=13) and AET (n=20) groups. The AET group completed 12 weeks of supervised aerobic exercise (treadmill) at ~70% VO_{2max}, 5 days/week for 60min/day. Skeletal muscle biopsies samples were obtained before and after the 12-week intervention period (in the fasted condition). Total GLO1 protein expression was quantified by Western blot from muscle tissue homogenates and via immunofluorescence in skeletal muscle histological preparations concomitant with myosin heavy chain fiber type profiling.

Results:

Baseline GLO1 expression was lowest in Type IIx fibers compared to Type IIa ($p = 0.002$) and Type I ($p = 0.26$). All fiber types had an increase in GLO1 expression post AET (Type I: 9.6% ± 14.6, Type IIa: 3.7% ± 16.6, Type IIx: 5.6% ± 21.6) however, no changes were statistically significant when compared to CON. In skeletal muscle homogenates, GLO1 protein expression was unchanged with AET.

Discussion and conclusion:

We and others have previously reported increased GLO1 expression with AET in older adults and adults with obesity and pre-diabetes. However, this is the first reporting of GLO1 protein expression with AET in obese adults with T2D. Our findings suggest that individuals with T2D are not responsive to GLO1 exercise adaptation. Numerous factors can affect GLO1 protein expression. Including, but not limited to NAMPT, SIRT 1, SIRT2, and NRF2. Future direction should focus on possible dysregulation amongst these pathways affecting inductive effects of AET on GLO1 protein expression.

Abstract 87: Cholesterol aggravates MASLD through the AGE-RAGE pathway

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Introduction:

Metabolic Dysfunction Associated Steatotic Liver Disease (MASLD) represents a spectrum of liver complications, ranging from steatosis to metabolic dysfunction-associated steatohepatitis (MASH), potentially progressing to cirrhosis and hepatocarcinoma. Lipotoxicity and advanced glycation and lipoxidation end products (AGEs/ALEs) synergistically contribute to disease progression. Lipotoxicity is marked by modified lipoproteins and free fatty acids (FFA). Both AGEs/ALEs and lipids bind to various liver cell surface receptors, triggering oxidative stress, inflammation, cell death, altered cellular metabolic activity, and fibrosis. However, the precise molecular mechanisms are not fully understood. Notably, lipids and AGEs/ALEs share some receptors. We hypothesized that lipids contribute to MASLD progression by modulating the AGE-RAGE pathway and aim to evaluate whether lipids modulate this pathway and contribute to MASH pathogenesis.

Materials and methods:

For in vivo experiments, C57BL/6 male mice, aged 8 weeks, were utilized. MASH was induced using a high-fat diet composed of 55% of calories from fat, 35% from carbohydrates, and 10% from protein, combined with a high-carbohydrate drink containing 25% fructose, administered over 43 weeks. During the final 12 weeks, the MASH group was divided into two subgroups: one continuing on the MASH diet and the other receiving the same diet with an additional 2% cholesterol (MASH+CHOL). The control group (CTL) was maintained on a commercial grain-based diet with 11% calories from fat, 63% from carbohydrates, and 26% from protein, alongside normal water for 43 weeks. In the in vitro experiments, HepG2 cells were incubated with a combination of FFAs (palmitic and oleic acid) or oxidized LDL (oxLDL) to induce steatosis. To investigate the effect of blocking RAGE on the steatosis phenotype, RAGE antagonists FPZ-M1 and TTP-488 were used. The study analyzed various outcomes, including levels of glyoxal (GO), carboxyethyl lysine (CEL), carboxymethyl lysine (CML), methylglyoxal-derived hydroimidazolone-1 (MG-H1), and methylglyoxal (MGO), AGE receptor gene expression, inflammatory markers, and glyoxalase-1 enzyme activity in lysed cells.

Results:

Supplementation with 2% cholesterol favored a worsening of the MASH stage, as shown by the increase in LDL cholesterol, liver total cholesterol, steatosis, inflammation, fibrosis and liver injury (AST/ALT) by modulation in the following mechanisms: increased expression of CML, RAGE/DIAPH1, GLO-1, CD36, FABP4, and NF-KB and levels of reactive oxygen species and superoxide dismutase and decreased in catalase level.

Discussion and conclusion:

These findings suggest that cholesterol supplementation may exacerbate the pathological processes associated with MASH, possibly through the induction of oxidative stress and inflammation mediated by AGE-ALE/RAGE pathway. As our animal studies have limited the elucidation of whether the events are in fact mediated by this pathway, we are exploring the interaction between lipids (free fatty acids and cholesterol) and the AGE-RAGE signaling pathway in further detail in the in*vitro* steatosis model.

Abstract 88: AGEs as a Mechanistic Link Between Alzheimer's Disease and Osteoporosis

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Introduction:

With extended lifespan, the incidences of chronic conditions associated with aging rapidly increase, such as Alzheimer's disease (AD) and osteoporosis. Accumulation of advanced glycation end-products (AGEs) can provide a pathophysiological linkage between these conditions, where hyperglycemia and elevated oxidative stress play an essential role. AGEs form through non-enzymatic glycation when glucose molecules react with amino acid residues on proteins and can affect various tissues/organs, including brain and bone. AGEs can lead to cognitive dysfunction bybinding to the receptor of AGEs, located on the blood–brain barrier, activating pro-inflammatory cytokines and producing reactive oxygen species. AGEs also have a distinct mechanism to skeletal fragility by disrupting bone metabolism, and degrading bone quality. Here, we posited that AGEs can serve as a biomarker for concurrent bone quality loss and cognitive dysfunction.

Materials and methods:

We used 5XFAD mice (34840-JAX) and wild-type (WT) littermate controls. From brain tissue homogenates, Aβ42 levels and AGE accumulation were measured by the Human Aβ42 ELISA kit and the Mouse AGE ELISA kit, respectively, then normalized by total protein content. From bone, total fluorescent advanced glycation end-products (fAGEs) was measured by the fluorescence intensity normalized to a quinine sulfate standard and then normalized to collagen protein content. Femora sections were used for Raman spectroscopy to measure two AGEs, carboxymethyl-lysine (CML) and pentosidine (PEN).

Results:

Results: 5XFAD mice had increased Aβ42 levels compared to WT mice ($p<0.01$). From bone tissue, 5XFAD mice showed accumulation of fAGEs, CML and PEN, compared to WT groups ($p<0.01$ for all). Notably, an increase in Aβ42 levels was correlated with increased accumulation of glycoxidation products in bone, CML and PEN, and glycation products, fAGEs ($p<0.001$ for all). Moreover, Aβ42 levels in the brain were strongly positive correlated with AGEs in the brain ($p<0.001$).

Discussion and conclusion:

We demonstrated the effects of the AD phenotype on bone quality and linked changes in bone to amyloid formation in the brain. We observed that amyloidosis correlated with an increase in CML, PEN and total fAGEs, suggesting a mechanistic link between elevated Aβ42 levels in the brain and AGE accumulation in bone. Moreover, levels of AGEs in the brain were positively correlated against Aβ42 levels in brain. We propose this association could be harnessed for diagnosis of bone health and for estimating amyloid load in AD. Considering the growing epidemic of Alzheimer's disease, our results offer both increased understanding and a possible clinical approach for skeletal fragility seen with Alzheimer's disease, and to monitor, manage, and possibly treat Alzheimer's disease itself.

Abstract 89: Investigation on the Maillard reaction pathways in fully aqueous emulsions through untargeted metabolomics

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Introduction:

Maillard reaction has been widely investigated in aqueous solutions, solid model systems and structured fluids, as oil-in-water emulsions and microemulsions to generate miscellaneous volatile and non-volatile compounds. Fully aqueous emulsions, i.e. W/W emulsions confer advantages such as the ability to efficiently compartmentalize reactants (spatial distribution), avoiding the use of synthetic surfactants and lipid (oil) compounds, as well as pre-fractionation of the end-products of the MR in either of the phases of the emulsion, subsequent to phase separation. Our previous findings indicate that W/W emulsions could serve as effective tools for controlling the location of both reactants and products, as well as MR pathways. We developed untargeted metabolomic experiments to decipher the chemical nature of the end-products formed and elucidate MR pathways in W/W emulsions.

Materials and methods:

Na_2SO_4-in-polyethylene glycol emulsions were prepared, and the reactants were partitioned either inside the droplets (co-encapsulation) or segregated (yet interfacially touching) between the interior and exterior phases of emulsions. For comparison purposes, single-phase solutions were also prepared. HILIC high resolution tandem mass spectrometry combined with chemometrics was used for the identification of end-products.

Results:

When the reactants were co-encapsulated, multiple reaction networks were observed with different final products derived by oxidation, acetylation and carboxymethylation on asparagine α-amino group. When considering Amadori product of asparagine, dehydration of sugar moiety was the predominant mechanism leading to the formation of degradation compounds part of polymerized structures. When the reactants were segregated, a more diversified molecular pattern explained the chemical reactions on indole ring: homologues series were formed putatively representative of α-dicarbonyls reaction on indole moiety of both tryptophan and Amadori compound of tryptophan.

Discussion and conclusion:

Molecular networks based on asparagine, tryptophan, and their Amadori compounds outlined reaction mechanisms behind polymerization reactions of melanoidins and other low molecular weight brown pigment. We demonstrated that in W/W emulsions the chemical nature of the end-products and their distribution are essentially driven by the reactant location and their partition coefficient.

Abstract 90: Intracellular concentrations of the glycolytic by-product methylglyoxal are high in leukocytes, increase after an oral glucose tolerance test and upon cellular activation

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Introduction:

Elevated plasma methylglyoxal (MGO), a reactive dicarbonyl compound, is linked with obesity and type 2 diabetes. We previously showed that increased postprandial MGO formation in plasma and tissues originates from exogenous glucose. During inflammation, immune cells display a shift toward glycolytic glucose metabolism. We evaluated levels and formation of MGO in immune cells.

Materials and methods:

MGO was quantified in human and mouse blood fractions using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). MGO content was estimated in the circulating leukocytes with flow cytometry using a fluorescent probe (mean fluorescence intensity/MFI) in 19 abdominally obese individuals undergoing a 75-g oral glucose tolerance test (OGTT). MGO levels were measured with UPLC-MS/MS in human leukocytes sorted with fluorescence-activated cell sorting. C57BL/6J and db/db mice were injected with universally labeled D (+) 13C glucose, and MGO formation was measured with UPLC-MS/MS. In neutrophils isolated from healthy donors MGO formation was investigated using flow cytometry upon activation with phorbol 12-myristate 13-acetate (PMA) or lipopolysaccharide (LPS).

Results:

MGO concentrations in monocytes (2873 ± 247 µmol/L), lymphocytes (2699 ± 1090 µmol/L), and neutrophils (1706 ± 534 µmol/L) were approximately 1,000-fold higher than in erythrocytes (2 ± 1 µmol/L), and 20,000-fold higher than in plasma (76 ± 9 nmol/L blood). During an OGTT, intracellular MGO levels increased continuously in human leukocytes with the highest formation after 2 hours: lymphocytes (+26%), monocytes (+18%), and neutrophils (+6.5%). In mice, leukocyte MGO levels were similarly high and 13C glucose administration led to an increase of 13C MGO in leukocytes, which was enhanced in db/db mice (5.7-fold higher levels at 60 minutes). In vitro, receptor-independent activation of primary neutrophils with PMA resulted in very rapid intracellular MGO formation (20 min) which was inhibited by blocking glycolysis with 2-deoxyglucose (1310047 ± 202602 MFI vs 232776 ± 139549 MFI). Receptor-dependent activation with LPS (17h) also induced glycolysis-dependent MGO formation (2287578 ± 59786 MFI vs 2705417 ± 123012 MFI). Culturing cells in glucose-free conditions did not affect PMA-induced MGO formation but inhibited LPS-induced MGO formation (2126879 ± 13550 MFI vs 1596834 ± 37208 MFI).

Discussion and conclusion:

Leukocytes contain high intracellular MGO levels, which is formed from exogenous glucose, and increases in a hyperglycemic state and upon inflammatory activation.

Abstract 91: Glucose-rich diet may promote endogenous glycation via methylglyoxal-independent mechanisms and reduce the longevity of Caenorhabditis elegans

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Introduction:

The formation of endogenous advanced glycation end-products (AGEs) is promoted by various pathogenic processes such as inflammation, oxidation and hyperglycemia. AGEs, which accumulate during type 2 diabetes (T2D), are suspected to be involved in the complications of this disease. However, whether AGEs formation is a causal mechanism or simply a pathological consequence is still under discussion.The aim of our study was to use the *Caenorhabditis elegans* model to analyse the association between dietary glucose exposure, glucose metabolism, oxidation, formation of AGEs and impact on health and lifespan.

Materials and methods:

C. elegans worms were grown in medium containing no glucose or different concentrations of glucose. Lifespan assays were performed with live or heat-inactivated bacteria to 1- check impact of glucose and bacteria on longevity and 2- choose the type of food to give to the worms for biological analysis. After 3, 6, 10 and 13 days of incubation, expression of antioxidant and metabolic genes was measured by RTqPCR whereas protein oxidation (carbonylation) and glycation (*N*-carboxymethyllysine or CML and methylglyoxal-derived AGEs) were monitored by western blot. Incubating the worms with glyoxal or methylglyoxal was used to control which AGEs were formed after exposure of the endogenous proteins to these 2 compounds.

Results:

The highest concentration of glucose decreased lifespan of *C. elegans.* In this glucose-rich diet, worms displayed more oxidized proteins and CML-modified proteins, whereas no methylglyoxal-derived AGEs epitopes were detected on worms'proteins. Furthermore, genes coding for isozymes with sorbitol dehydrogenase (SODH) activity from the polyol pathway were modulated. CML formation, possibly induced by glyoxal, was associated with different biological alterations which were highlighted by transcriptional modulation such as cytoplasmic and mitochondrial unfolded protein response (UPR) and lysosomal functions.

Discussion and conclusion:

This study has demonstrated induction of oxidation and glycation of endogenous proteins, which were possibly derived from the modulation of the polyol pathway and the formation of glyoxal. These results open perspectives on the use of the *C. elegans* model to better understand the health effects of endogenous glycation.This organism is certainly a valuable model for determining whether endogenous glycation is merely a consequence of certain pathologies such as TD2 or whether and how it is also involved in the development of their complications.

Abstract 92: Peptide-based glycation models as a tool to address the mechanisms behind glycation in plants

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Introduction:

Protein glycation (often also referred as Maillard reaction of proteins) is usually referred to as an arrow of post-translational modifications targeting side chains of lysine, arginine and, to less extent, cysteine residues in proteins. Carbonyl compounds – sugars, their derivates and α-dicarbonyls, act as potent glycation agents, yielding broad patterns of early and advanced glycation end products (AGEs). To date, the Maillard reaction of proteins is comprehensively described in the food chemistry and clinical aspects. In this context, resulting advanced glycation end products (AGEs) were repeatedly reported as important modulators of human metabolism and pathology markers. Later on, it was shown that the phenomenon of glycation is universal for all living organisms and is characteristic not only for animals and humans, but also microorganisms and plants. However, in comparison to humans, the patterns of plant protein glycation are characterized with much higher diversity, complexity, dynamics and strong predominance of AGE formation over early glycation (i.e. formation of Amadori and Heyns products). This high complexity of glycation patterns might be attributed to tremendous diversity of plant carbonyl compounds. The accumulated so far data indicate prospective signaling and/or regulatory role of these glycative modifications. Therefore, understanding the complexity of the pathways and mechanisms of plant glycation might give access to prospective glycation-related mechanisms.

Materials and methods:

To access the complex patterns of plant glycation, the whole network of the Maillard reaction, which relays on multiple glycation agents, might be dissected in individual reactions. For this, we employ *in vitro* peptide-based glycation model, which relies on reaction of partly protected decapeptides with lysine or arginine residues in mid position with individual sugars and their derivatives. Employment of the combined chromatography-mass spectrometry-based peptidomics and metabolomics platforms allowed parallel identification of peptide-based glycation products and dynamics of related metabolites.

Results:

With this approach, we addressed a broad selection of plant sugars and their derivatives which demonstrated essential variations in reactivity. Besides this, we found that the patterns of glycation products were strongly specific for particular glycation agents. Consideration of these results in the context of stress-related dynamics of plant metabolome allowed annotation of specific stress induced plant metabolites as case-specific glycation agents.

Discussion and conclusion:

This result delivers a new confirmation for the prospective signaling and regulatory role of protein glycation in plants. Thereby, amide AGEs appeared to be the most promising candidates for the new glycation-related plant signaling molecules.

Abstract 93: Evolution of a novel carboxymethyl lysine (CML) oxidase that can both detect CML and repair CML-damaged proteins

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Introduction:

Advanced glycation endproducts (AGEs) are a set of heterologous chemical modifications formed within the body as a result of the Maillard reaction. AGE accumulation is a hallmark of mammalian aging and they are implicated in the pathogenesis of numerous aging-associated diseases. Nε-carboxymethyl-lysine (CML), a Maillard reaction product of lysine, is the best characterized AGE and is implicated in the pathogenesis of various diseases of aging including diabetic retinopathy, diabetic kidney disease, Alzheimer's, and microvascular disease. Previous attempts to therapeutically target CML focus on preventing its formation or blocking subsequent AGE signaling via the receptor of AGEs (RAGE).

Materials and methods:

To identify an enzyme that can repair CML, we characterized a variety of oxidases selected for specific sequence criteria. We undertook an enzyme engineering campaign to enhance the activity of our CML oxidase. The enzyme activity was refined over several generations with a directed evolution approach using a combination of random and site-directed mutagenesis.

Results:

Here, we report the identification of a previously uncharacterized oxidase with native activity on free CML. We achieved a remarkable 100-fold improvement in activity on free CML. We were also able to evolve the unique ability to repair CML modifications on peptides and proteins. Using ELISA assays with antibodies specific for CML modifications, we show that our novel CML oxidase can remove a majority of CML modifications from glycated model proteins.

Discussion and conclusion:

The ability to remove CML modifications from proteins offers a unique repair-based approach to treating CML-associated diseases. In addition, our CML oxidase can be used as a simple research tool to detect CML modifications due to the production of hydrogen peroxide which can be readily detected using a horseradish peroxidase-linked fluorescent assay. Overall, we describe the discovery and engineering of a novel CML oxidase that can be used as a research or diagnostic tool for detecting CML modifications and that has great potential for use as a therapeutic to treat aging and diabetes-associated diseases.

Abstract 94: Serum Advanced Glycation End Products (AGEs) Correlation to Diabetes and Tendon Properties

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Introduction:

Advanced glycation end products (AGEs) accumulate in the serum of those with type 2 diabetes and hyperglycemia. Individuals with type 2 diabetes are nearly four times more likely to live with debilitating tendon complications than those without diabetes. Our previous work linked serum advanced glycation end-products (AGEs) to reduced tenocyte function and poor tendon healing. We hypothesized that serum factors other than blood glucose could be used to predict in vivo tendon properties.

Materials and methods:

We investigated the relationships between in vivo imaging-based tendon properties and serum AGEs across non-diabetic controls (n=8, Age: 31±3y, BMI: 23±1, HbA1c: 5.2±0.1%), pre-diabetes (n=4, Age: 38±11y, BMI: 29±3, HbA1c: 5.5±0.1), and type 2 diabetes (n=6, Age: 49±4y, BMI: 34±3, HbA1c: 6.6±0.3). Patellar tendon modulus was evaluated using ultrasound methodologies and strain tracking. Patellar tendon cross-sectional area and signal intensity were assessed using T1-weighted MRI imaging. HbA1c, insulin, metabolic panel, and lipid panels were conducted using serum drawn after a 12 hour fast. AGE loads were asssessed at the Univerisity of Michigan.

Results:

Tendon modulus tended to be correlated with serum carboxymethylysine (CML) ($p=0.09$, $r=-0.47$) and was correlated with age ($p=0.003$, $r=-0.49$). Tendon volume was correlated to all AGEs measured ($p<0.05$, $r=0.43$-0.75). Serum CML concentrations were associated with increasing age, BMI, insulin, and markers of inflammation (IL-6 and TNF-a).

Discussion and conclusion:

Tendon pathology is often associated with increased tendon volume and reduced function. We demonstrated that several serum and clinical variables, i.e., serum AGEs, leptin, body mass index (BMI), age, and lipids are associated with in vivo tendon biomechanics and morphology. AGEs and other serum variables may serve as early predictors of tendon pathology in those with diabetes and metabolic disorders. We will expand these observations to a larger cohort and investigate the relationship of serum AGE in individuals with tendon pathology. With refinement, serum parameters could estimate injury risk, enhance early diagnosis, and reduce post-injury rehabilitation time.

Abstract 95: Impact of RAGE inhibitor administration on tendon biomechanical properties in a mouse model of type 2 diabetes

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Introduction:

Disruption of tendon extracellular matrix homeostasis and altered biomechanical properties result in substantial clinical challenges for millions of individuals with diabetes. Compounding the problem, improving blood glucose levels does not normalize tendon properties in those with diabetes. Advanced glycation end-products (AGEs) accumulate in the serum of individuals with diabetes via hyperglycemia, the intake of AGE-rich foods, and diminished kidney AGE clearance. Our primary aim was to determine if administration of a receptor for AGEs (RAGE) inhibitor would improve patellar tendon biomechanical properties in a mouse model of type 2 diabetes.

Materials and methods:

19 db/db mice with naturally elevated serum AGEs and impaired tendon function were treated daily with a RAGE inhibitor [Azeliragon (AZ), n=9] or vehicle (n=10) for three weeks. Structural and material properties were calculated from the ramp-to-failure. Stiffness and modulus were calculated from the linear portion of the load-displacement and stress-strain curves, respectively. This project was supported by NIH 1R01AR081967-01A1 to CCC and an IU School of Medicine Center for Diabetes and Metabolic Diseases Pilot and Feasibility Award. The Purdue University Animal Care and Use Committee approved this study (Protocol #: 1905001903).

Results:

Vehicle and AZ treatment groups were compared using a Mann-Whitney U test. Values were considered significant at an α level of $p<0.05$. All data are expressed as mean ± SE and analyzed using Prism 9.5.1 (GraphPad). Serum glucose was not different ($p>0.05$) between the groups (Vehicle: 787±80 mg/dl, AZ-Treated: 830±63 mg/dl). Further, serum insulin was not different between groups (Vehicle: 10±1 µg/L, AZ-Treated: 14±3 µg/L). Patellar tendon stiffness and modulus were greater ($p<0.05$) in mice receiving AZ (stiffness: 9.6±1.2 N/mm, modulus: 78.2±8.2 MPa) compared to vehicle (5.8±0.9 N/mm, modulus: 49.0±8.3 MPa). Maximum stress tended to be greater in the AZ group (Figure 2a, vehicle: 14.6±2.4, AZ: 23.3±2.9 N/mm2, $p=0.156$). Maximum load was not different between groups ($p>0.05$. Figure 1c). Maximum strain (vehicle: 0.9±0.1, AZ: 0.8±0.05) and toughness (vehicle: 6.1±1.4, AZ: 6.5±1.2 J·m−3) were not different between groups (Figure 2b and c, $p>0.05$).

Discussion and conclusion:

While additional work is needed to define the role of RAGE in regulating tendon properties, our preliminary results provide a premise for detailed mechanistic studies and the framework to evaluate therapeutic approaches to prevent tendon complications in people with diabetes through RAGE inhibition. Our data suggests that RAGE inhibition improves patella tendon biomechanics in a mouse model with elevated serum AGEs.

Abstract 96: Impact of serum advanced glycation end-products and RAGE inhibitor administration on patellar tendon healing in a mouse model

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Introduction:

We propose excess serum advanced glycation end products (AGEs) via activation of the receptor for AGEs (RAGE) is a novel mechanism contributing to poor tendon healing in diabetic individuals. AGEs accumulate in the serum of diabetic individuals via overnutrition and impaired clearance mechanisms. We determined if elevated serum AGEs would limit the recovery of tendon biomechanical properties. Further, we determined if administration of a RAGE inhibitor [Azeliragon (AZ)] could improve tendon mechanics.

Materials and methods:

Nine-week-old mice were injected daily with bovine serum albumin(BSA) with a control dimethyl sulfoxide (DMSO) (BSA-DMSO), n=6), BSA and 100µg/day AZ (BSA-AZ, n=5), 200 µg/mL glycated BSA (AGE) (AGE-DMSO, n=4), or (AGE) with AZ (AGE-AZ, n=6). A circular defect was created in both patellar tendons. After three weeks, the patellar tendon of one limb was extracted and tested for biomechanical characteristics.

Results:

Tendon stiffness and modulus were lower in AGE-treated mice ($p<0.05$, 10.8±1.4 N/mm and 28.0±7.0 MPa) compared to BSA-only (17.6±1.3 N/mm and 63.5±9.0 MPa). Further, tendon stiffness and modulus in AGE-treated mice given AZ were not different from AGE-BSA ($p>0.05$, 12.7±1.8 N/mm and 47.6±10.4 MPa). We found no statistically significant difference between groups in maximum load, stress, strain, or toughness ($p>0.0$).

Discussion and conclusion:

In this initial pilot cohort, we demonstrate that increasing serum AGEs in healthy mice impairs recovery of tendon biomechanical properties after injury. Treatment with AZ in the presence of elevated AGEs tended to improve tendon modulus ($p=0.139$). This data links serum AGEs to impaired tendon healing and supports our hypothesis that elevated serum AGEs, as seen with diabetes, could contribute to delayed tendon healing.