14th Triennial International Symposium on the Maillard Reaction (IMARS-14) - Protein glycation in food, health and disease
20th – 24th September 2021

Virtual conference on-line hosted from Doha, Qatar, by:

IMARS President, Dr Paul J Thornalley
Qatar Biomedical Research Institute (QBRI),
Hamad Bin Khalifa University (HBKU), Qatar Foundation,
Doha, Qatar

and

**Chair, IMARS-14 Organizing Committee,
Professor Naila Rabbani**
Department of Basic Medical Science, College of Medicine,
QU Health, Qatar University, Doha, Qatar

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Welcome Message from the President of IMARS

As President of IMARS, I would like to welcome you to the 14th Triennial International Symposium on the Maillard Reaction (IMARS-14) - Protein glycation in food, health and disease, 20th – 24th September 2021. IMARS-14 is a virtual on-line conference – a first for IMARS conferences. Although travel restrictions due to the current COVID-19 pandemic have prohibited us from meeting face-to-face at the conference, we can still come together on-line to present, share and discuss our latest research. I and other delegates look forward to viewing and listening to your presentations, welcome your questions and comments, and hope you have a great virtual conference experience.

IMARS is *the home of the glycation research community.* Together, we have a wealth of experience and know-how in techniques, models and study design in glycation research and data analysis, interpretation and impact. Please take advantage of this during your time at the conference. Submit questions and comments to presenters and chat on-line during the poster discussion session. Visit the virtual reception, Seminar Hall, Poster Hall and Trade Exhibition. Gain new insights, discover new concepts and explore new collaborations.

I will also be presenting and inviting input into the Doha Glycation Declaration 2021 – a statement of our vision for the future of glycation research. I pay tribute to the pioneers of the glycation research and invite you to help shape the future of glycation research. The Doha Glycation Declaration 2021 is included later in this booklet.

Enjoy the conference and let's share our glycation research.

Kind regards,

Prof Paul J Thornalley, President of IMARS & Member #IMARS14 Organizing Committee, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar. Email: Maillard.Society@gmail.com

As Chair of the Organizing Committee of IMARS-14, I welcome you to the virtual conference. The virtual conference website is a new experience for IMARS members and supporters. I hope you like it and enjoy your time in conference and virtual poster and exhibition halls. This year we encouraged local medical students to attend, present and write a conference report for IMARS Highlights as an outreach activity. I would like to thank the local and international organizing committee for their support.

Kind regards,

Professor Naila Rabbani, Chair, #IMARS14 Organizing Committee, Department of Basic Medical Sciences, College of Medicine, QU Health, Qatar University, Doha, Qatar

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Session 4 on Dicarbonyl stress in obesity and diabetes is presented in association with European Association for the Study of Diabetes (EASD) Reactive Metabolites Study Group (RMSG)

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Communication and engagement
Miss Aisha Nasser J M Al-Saei *(Research Assistant, College of Medicine, Qatar University, Qatar)*
We thank Miss Aisha Al-Saei for assisting in compiling, arranging and editing the conference Agenda and abstract booklet.

20th September 2021   **Day 1**

AGENDA

Time	**OPENING CEREMONY**

Time	
16-00	Reading from the Qur'an **Mr Ammar Humaidi** (*4th Year medical student, College of Medicine, Qatar University*)
16-05	Welcome and opening remarks **Prof Paul J Thornalley** President of IMARS (*QBRI/HBKU*) and **Prof Naila Rabbani** Chair IMARS-14 Organizing Committee (*Qatar University*)
16-20	Welcome by our sponsors, Qatar National Research Fund (QNRF) **Dr Fatemeh Darakhshan** (*Program Manager, Biomedical Science & Health, QNRF*)
16-30	**Prof Paul J Thornalley** (*QBRI/HBKU*) **Doha Glycation Declaration 2021- Grand challenges in glycation research** Invitation for input

Time	Speaker name	Speaker topic

Session 1

	Breakthrough research: Unfolded protein response	
	Moderators: Prof Naila Rabbani (*Qatar University, Qatar*) and Prof Paul J Thornalley (*QBRI/HBKU*)	
16-45	**Keynote Speaker:**	
	Prof Kazutoshi Mori, Lasker laureate (*Kyoto University, Japan*)	Dynamics of function and regulation of the endoplasmic reticulum
17-30		End of Session

Notes

20th September 2021   **Day 1**

AGENDA

Time	Speaker name	Speaker topic
Session 2		

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Diabetes and diabetic complications

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Moderators: Prof Naila Rabbani (*Qatar University, Qatar*) and Prof Shahrad Taheri (*Hamad Medical Corporation, Qatar*)

Time	Speaker name	Speaker topic
17-30	**Prof Abdul-Badi Abou-Samra** (*Metabolic Research Institute, Hamad Medical Corporation, Qatar*)	Qatar Diabetes Prevention Program (QDPP)
18-00	**Prof Mark Cooper** (*Monash University, Australia*)	Transactivation of the Receptor for Advanced Glycation Endproducts mediating pro-inflammatory signaling in diabetes
18-30	**Prof Rayaz Malik** (*Weill-Cornell Medical College, Qatar, Qatar*)	Challenging the Dogma in Diabetic Neuropathy
19-00	**Prof Ann Marie Schmidt** (*New York University, USA*)	RAGE/DIAPH1: Molecular Mechanisms and Therapeutic Strategies in Obesity and Diabetic Complications
19-30	**Assoc Prof Melinda Coughlan** (*Monash University, Australia*)	Targeting the gut to reduce AGE-mediated damage in diabetes
20-00	Day 1 – Plenary session close	

Notes

21st September 2021   **Day 2**

AGENDA

Time	Speaker name	Speaker topic
15-00 – 16-00	**Pre-session on-line poster discussion session 1 (Posters 1 – 22)**	

Session 3

Glycation in food: innovation for a healthy diet

Moderators: Prof Hiba Bawadi *(Qatar University, Qatar)* & Prof Monika Pischetsrieder *(University of Erlangen-Nuremberg, Germany)*

16-00	**Prof Vincenzo Fogliano** (*University of Wageningen, The Netherlands*)	Glycation and development of healthier foods
16-30	**Prof Thomas Henle** (*Technical University of Dresden, Germany*)	Manuka honey and its unique glycation chemistry
17-00	**Prof Fred Tessier** (*University of Lille, France*)	Does the quote "*Sola dosis facit venenum*" apply to the physiological effects of glycation adducts?

Short talks

17-30	**Dr Gosia Teodorowicz** (*University of Wageningen, The Netherlands*)	Immunogenicity and allergenicity of glycated cows' milk proteins
17-40	**Dr Clemens Kanzler** (*Technische Universität Berlin, Germany)*	Structure of melanoidins formed in the Maillard reaction of methylglyoxal with L-alanine or L-lysine
17-50	**Ruth Fabiola Peña-Correa** (*University of Wageningen, The Netherlands*)	Fluidized bed roasting drives Maillard reactions toward A more aromatic cocoa
18-00		End of Session

Notes

21st September 2021   **Day 2**

AGENDA

Time	Speaker name	Speaker topic

Session 4

Dicarbonyl stress in obesity and diabetes
(with EASD Reactive Metabolites Study Group)

Moderators: Prof Reiko Inagi (University of Tokyo, Japan) and Prof Vincent Monnier (Case Western Reserve University, USA)

Time	Speaker name	Speaker topic
18-00	**Prof Casper G. Schalkwijk** (*Maastricht University, The Netherlands*)	Methylglyoxal stress in obesity and (risk of) type 2 diabetes
18-30	**Prof Jacob Haus** (*University of Michigan, USA*)	Dicarbonyl stress in insulin resistance and effects of exercise interventions
19-00	**Prof Bernd Stratmann** (*Ruhr-University Bochum, Germany*)	Dicarbonyl stress in diabetic vascular disease
	Short talks	
19-30	**Dr Kim Maasen** (*Maastricht University, The Netherlands*)	Higher habitual intake of dietary dicarbonyls is associated with higher concentrations of corresponding plasma dicarbonyls and with skin autofluorescence: the Maastricht Study
19-40	**Prof Naila Rabbani** (*Qatar University, Qatar*)	Dynamics of hexokinase-2 linked glycolytic overload driving dicarbonyl stress and endothelial cell dysfunction in high glucose concentration *in vitro*
19-50	**Dr Mathias Van den Eynde** (*Maastricht University, The Netherlands*)	Pyridoxamine reduces glycation and markers of endothelial function in a placebo-controlled intervention trial with abdominally obese individuals
20-00		Day 2 – Plenary session close

20-00 – 20-30 Virtual Trade Exhibition
(Exhibitors available on-line for inquiries and discussion)

<u>Notes</u>

22nd September 2021   **Day 3**

AGENDA

Time	Speaker name	Speaker topic
15-00 – 16-00	**Pre-session on-line poster discussion session 2 (Posters 23 – 44)**	

Session 5

Glycation in through the life course – from maternal bonding to aging

Moderators: Prof Ann Marie Schmidt *(New York University, USA)* and Assoc Prof Melinda Coughlan *(Monash University, Australia)*

Time	Speaker name	Speaker topic
16-00	**Prof Yasuhiko Yamamoto** *(Kanazawa University, Japan)*	RAGE and maternal bonding – an expected mechanism relationship
16-30	**Prof Vincent Monnier** *(Case Western Reserve University, USA)*	Ascorbic acid as a glycating agent in the aging lens
17-00	**Prof Allen Taylor** *(Tufts University, USA)*	Glyoxalase 1 activity declines with age in many tissues
	Short talks	
17-30	**Selena Le Bagge** *(University of Queensland, Australia)*	Short term intraperitoneal administration of mammalian cell derived human soluble receptor for advanced glycation end products (sRAGE) prevents type 1 diabetes onset in mice
17-40	**Dr Michael Howsam** *(University of Lille, France)*	High hydrostatic pressure processing of human milk avoids the formation of Maillard reaction products and preserves oligosaccharides
17-50	**Armand Linkens** *(Maastricht University, The Netherlands)*	Effect of a 4-week diet low and high in AGEs on insulin sensitivity and secretion, vascular function, and markers of low-grade inflammation and endothelial dysfunction of abdominally obese individuals – preliminary results from the deAGEing trial
18-00		End of Session

Notes

22nd September 2021   **Day 3**

AGENDA

Time	Speaker name	Speaker topic

Session 6

Glycation in plants – physiology, function and food security.
Glycation in the COVID-19 response

Moderators: Dr Talaat Youssef (*Environmental Science Center, Qatar University, Qatar*) and Prof Vincenzo Fogliano *(University of Wageningen, The Netherlands)*

Glycation in plants – physiology, function and food security

Time	Speaker name	Speaker topic
18-00	**Prof Chikahiro Miyake** (*Kobe University, Japan*)	Production mechanism of methylglyoxal and its reactivity with oxygen to produce reactive oxygen species in illuminated chloroplasts of plant leaves
18-30	**Prof Andrej Frolov** (*St. Petersburg State University, Russia & Leibnitz Institute of Plant Biochemistry, Halle, Germany*)	Glycation of plant proteins – a step forward to understanding the biological role
19-00	**Prof Sneh L. Singla-Pareek** (*International Centre for Genetic Engineering and Biotechnology, India*)	Glyoxalases: the antidote for methylglyoxal and plant stress

Short talks

Time	Speaker name	Speaker topic
19-30	**Dr Alena Soboleva** (*St. Petersburg State University, Russia & Leibnitz Institute of Plant Biochemistry, Halle, Germany*)	Drought-related changes in pea root nodule metabolome

Glycation in the COVID-19 response

Time	Speaker name	Speaker topic
19-40	**Israa ElBashir** (*Qatar University, Qatar*)	Evaluation of MG antiviral activity against SARS-CoV-2
19-50	**Prof Paul J Thornalley** (*QBRI/HBKU, Qatar*)	Anti-inflammatory activity of glyoxalase-1 inducer, *trans*-resveratrol and hesperetin, in human small airway epithelial cell primary cultures support application for prevention of COVID-19.
20-00		Day 3 – Plenary session close

20-00 – 20-30 Virtual Trade Exhibition
          (Exhibitors available on-line for inquiries and discussion)

23rd September 2021   **Day 4**

AGENDA

Time	Speaker name	Speaker topic

15-00 – 16-00   **Pre-session on-line poster discussion session 3 (Posters 45 – 67)**

Session 7

Glycation analytics and chemistry

Moderators: Prof Fred Tessier *(University of Lille, France)* and Prof Thomas Henle *(Technical University of Dresden, Germany)*

16-00	**Prof Monika Pischetsrieder** *(University of Erlangen-Nuremberg, Germany)*	Detection of reactive carbonyl intermediates of glycation
16-30	**Prof Ryoji Nagai** *(Tokai University, Japan)*	Measurement of AGEs using LC-MS/MS, immunoassay and skin autofluorescence
17-00	**Prof Varoujan Yaylayan** *(McGill University, Canada)*	Mechanochemistry: Maillard Reaction "Through the Looking Glass"
	Short talks	
17-30	**Prof Marcus Glomb** *(Martin-Luther University of Halle, Germany)*	Update to the N_6-carboxymethyl lysine story
17-40	**Prof Michael Hellwig** *(Technical University Braunschweig & Technical University Desden, Germany)*	Enzymatic decarboxylation of N_ϵ-carboxymethyl-lysine by ornithine decarboxylases reveals underground metabolism as a route for in vivo processing of glycated amino acids
17-50	**Dr Alberto Fiore** *(University of Abertay, United Kingdom)*	Polyphenols as trapping agents of reactive carbonyl species: new strategy to reduce harmful compounds in e-cigarette emissions
18-00		End of Session

Notes

23rd September 2021   **Day 4**

AGENDA

Time	Speaker name	Speaker topic

Session 8

Glycation in kidney disease, cancer and mental health

Moderators: Dr Mariam Al-Muftah *(QBRI/HBKU)* and Prof Casper G. Schalkwijk *(Maastricht University, The Netherlands)*

Time	Speaker name	Speaker topic
18-00	**Prof Reiko Inagi** (*University of Tokyo, Japan*)	Organelle stress and metabolic derangement in kidney disease
18-30	**Prof Matthew Vander Heiden** (*MIT, USA*)	Methylglyoxal metabolism is a targetable liability of glycolytic metabolism in cancer
19-00	**Prof Makoto Arai** (*University of Tokyo, Japan*)	Glycation and mental health: schizophrenia
	Short talks	
19-30	**Prof Paul J Beisswenger** (*Dartmouth University, USA*)	Multiple outcome studies confirm predictive value of AGEs for diabetic kidney disease
19-40	**Dr Muhanad Alhujaily** (*Bisha University, Kingdom of Saudi Arabia*)	The spliceosome is a target for glycation in methylglyoxal-induced apoptosis and is shielded by glyoxalase 1 in multidrug resistance-linked cancer chemotherapy.
19-50	**Leigh Donnellan** (*University of South Australia, Australia*)	Methylglyoxal targets proteins involved in mitotic fidelity
20-00		Day 4 – Plenary session close

20-00 – 20-30 Virtual Trade Exhibition
(Exhibitors available on-line for inquiries and discussion)

Notes

24th September 2021   **Day 5**

AGENDA

Time	Speaker name	Speaker topic

Session 9

Imaging, diagnostic algorithms and therapeutics

Moderators: Prof Ryoji Nagai *(Tokai University, Japan)* and Prof Bernd Stratmann *(University of the Ruhr-Bochum, Germany)*

Time	Speaker name	Speaker topic
16-00	**Prof Chunyong Ding** *(Shanghai Jiaotong University, China)*	Design and synthesis of near-infrared fluorescent probe targeting tumour metabolite methylglyoxal for visualization study
16-30	**Prof Naila Rabbani** *(Qatar University, Qatar)*	Glycation and machine learning – diagnostic algorithms for diabetes, arthritis and autism
17-00	**Prof Paul J Thornalley** *(QBRI/HBKU, Qatar)*	Glycation based therapeutics: Glo1 inducers and Glo1 inhibitors. COVID-19 repurposing
18-00		End of Session

Notes

24th September 2021   **Day 5**

AGENDA

Time	Speaker name	Speaker topic

Finale and Close

Methods and models in glycation research – Qatar glycation collaboration

Moderators: Moderators: Prof Naila Rabbani *(Qatar University, Qatar)* and Prof Paul J Thornalley *(QBRI/HBKU)*

Time	Speaker name	Speaker topic
17-30	**Dr Sowndramalingam Sankaralingam** (*Qatar University, Qatar*)	Measurement of glyoxalase activities
17-45	**Maryam Al-Motawa** (*QBRI/HBKU, Qatar*)	Genetics of glycation - glycated hemoglobin, GLO1 single nucleotide polymorphisms and copy number variation
18-00	**Prof Paul J Thornalley (**(*QBRI/HBKU, Qatar*)	Assay of glyoxalase metabolites: methylglyoxal, S-D-lactoylglutathione and D-lactate
18-15	**Prof Naila Rabbani** (*Qatar University, Qatar*)	Quantitation of glycation adducts by stable isotopic dilution analysis LC-MS/MS
18-30	**Dr Patrick Wijten** (*QBRI/HBKU, Qatar*)	Proteomics of glycation
18-45	**Dr Mingzhan Xue** (*QBRI/HBKU, Qatar*)	Application of functional genomics in studies of dicarbonyl stress
19-00	**Dr Alberto da la Fuente** (*QBRI/HBKU, Qatar*)	Mathematical modelling of dicarbonyl stress
19-15	**Dr Hebah Al Khatib** (*Qatar University, Qatar*)	Glycation in drug repurposing for COVID-19
19-30	**Dr Essam Abdelalim** (*QBRI/HBKU, Qatar*)	Inducible pluripotent stem cells (iPSCs) application in diabetes and glycation research

24th September 2021   **Day 5**

AGENDA

Time	Speaker name	Speaker topic

FINALE AND CLOSE

19-45	**Prof Paul J Thornalley** (IMARS President, *QBRI/HBKU, Qatar*)	• Prize awards for best presentations • Grand challenges in glycation research - Doha Glycation Declaration 2021 • IMARS – Change of President

Day 5 - Conference Close and Farewell

IMARS-14 conference logo © Jarape

Draft

Doha Glycation Declaration 2021

"*We, the undersigned - glycation researchers at IMARS-14, identify the following areas for priority advance in glycation research:*

1. Research in glycation-related analytical techniques and chemistry
2. Research in food processing for healthier, safe and nutritious food
3. Research in glycation-resistant crops for improved food security in climate change
4. Research on clinical diagnostics for improved diagnosis, risk prediction and therapeutic monitoring of health conditions and disease
5. Research on therapeutics for improved treatment of disease – including COVID-19"

Your suggestions for amendment and additions are welcome. Your support to record our vision for the future of glycation research is heartily invited.

Contact email: Maillard.Society@gmail.com

IMARS-14 ABSTRACTS

Other

Keynote presentation

PRESENTING AUTHOR: Lasker laureate, Professor Kazutoshi Mori

Title: Dynamics of Function and Regulation of the Endoplasmic Reticulum
Authors: Kazutoshi Mori
Affiliations: Department of Biophysics, Graduate School of Science, Kyoto University, Japan

The endoplasmic reticulum (ER), where newly synthesized secretory and transmembrane proteins are folded and assembled, has the ability to discriminate folded proteins from unfolded proteins and controls the quality of synthesized proteins. Only correctly folded molecules are allowed to move along the secretory pathway, whereas unfolded proteins are retained in the ER.

The ER contains a number of molecular chaperones and folding enzymes (ER chaperones hereafter), which assist productive folding of proteins, and therefore newly synthesized proteins usually gain correct tertiary and quaternary structures quite efficiently. Yet unfolded or misfolded proteins even after assistance of ER chaperones are retrotranslocated back to the cytosol, ubiquitinated and degraded by the proteasome. This disposal system is called ER-associated degradation (ERAD). Thus, the quality of proteins in the ER is ensured by two distinct mechanisms, productive folding and ERAD, which have opposite directions.

Under a variety of physiological and pathological conditions collectively termed ER stress, however, unfolded or misfolded proteins accumulate in the ER, which in turn activates ER stress response or Unfolded Protein Response (UPR). The UPR is mediated by transmembrane proteins in the ER, and three ER stress sensors/transducers, namely IRE1, PERK and ATF6, operates ubiquitously in mammals. Thanks to these signaling pathways, translation is generally attenuated to decrease the burden on the folding machinery; transcription of ER chaperones is induced to augment folding capacity; and transcription of components of ERAD machinery is induced to enhance degradation capacity, leading to maintenance of the homeostasis of the ER. If ER stress sustains, cells undergo to apoptosis.

I will talk on the mechanism, evolution, and physiological importance of the UPR as well as its involvement in development and progression of various diseases.

Invited Speaker 1

Title: The Qatar Diabetes Prevention Program (QDPPP).
Authors: Abdul-Badi Abou-Samra
Affiliations: *Qatar Metabolic Institute, Hamad Medical Corporation, Doha, Qatar*.

Qatar is among of the countries with high prevalence of type 2 diabetes mellitus (T2D); this places a significant burden on future health, economy and development. The Qatar Diabetes Prevention Program (QDPP) is a multi-institutional research collaboration, combining the range of scientific and clinical expertise in Qatar, to predict, prevent and reverse T2D in Qatar by targeting those at increased diabetes risk (pre-diabetes [PreD] and gestational diabetes mellitus [GDM]), or those with early T2D.   QDPP is a flagship program leveraging existing national initiatives to answer key questions critical to population health while driving development across the health sector to deliver targeted interventions. QDPP will harness and integrate into various national resources and initiatives.

i.   The Qatar National Diabetes Strategy plans a systematic national screening for T2D within the next 2 years. The screening identifies individuals with PreD, and those with early T2D.

ii. A universal GDM screening has been implemented by the national GDM guideline; this showed that nearly 40% of pregnant women develop GDM.

iii. Qatar is unique in that pre-marital screening is mandatory for those intending marriage; this identifies young women at risk of GDM and T2D.

iv. The Primary Health Care Corporation (PHCC) wellness centers facilitate the lifestyle interventions proposed in QDPP.

v.   The Qatar Biobank (QBB) cohort includes individuals with PreD (about 20%) and newly-diagnosed early T2D. QBB participants are extensively phenotyped, including genomic and biochemical profiling.

vi. A national eHealth and Data Management Strategy is being developed for Qatar which focuses on facilitating public and healthcare providers interaction with the Qatar health system.

QDPP leverages the potential of the above-mentioned activities and cohorts to acquire a comprehensive understanding of T2D in Qatar and to identify the most effective approaches to prevent the disease, based on behavioral, lifestyle and medical interventions while discovering genetic and molecular markers of risk and treatment response.

The QDPP cluster includes seven sub-projects: four are clinical trials, three of which target individuals at risk for T2D and a fourth that targets subjects with early T2D; two sub-projects explore the QBB cohort to identify genetic, proteomic and metabolomic markers for PreD and progression to T2D; and one cross-cutting project incorporates eHealth to support the four clinical trials.

QDPP research shall provide for Qatar, in a high-risk population, important answers regarding approaches to prevent progression of diabetes risk factors and pre-diabetic condition into T2D and best approach to reverse early T2D. The QDPP research will have a major positive long-term impact on the health and economy of Qatar.

Invited Speaker 2

Title: Transactivation of the Receptor for Advanced Glycation End-products Mediating Pro-inflammatory Signaling in Diabetes

Authors: Mark E Cooper[1*] and Merlin C Thomas[1]

Affiliations: [1]Department of Diabetes, Central Clinical School, Monash University, Melbourne, Australia

Upregulation and activation of the Receptor for Advanced Glycation End-products (RAGE) has been clearly implicated in the development of diabetic complications, including accelerated atherosclerosis, kidney and eye disease. In each case, dysfunction has been previously ascribed to activation of RAGE by ligands including Advanced Glycation End-products (AGEs) and calgranulins/S100 proteins that trigger Nuclear Factor κB (NF-κB)-driven pro-inflammatory gene expression. However, we have recently demonstrated a ligand-independent mechanism for activation of cytosolic tail of RAGE following activation of co-complexed G-protein coupled receptors leading to pro-inflammatory signaling. This *transactivation* is independent to the liberation of RAGE-ligands or the ligand-binding extracellular domain of RAGE, so is not blocked by soluble RAGE or RAGE neutralizing antibodies. A number of different GPCRs can transactivate RAGE and trigger proinflammatory signaling, including the AT1 receptor, CCR2 and CXCR2, meaning that these physiological signaling pathways can feed into pathogenic signaling following upregulation of RAGE following injury, inflammation or hypoxia. The importance of RAGE transactivation pathway is demonstrated by the observation that *Ager* knockout (KO) mice lose their protection against diabetic complications following restoration of the cytosolic tail of RAGE, suggesting that ligand-independent transactivation is the major pathway of RAGE activation in vivo. This is also consistent with recent data suggesting that RAGE ectodomain is effectively ligated *in vivo* via the association of the RAGE ectodomain with charged glycans in the surrounding matrix. Blocking RAGE transactivation is therefore a key target for the prevention and treatment of diabetic complications. As proof of concept, we show that selective inhibition of RAGE transactivation attenuates atherogenesis and kidney damage in diabetic mice. This pathway is broadly significant as inhibition of RAGE transactivation also inhibits smoke induced inflammation in mice.

Invited Speaker 3

Title: Challenging the Dogma in Diabetic Neuropathy

Authors: Professor Rayaz Malik
Affiliations: Weill Cornell Medicine-Qatar, Doha, Qatar

Peripheral Neuropathy (PN) can occur in children with T1DM, subjects with impaired glucose tolerance and adults with T1DM and T2DM. Hyperglycemia and cardiovascular risk factors are associated with the development and progression of DPN. Currently advocated tests of DPN e.g., neurological examination, vibration perception and monofilament testing identify established neuropathy and miss early neuropathy and have proven to be inadequate in multiple clinical trials of new therapies for DPN.

Early recognition using sensitive and objective measures of small fibre neuropathy with timely management of glycaemia and vascular risk factors is key to preventing the development and progression of DPN. Furthermore, the lack of FDA approved disease modifying therapies may be attributed to an inability to translate highly positive experimental studies through the deployment of inadequate neuropathy endpoints to assess efficacy. New biomarkers for nerve repair and better patient stratification are key to securing FDA approval of new therapies and advance the management of DPN.

Corneal confocal microscopy is a rapid, non-invasive small fibre imaging technique which can identify early small fibre damage and has been used as an endpoint in clinical trials to show early nerve repair after an improvement in glycemia, lipids, exercise, and bariatric surgery. CCM may allow enrichment of participants in clinical trials and identify early nerve repair following intervention with new treatment options for DPN.

Invited Speaker 4

Title: RAGE/DIAPH1: Molecular Mechanisms and Therapeutic Strategies in Obesity and Diabetic Complications

Authors: Ann Marie Schmidt
Affiliations: Diabetes Research Program, Department of Medicine, New York University Grossman School of Medicine, New York, New York, U.S.A.

The Receptor for Advanced Glycation End Products (RAGE), discovered on account of its ability to bind the advanced glycation end products, is a multi-ligand receptor that also binds pro-inflammatory S100/calgranulins and amphoterin (also known as high mobility group box 1 (HMGB1)). RAGE is also known as a receptor for DAMPs, or Damage Associated Molecular Pattern molecules. In animal models, through studies testing genetic modulation of RAGE and pharmacological interventions, RAGE has been shown to play key roles in the pathogenesis of macro- and microvascular complications of diabetes. In obese non-diabetic human and murine adipose tissue, the observed accumulation of AGEs led to studies which revealed that RAGE contributes to high fat diet-induced obesity in mice through suppression of energy expenditure, at least in part through AGE-dependent downregulation of thermogenic gene expression in adipocytes. The cytoplasmic domain of RAGE binds to the formin DIAPH1 and biochemical, molecular, signal transduction and *in vivo* studies suggest that DIAPH1 is important for RAGE functions. Studies are accruing to illustrate that in obesity and diabetes, mice devoid of *Diaph1* demonstrate similar protections to those noted upon *Ager* deletion in murine models of these disorders.

Recent reports are uncovering complex roles for RAGE/DIAPH1 in the innate immune response and the interface with diabetes- associated complications. Experiments illustrating that deletion of *Ager* enhances regression of atherosclerosis in diabetic mice uncovered potential roles for RAGE-dependent regulation of macrophage *Irf7* (Interferon Regulatory Factor 7) in exacerbation of maladaptive cholesterol metabolism and inflammatory signaling in these cells; processes predicted to contribute to atherosclerosis. As IRF7 is a critical regulator of the anti-viral innate immune response, its upregulation by RAGE and its ligands (such as AGEs) suggests critical intersections between innate immune responses and inflammatory mechanisms that characterize chronic diseases such as obesity and diabetes.

Finally, novel therapeutic opportunities have been uncovered based on experimental evidence indicating that the multiple ligands of RAGE may bind at distinct sites on the three extracellular immunoglobulin-like domains. On account of these observations, our recent work has focused on the development of novel antagonists that interrupt the binding of the RAGE cytoplasmic domain with DIAPH1. First studies illustrate that these high affinity antagonists reduce diabetes-like kidney disease in types 1 and 2 diabetes models; improve wound healing in type 2 diabetic-like mice; and reduce myocardial infarction induced by transient ligation and reperfusion of the left anterior coronary artery in type 1-like diabetic mice. Work is underway to identify candidates for testing in clinical trials.

In summary, dissection of the biology of the AGE-RAGE-DIAPH1 pathway has uncovered key roles for glycation and its receptor axis in chronic disorders typified by immunometabolic dysfunction and identified novel opportunities for therapeutic intervention.

Invited Speaker 5

Title: Targeting the gut to reduce AGE-mediated damage in diabetes.
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Intake of processed foods, high in advanced glycation end products (AGEs) has increased markedly over the past decades, coinciding with increased microvascular diseases such as chronic kidney disease (CKD) and diabetes. Here, we show in rodent models that long-term consumption of a heat-treated, high AGE diet drives intestinal barrier permeability and an increased risk of CKD. Consequently, a high AGE diet leads to innate immune complement activation and local kidney inflammation and injury via the potent proinflammatory effector molecule complement 5a (C5a). Inhibition of the complement pathway, by pharmacological targeting of the C5a receptor (C5aR1) improved kidney injury. In a mouse model of diabetes, targeting the gut microbiota using a high resistant starch fiber diet led to a redistribution of the gut commensal consortium, maintained gut barrier integrity and decreased severity of kidney injury via suppression of complement. These studies provide mechanistic insight into the role of high AGE diets on inflammation and risk for diabetic kidney disease and provide the basis for future translational studies using gut-targeted therapies.

Invited Speaker 6

Title: Glycation and development of healthier foods.
Authors: Vincenzo Fogliano.
Affiliations: Food Quality & Design group, Wageningen University, The Netherlands.

Non enzymatic glycation is surely harmful *in vivo*, however the physiological relevance of MR has multiple outcomes. Is the consumption of food rich in melanoidins and dietary advanced glycosylation end-products (dAGEs) harmful or beneficial for human health? This is still an unanswered burning question. Melanoidins, the polymer constituting the final product of MR in food, have positive functions particularly within the gastrointestinal tract, whereas the intake of small molecular weight dAGEs has controversial physiological consequences. The restriction of dAGEs has been proposed as a strategy for alleviating complications of diabetes and for a healthy lifestyle. Although this is plausible, further efforts must be taken to discover solid evidence to elucidate the mechanisms. Many evidence suggests that the damages produced by the heavily processed foods used in the Western diet could be due to the increased intake of some dAGEs but also to other concomitant factors. The current processing and formulation strategies lead to a high concentration of melanoidins and dAGEs in foods having also high energy density and whose consumption is harmful for human health. For this reason, observational studies cannot disentangle the specific physiological effects of melanoidins and dAGEs from the general negative effects linked to heavily processed, refined, calorie-dense foods which are now also indicated with the notation ultra-processed foods. In this lecture some examples of how glycation can be used in food design to obtain foods promoting specific health functions.

Invited Speaker 7

Title: Manuka honey and its unique glycation chemistry
Authors: Thomas Henle
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Manuka honey is a monofloral honey originating from the nectar of the manuka tree (Leptospermum scoparium) in New Zealand. The nectar of manuka blossoms contains high amounts of dihydroxyacetone (DHA), which is non-enzymatically converted to methylglyoxal (MGO) during honey maturation. Commercial manuka honeys may contain high concentrations of 1,2-dicarbonyl compounds, ranging from 300 to 800 mg/kg for 3-deoxyglucosulose (3-DG) and up to 1000 mg/kg for MGO, respectively. Due to high concentrations of MGO, manuka honey has an exceptional antibacterial activity, which is considered as a characteristic property from a commercial point of view. During storage, glycation reactions between MGO and amino acids or proteins present in the honey matrix lead to the formation of amino acid derivatives such as the flavor compound 2-acetylpyrroline (formed from proline) as well as peptide-bound adducts such as N-ε-carboxyethyllysine (CEL) and MG-H1, which can be used to prove the reliability of labeled MGO levels in honeys and possibly enable the detection of fraudulent MGO or DHA addition to honey.
With a usually ingested daily amount of 10-20 g honey, dietary intake may reach values up to 10 to 20 mg per day each for 3-DG and MGO. Manuka honey high in 3-DG and MGO, therefore, can be used as a model food to study the metabolic transit of 1,2-dicarbonyl compounds in humans. No influence on the excretion of MGO or its physiological metabolite D-lactate in urine was observed following oral administration of MGO, indicating that dietary MGO is rapidly degraded during the digestion process in the intestine and, therefore, exerts no influence on the MGO level in vivo. During simulated gastrointestinal digestion, a rapid decrease of MGO in the presence of proteins, accompanied by the formation of MG-H1was observed, indicating that dietary MGO induces the endogenous formation of amino acid adducts during the digestion process. In contrast to MGO, administration of 3-DG-containing honey led to a significant increase of 3-DG and its metabolites 3-deoxyfructose (3-DF) and 2-keto-3-deoxygluconic acid (3-DGA) in the urine of the subjects, with 3-DF as main product of the metabolism of dietary 3-DG.

Invited Speaker 8

Title: Does the quote "*Sola dosis facit venenum* » apply to the physiological effects of glycation adducts?

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There have been many studies over the past 40 years on the effects of dietary glycation products (dAGEs) and other Maillard reaction products and over time the results often appear contradictory. This has brought about controversial interpretations, conclusions and recommendations. In the early 16th century, Paracelsus was the first scientist to observe that "*only the dose makes the poison*". Even if this concept is nowadays challenged by modern toxicology, the dose effect remains valid in many cases.

The absorption kinetics, the metabolism, the elimination and the physiological effects of dAGEs are observed and measured using cell culture, *in vitro* digestive model systems, animal models and, human studies. Is it possible then that the exposure doses used in the different model systems are responsible for the discrepancies between studies?

Here, we present 5 hot topics for which no consensus has been reached.

- First of all, the low digestibility of glycated proteins is a commonly-shared observation within our group and other scientists who have tested different forms of such proteins in *in vitro* digestive systems. However, animal and clinical studies do not confirm this observation. In this case the controversy appears to be more related to the limits of the *in vitro* models than the doses used.

- The next matter is the difference in the kinetic of absorption of dietary carboxymethyllysine (dCML) observed between mice and humans which might be explained by a difference of exposure. In addition, the low correlation between dietary CML and circulation free CML observed in humans could be due partly to the narrower range of exposure observed among humans (20 to 200 µg/kgBW/day) compared to animals (40 to 70 000 µg/kgBW/day).

- One of our mice experiments has shown that a long-term dCML exposure induces an endothelial dysfunction and accelerates the development of arterial aging. However the results of the epidemiologic Maastricht Study did not confirm such effect of dCML. Here again the difference between the highest exposure observed in human and the one tested in mice can alone explain the apparent discrepancy.

- A difference of RAGE-mediated inflammatory and oxidative responses from one study to another can also be due to a difference of dose tested to a cell line. Indeed, concentrations of glycated proteins applied to different cell cultures vary greatly from one study to another (from 10 to 1000 µg/mL).

- Finally, whenever a biological explanation is given to justify the toxicity of dAGEs, the interaction between dAGEs and RAGE is usually presented as the well proven and only mechanism. However, this hypothesis is far from being verified even with the most sophisticated transgenic mouse models. The dAGEs-RAGE interaction hypothesis does not seem to hold water when we consider that dAGEs reach the circulation only as free dAGEs (not protein bound dAGEs), and that free dAGEs are not ligands of RAGE. The only possible interaction between protein- or peptide-bound dAGEs and RAGE may be observed in the gut. But here again the activation of inflammatory and oxidative pathways on enterocytes still needs to be definitely proven.

The 5 examples of controversies presented above indicate clearly that the dose makes the biological effect or at least its statistical significance. However it is not the only parameter that could explain the difference among studies. The type of diet and protein tested, the degree and type of glycation and the duration of exposure are among other crucial parameters that can affect the results and the interpretation.

In view of all of this and without strong scientific evidence we are far from being in a position to offer either nutritional recommendations to the population or dAGE limits in food to the food industry.

Invited Speaker 9

Title: Methylglyoxal stress in obesity and (risk of) type 2 diabetes

Authors: Casper G. Schalkwijk
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There is now considerable scientific evidence from experimental and preclinical research that increased formation of the reactive dicarbonyl methylglyoxal by hyperglycemia enhances the development of vascular complications in diabetes. Inflammation and hypoxia may also be major determinants of methylglyoxal formation, since under these conditions the expression and/or activity of glyoxalase 1, the rate-liming enzyme in the glyoxalase pathway, is impaired. This explain why methylglyoxal is associated not only with diabetes and its complications, but also with several other age-related chronic inflammatory diseases such as atherosclerosis, obesity, hypertension and disorders of the central nervous system. Methylglyoxal is the major precursor of non-enzymatic glycation of proteins and DNA and methylglyoxal and methylglyoxal-derived AGEs can impact on organs and tissues affecting their functions and structure.

There is also emerging evidence that methylglyoxal is a modulator of insulin resistance. The accumulation of methylglyoxal in adipose tissue increases adipose tissue inflammation, and may contribute to insulin resistance and the development of type 2 diabetes. In addition, structural and functional abnormalities of the insulin molecule by methylglyoxal may contribute to the pathogenesis of insulin resistance. Methylglyoxal also interferes in the complex molecular pathways of insulin signalling in muscle cells, endothelial cells and B-cells and is as such directly linked to insulin resistance. Thus, methylglyoxal is not only a reflection of the flux of hyperglycemia in diabetes but may itself induce insulin resistance and thus predispose to type 2 diabetes. Although interventions to treat methylglyoxal-associated complications are not yet available in the clinical setting, several strategies to lower methylglyoxal have been developed over the years. Targeting methylglyoxal burden may provide new therapeutic applications to mitigate diseases in which methylglyoxal plays a crucial role such as type 2 diabetes and its complications.

Invited Speaker 10

Title: Dicarbonyl stress in insulin resistance and effects of exercise interventions
Authors: Jacob M. Haus and Edwin R. Miranda
Affiliations: School of Kinesiology, University of Michigan

Glyoxalase I (GLO1) is the primary enzyme for detoxifying the reactive dicarbonyl methylglyoxal. Pre-clinical models demonstrate that loss of GLO1 promotes the development of diabetes in obesogenic conditions which may be related to the altered redox state and subsequent accumulation of acetylation on GLO1. However, there is limited clinical data examining the relationship between obesity and GLO1 expression especially in tissues of high relevance to glucose regulation such as skeletal muscle. Our investigations reveal that GLO1 is attenuated in the skeletal muscle of individuals with obesity and insulin resistance, and we examine the role of acute and chronic aerobic exercise interventions on GLO1 acetylation, protein abundance and gene expression. Further we explore the hypothesis that the pseudo-hypoxic state that limits NAD+ bioavailability, and SIRT1 activity, with obesity and insulin resistance, may promote GLO1 acetylation and degradation. The loss of GLO1 in muscle may be an early molecular event during obesogenesis that leaves the muscle susceptible to dicarbonyl stress and eventually culminates in insulin resistance.

Invited Speaker 11

Title: Dicarbonyl stress in diabetic vascular disease
Authors: Bernd Stratmann
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Methylglyoxal (MGs) and its advanced glycated end products (AGEs) are associated with diabetes late complications including vascular dysfunction and end organ damage. Research in this area has proven a central role for reactive glucose metabolites like MG resulting from hyperglycemia or reduced detoxification via the glyoxalase system. MG accumulates quickly in various tissues and due to its high reactivity forms AGEs more rapidly than glucose does. Thus, MG is a highly potent precursor of AGEs.

Cellular apoptosis, oxidative stress, inflammation, and AGE formation are responsible mechanisms resulting in vascular endothelial cell malfunction and finally in endothelial dysfunction (ED). ED is regarded as the first step in the initiation, progression and clinical outcome of vascular complications.

The vascular endothelium is characterised by a distinctive balance between autocrine and paracrine mechanisms that regulate vascular homeostasis, resulting in regulation of vascular tone, cell-cell interactions, permeability and coagulation by extracellular matrix components and soluble factors as concrete response to stimuli. Disturbances result in vasoconstriction by either downregulation of eNOS or upregulation of endothelin-1, leading to vascular inflammation, impaired coagulation/fibrinolysis and finally atherosclerosis. On the level of endothelial cells, MG dose-dependently induces autophagy, thereby reducing angiogenesis, proliferation, migration and tube formation. Beside this effect, apoptosis is induced reducing the number of endothelial cells.

In addition, MG was detected in arterial walls and aorta of spontaneously hypertensive rats with aging and vascular contractile dysfunction in high blood pressure in increased levels suggesting direct effects. By altering protein structure, function and lifetime of target proteins, MG may impair ED at distinct levels - directly and indirectly, adumbrating alleviation of MG as a promising therapeutic approach. MG scavengers have been found to reduce vascular damage as well as Glo1 overexpression, supporting the link between reactive glucose metabolite and vascular phenotype. Whereas MG scavengers like aminoguanidine only resulted in disappointing effects in clinical attempts, overexpression of Glo1 in endothelial cells completely prevented hyperglycemia-induced AGE-formation, proving that MG is an important AGE precursor in endothelial cells. On the other hand, knockdown of Glo1 in human aortic endothelial cells increased MG levels and induced changes in the expression of genes linked to coronary artery disease and provoked collagen expression, endothelial inflammation and apoptosis.

Negative effects of MG on vasculature are not limited to macrovascular disease but are also documented in microvascular disease like retinopathy, neuropathy and nephropathy. Alleviation of dicarbonyl stress thus is a future attempt to overcome diabetes induced vascular disease. Most promising is the use of Glo1 inducers (e.g. Glo1 inducer combination of trans-resveratrol and hesperetin (tRES-HESP)) through the activation and binding of Nrf2 to the Glo1 functional ARE. TRES-HESP has been evaluated in a Phase 1 clinical trial and is now available for evaluation in Phase 2. In highly overweight subjects, tRES-HESP improves arterial dilation and decreases the vascular inflammation marker VCAM-1, thereby reducing symptoms of ED.

Without doubt reactive glucose metabolites are triggers of vascular and end organ damage in diabetes. Due to their high reactivity these glucoradicals are potent modifiers of vascular and organ function.

Invited Speaker 12

Title: RAGE and maternal bonding – an expected mechanism relationship
Authors: Yasuhiko Yamamoto[1], Ai Harashima[1], Seiichi Munesue[1], Kumi Kimura[1], Nontaphat Leerach[1], Yu Oshima[1], Hisanori Goto[1], Mariko Tanaka[1], Haruhiro Higashida[2]
Affiliations: [1]Department of Biochemistry and Molecular Vascular Biology, Kanazawa University Graduate School of Medical Sciences, Japan; [2]Department of Basic Research on Social Recognition and Memory, Research Centre for Child Mental Development, Kanazawa University, Japan

Glucose is the major source of energy for living processes in mammals. Nonenzymatic glycation reactions occur between glucose/glucose metabolites and biological macromolecules. That is to say, glycation is an unavoidable background reaction in all living systems, unfortunately leading to aging and aging-related diseases.

The receptor for advanced glycation end-products (receptor for AGEs, RAGE) is a widely known pattern-recognition receptor. Various ligands (*e.g.*, AGEs, high mobility group box 1, S100 proteins, lipopolysaccharides, and amyloid-β) can induce intracellular RAGE signalling pathways that lead to pathological processes, including inflammation, diabetes, aging, and cancer growth/metastasis. From an evolutionary perspective, all mammals have a homolog of RAGE, but other vertebrates such as birds, amphibians, fish, and reptiles don't have it at the genomic level. This arouses our interest in the reason why mammals require this RAGE.

One characteristic of all mammals is lactation; all mammals secrete oxytocin (OT) to stimulate nursing-associated milk letdown. OT in the brain has attracted increasing attention because it has been shown to be important in human behaviours such as sexual arousal, recognition, trust, anxiety, and mother-infant bonding. For OT to exert its function, it must pass from the circulatory system through the blood-brain barrier (BBB) portion of the neurovascular unit into the brain. There has been no direct evidence for this OT transport process and for molecular mechanisms underlying the process. Our team has demonstrated that RAGE on endothelial cells of the BBB can bind OT and transport OT from the blood into the brain, resulting in the regulation of brain OT levels. OT cannot compete with the interaction of RAGE with other ligands or induce RAGE intracellular signalling.

It is also known that there are soluble forms of RAGE (sRAGE) including endogenous secretory RAGE (esRAGE), a product of an alternatively spliced mRNA, and an ectodomain-shed form of RAGE in addition to full-length membrane-bound RAGE. We examined whether sRAGE affected RAGE-dependent transport of OT into the brain. As a result, sRAGE did not inhibit the OT transport and sRAGE itself was found to be transported into the brain through the BBB by RAGE.

This discovery of RAGE-mediated OT transport will open a new avenue for the link between energy metabolism, glycation, aging, and OT for brain function and social behaviours in mammals. These above-mentioned pathophysiological roles of RAGE will be presented and discussed.

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Invited Speaker 13

Title: Ascorbic acid as a glycating agent in the aging lens

Authors: Vincent M Monnier[1,2], Keith Zientek[3], Larry David[3], James Dai[1], Xingjun Fan[4], Sichuan Dong[5] David R Sell[1]

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Background and aims: Ascorbic acid (ASA, Vitamin C) is an enzyme cofactor and antioxidant that is essential for mammalian cells. In absence of catalytic metals it is very stable but its oxidized form, dehydroascorbic acid (DHA), is a source of highly reactive glycation products. While interstitial concentrations of ascorbate are very low (~50uM) it is taken up by active transporters and stored in millimolar concentrations in lens, brain, and adrenal gland to fight oxidant stress in conjunction with glutathione. In lens ascorbate acts as a UVB filter and antioxidant, and its absence leads to cataract. In aging, both the lens and the brain suffer from protein aggregation and deposits that are the results of complex insults involving deamidation, crosslinking, truncation, oxidation and the advanced Maillard reaction. Recent studies from our laboratory have implicated ascorbate as a major source of MG-H1 formation, both in lens and brain, whereby the three carbons of the hydroimidazolone ring are donated by carbons C4-C6 of the ascorbate molecule.

Materials and methods: To understand the impact of ascorbylation on crystallin stability, we incubated bovine crystallins with 10 mM ASA or DHA with or without metal chelator DTPA. After 3-7 days solutions were centrifuged and both the precipitate and supernatants were analyzed, using proteomics, for CML, CEL and MG-H1 hot spots specifically present in precipitated but not soluble lens crystallins.

Results: Precipitation of beta-crystallins from ASA was not suppressed by DTPA implicating glycation in crystallin precipitation. DTPA suppressed precipitation of gamma crystallins by both ASA and DHA implicating oxidation. A total of 8 highly specific sites were present only in protein pellets that included CML in CRYAA (K88), CRYBB1 (K23) , CRYBB2 (K168) and CRYGD (K163); CEL in CRYBB2 (K168) and MG-H1 modification sites CRYBB2 (R188), CRYGC (R147), and CRYGD (R10) which is in highly conserved regions of the protein. In CRYBB2 the cataract prone R188H mutation was shown to promote protein destabilization (Zhang et al, BBA 2010).

Conclusion: The significance of these modifications for protein stability in the human lens is under investigation. In further studies, small molecule screens for drugs with ability to prevent ascorbate/H_2O_2 mediated aggregation of lens beta and gamma crystallins led to the identification of alpha-crystallin chaperone mimetic drugs that bind to sites shared with the anti-aggregation mini-chaperone peptide 70-88 from αA-crystallin. The significance of these findings for age-related cataract will be discussed.

Invited Speaker 14

Title: Glyoxalase 1 activity declines with age in many tissues

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Rationale: Consuming higher glycemia diets results in accumulation of cytotoxic advanced glycation end products (AGEs) and is associated with increased risk for age related macular degeneration, CVD and diabetes. AGEs result from the non-enzymatic modification of biomolecules by sugars or their metabolites through a process called glycation. AGEs compromise many protein functions as well as the activity of proteolytic capacities that might otherwise eliminate the damaged proteins; putting organisms a double jeopardy. The glyoxalase (GLO) system is the primary mechanism for detoxifying the reactive intermediates of glycation and we are interested in determining if GLO1 activity can be enhanced and organism function retained during aging, especially upon consuming higher glycemia American diets. GLO is comprised of the combined activities of GLO1 and GLO 2. GLO1 is the rate-limiting enzyme. There is limited information about GLO1 activity and aging.

Purpose: In this study we evaluate the role of GLO1 in ocular and non-ocular tissues with age.

Method: We examined the expression of GLO1 by Western blotting and immunohistochemistry, and its activity spectophotometrically, as the initial rate of formation of S-D-lactoylglutathione in non-ocular (liver, brain, heart and kidney) and ocular tissues (retina, RPE/choroid and lens) from 4-, 12- and 24-months old wild type C57BL/6J mice.

Results: Glyoxalase 1 was detected in all tissues at 4-months and the activity correlated with protein level determined by Western blotting. Two bands were detected using antibodies that specifically recognize GLO1. The differential electrophoretic profiles of these GLO1-positive bands might indicate posttranscriptional changes. GLO1 activity was the highest in the retina. In comparison, liver, kidney, brain and heart showed 46%, 27%, 22% and 11% of GLO1 specific activity, respectively. The enzymatic assay also revealed that GLO1 activity is ~10 fold higher in the retina compared to lens or RPE/choroid. Morphological examination of retinal tissues revealed spatial differences of GLO1 in retina. GLO1 protein was present in all cell types within the retina, with high levels within cell bodies of the inner nuclear layer and ganglion cell layer. Photoreceptor cell bodies in the outer nuclear layer had lower levels. The RPE also had high levels of GLO1 protein, whereas the choroid and sclera had lower amount of GLO1 protein. Aging impacts in all tissues with a significant decline in protein and specific activity suggesting that the age-related decline in GLO1 is systemic.

Conclusions: Although GLO1 is a ubiquitous protein, the levels of this enzyme are regulated in an age- and tissue-dependent manner. The age-related decline of GLO1 activity takes place in all the tissues analyzed. This could have a detrimental impact in tissue fitness, especially in tissues highly susceptible to glycation-derived damage due to low regeneration capacity such as retina,

Invited Speaker 15

Title: Production mechanism of methylglyoxal and its reactivity with O_2 to produce ROS in illuminated chloroplasts of plant leaves
Author: Chikahiro Miyake
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Photoautotrophs are in grave danger of oxidative stress by the attacks of sugar-derived RCs in CO_2 assimilation, which magnitude is higher than autotrophs. For examples, CO_2 assimilation of C3-plants accumulated sugars to several hundred mM in the cells (Qui et al. 2008). Furthermore, the stimulation of CO_2 assimilation at high CO_2 conditions increased the glycation of proteins (Qui et al. 2008). Advanced glycation end-products (AGEs) accumulated in their leaves in response to CO_2 assimilation (Bechtold et al. 2009). Generally, CO_2 assimilation rate is about 20-30 folds larger than respiration rate (von Caemmerer and Farquhar 1981). The triose phosphate isomerase (TPI) reaction producing both MG and GLO also proceeds in the Benson-Bassham Calvin cycle of chloroplasts. Therefore, the productions of these sugar-derived RCs are expected to be stimulated at the high activity of CO_2 assimilation. In my presentation, the molecular mechanisms of sugar-derived RCs production and their reactivities in C3-plants are introduced. P700 oxidation system (Miyake 2020) is proposed to suppress RCs-dependent ROS production in CO_2 assimilation.

(1) Production mechanisms of MG/GLO: The addition of 3-phosphoglycerate (3-PGA), the reaction product of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco), to intact chloroplasts in the illuminated conditions enhanced the production of MG/GLO (Takagi et al. 2014). 3-PGA is metabolized to dihydroxy acetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP), which are the substrates for TPI. The production rates of MG/GLO increased with 3-PGA dependent photosynthesis in intact chloroplasts. Furthermore, the transition of partial pressure of CO_2 (pCO_2) from 40 to 90 Pa increased CO_2 assimilation rate with the productions of MG/GLO in wheat leaves. These results show the increased pCO_2 activated the turnover of the Benson-Bassham Calvin cycle in chloroplasts, leading to the enhanced productions of MG/GLO by TPI reaction.

(2) Reactivity of MG specific to photosynthesis organisms: The addition of MG to illuminated intact chloroplasts stimulated O_2-uptake (Saito et al. 2011). Furthermore, the steady state of chlorophyll fluorescence was quenched by the addition of MG to the intact chloroplasts. That is, MG stimulated the electron flux in photosynthetic electron transport by accepting electrons at photosystem I (PSI), where MG was photo-reduced to the MG-radical. Then, MG radical reduced O_2 to O_2^-. The electron carriers at the acceptor side of PSI: phylloquinone (E'm -800 mV), F_X (E'm -705 mV), and F_A/F_B (E'm -520 to -580 mV) have the potential to reduce MG (E'm -330 mV). MG-dependent photoreduction rate of O_2 was close to the electron flux in CO_2 assimilation rate (Saito et al. 2011). The apparent Michaelis constant of MG to reduceO_2 was about 0.1 mM, which value was lower than the concentration of MG observed in intact chloroplasts and intact leaves (Takagi et al. 2014). These facts show CO_2 assimilation in photosynthesis inevitably could produce sugar-derivative RCs, which could oxidatively inactivate photosynthetic functions.

(3) Hypothesis: Suppression mechanism of MG-dependent photoreduction of O_2 to O_2^- at photosystem I: All oxygenic photosynthesis organisms have the molecular mechanism to suppress the production of O_2^- in PSI, P700 oxidation system (Miyake 2020). I discuss the possibility of this system to suppress the photoreduction of MG in photosynthesis.

Invited Speaker 16

Title: Glycation of plant proteins – a step forward to understanding the biological role
Authors: Tatiana Bilova,[1,2] Ahyoung Kim,[1] Tatiana Leonova,[1,3] Alena Soboleva,[1,3] Julia Shumilina,[1,3] Nadezhda Frolova,[2,5] Alena Kusnetsova,[1,3] Natalia Vashurina,[3] Christian Ihling,[4] Manuel Matamoros,[6] Vladimir A. Zhukov,[7] Kirill Demchenko,[8] Claudia Birkemeyer,[5] Andrea Sinz,[4] Ludger A.Wessjohann,[1] <u>Andrej Frolov</u>[1,3]
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Background and aims: Glycation is usually referred to as an array of post-translational modifications of protein lysyl and arginyl residues with reducing carbohydrates and α-dicarbonyl products of their degradation. In mammals, resulting advanced glycation end products (AGEs) accumulate with ageing and under hyperglycemic conditions or/and oxidative stress. Recently, plant proteins were shown to be highly and site-specifically glycated. Thereby, corresponding AGEs accumulated with ageing and accompanied at least some environmental stress responses. Although this sight specificity of age-dependent glycation was proved for different plant tissues and organs, its biological role is still far from understanding. Therefore, in our work we address plant glycated proteome and try to get the first insight in the functional domain of plant protein glycation.
Materials and methods: At the first step, sufficient information on plant protein glycation patterns needs to be accumulated. For this, AGE-related modifications accompanying ageing and stress response needs to be assessed in different plant organs and tissues. This can be accomplished with the general bottom-up proteomics approach, based on LIT/Q-Orbitrap- or QqTOF-MS. The functional role of identified glycation sites can be probed by the methods of computational chemistry and bioinformatics.
Results: The body of data, accumulated to date, clearly indicate glycation hot spots as the marker of plant ageing or/and development: such AGE-modification sites were found not only in leaves, but also in such specialized structures as legume nodules. Thereby both effector and regulatory proteins are found to be glycated. Interestingly, different functional groups of proteins are modified in different way that might indicate different glycation intermediates, different glycation pathways and different regulatory mechanisms. In contrast, stress response can be accompanied not only with enhancement, but also suppression of glycation, which is often featured with down-regulation of corresponding proteins. For example, whereas in leaves drought-related accumulation of specifically glycated proteins is observed, in root nodules a reversed situation is observed.
Conclusion: The accumulating data allow assuming some biological role behind glycation of plant proteins. This can be mediated via modification of regulatory proteins, predominantly – arginine-rich transcription factors and kinases. Modification of effector proteins can be another way of regulation. Finally, glycation can be the marker of protein degradation via both proteasomal pathway and by autophagy.

Invited Speaker 17

Title: Glyoxalases: The antidote for Methylglyoxal and plant stress

Authors: Sneh Lata Singla-Pareek
Affiliations: Plant Stress Biology, International Centre for Genetic Engineering and Biotechnology, New Delhi, India

Methylglyoxal (MG) imposes adverse effects on plant growth by altering cellular processes through glycation reactions. It readily modifies proteins, nucleic acids and lipids forming advanced glycation end products which in turn, affects their biochemical properties and functions. However, besides exhibiting cytotoxicity, MG also serves physiological role in the cell. It can act as a stress signal molecule in plants. Thus, a delicate balance in MG levels is essentially required for maintaining cellular processes.

All living organisms have evolved various enzymatic mechanisms to get rid of MG from the cell as against their generation, which is primarily spontaneous from triose sugars. Glyoxalase pathway comprising of Glyoxalase I (GLYI) and Glyoxalase II (GLYII) enzymes acts as the main route of MG metabolism and consequently, maintains its homeostasis in the cell. Besides, conventional glyoxalase system, which catalyzes a two-step reaction, glyoxalase III (GLYIII) enzymes also exist in plants that directly convert MG to D-lactate in a single step. Interestingly, levels of this cytotoxin rise under stress conditions due to changes in the metabolic activity of plants. Therefore, controlling MG levels offers a promising strategy for stress mitigation in plants.

Towards this, our group has developed plant varieties overexpressing glyoxalase pathway genes, for raising stress tolerance. By reducing stress-mediated increase in MG and subsequently ROS levels, and maintaining photosynthesis under stress, these glyoxalase-overexpressing plants demonstrate significantly improved stress fitness. In fact, maintaining MG homeostasis through glyoxalases can confer general stress tolerance in plants. However, our studies also show that MG is required for stress signaling as it can cause global changes in gene expression, specifically perturbing signaling pathways and transcription factor gene expression thus, rewiring cellular responses under stress.

As plants have multiple genes encoding GLYI and GLYII enzymes, we have studied biochemical and molecular properties of these multiple members to explore their physiological significance. These members not only differ in their catalytic efficiency and other biochemical properties but also in their regulation, with some forms being more stress-inducible (OsGLYI-11.2) while others like OsGLYI-8, being constitutive proteins that are localized in the nucleus and possess higher catalytic efficiency. In addition, several members (like OsGLYI-6 or OsGLYII-1) lack MG detoxification ability and have acquired other functions. Further, our studies on GLYIII enzymes reveal that these enzymes, apart from MG detoxification ability, inherit other functions as well. Overall, we believe that MG levels are tightly controlled in the cell as evident from the presence of multiple pathways for its removal. Therefore, for generating climate-ready plants with significantly enhanced stress tolerance, gene pyramiding with MG metabolizing genes appears as a promising strategy.

Invited Speaker 18

Title: Detection of reactive carbonyl intermediates of glycation
Authors: Monika Pischetsrieder, Andrea Auditore, Sabrina Gensberger-Reigl, Joachim Stützer, Ingrid Weigel, Anna Becker, Susanne Sauer
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Both, in foods as well as *in vivo*, the formation of advanced glycation end-products (AGE) is mainly mediated by reactive carbonyl compounds (RCC) and only to a lesser extent directly by sugars. The qualitative and quantitative profiles of RCCs, which are formed as glycation intermediates from sugars, strongly determine the structures and concentrations of the resulting AGEs. The RCC profiles of a food, a drug or a physiological environment are highly variable and dependent on the external conditions, such as temperature, pH and the presence of metal cations. Therefore, realiable qualitative and quantitative RCC profiling methods are important to determine the RCC fingerprint of a sample aiming to predict its glycation potential and its physiological activity.

Dicarbonyl profiling is widely performed by LC-UV/MSMS after derivatization with *o*-phenylene diamine covering the most important glucose degradation products 3-deoxyglucosone (3-DG), 3-deoxygalactosone (3-DGal), 3,4-dideoxyglucosone-3-ene (3,4-DGE), glucosone, methylglyoxal and glyoxal. Recently, we introduced additionally a validated LC-UV/MSMS method for profiling of monocarbonyl compounds using 2,4-dinitrophenylhydrazine for derivatization. The most prominent glucose degradation products with monocarbonyl structures were thus identified as formaldehyde, acetaldehyde, 5-hydroxymethylfurfural and furfural.

3,4-DGE, which contains a Michael system, is highly reactive and therefore potentially harmful. Human exposure to 3,4-DGE can occur during peritoneal dialysis, intravenous infusion or by nutrition. Additionally, 3,4-DGE can be formed under physiological conditions from 3-DG and may therefore be a reactive intermediate of serum 3-DG. In contrast to 3-DG and the short chain RCCs methylglyoxal and glyoxal, 3,4-DGE shows a high cytotoxicity and glycation activity. Moreover, a strong activation of peptidergic nociceptors by 3,4-DGE was observed in the murine peritoneum *in vitro*, which may be linked to pain sensation.

In order to identify possible detoxification mechanism, but also possible targets of 3,4-DGE *in vivo*, a serum model was established which differentiates the contribution of the most important serum components. Thus, it was shown for example that 3,4-DGE leads to a depletion of serum glutathione, which may be linked to oxidative stress. On the other hand, it also reacts readily with HSA, which may be an efficient detoxification pathway or could also lead to the impairment of HSA function.

Invited Speaker 19

Title: Measurement of AGEs using LC-MS/MS, immunoassay, and skin autofluorescence

Authors: Ryoji Nagai, Nana Katsuta, Yuki Tominaga, Ikuho Ban, Sayuri Kato, Yoshitaka Hiraoka, Ryosuke Tsuzuki, Seitaro Tanaka, Hikari Sugawa, Mime Nagai

Affiliations: Graduate School of Agriculture, Tokai University

The progression of the Maillard reaction was estimated by evaluating the development of brown color and fluorescence intensity. Although this reaction has garnered considerable interest in the medical field following the development of antibodies against glycated proteins, the epitope structures of these antibodies have not been elucidated. N^ε-(carboxymethyl)lysine (CML) has been identified as a major antigenic AGE structure. Furthermore, our previous study showed that the monoclonal anti-AGE antibody, 6D12, recognizes not only CML but also N^ε-(carboxyethyl)lysine (CEL). Our subsequent study showed that specific antibodies, such as those against CML and CEL, can be obtained by conjugation to carrier protein with EDC or glutaraldehyde (1). However, since the measurement of AGEs by antibodies in physiological samples is difficult owing to the presence of interfering substances and autoantibodies against AGEs, instrumental analysis is a suitable method for evaluating the levels of AGEs *in vivo*. We previously reported that N^ε-(carboxymethyl)arginine (CMA) is produced significantly in glycated collagen by monoclonal antibody (2). A subsequent study using an antibody-affinity column clarified that the GER sequence on the collagen aids CMA production. Furthermore, LC-MS/MS analysis showed that the level of CMA in mice skin was higher than those of other AGEs, such as CML and N_δ-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1) (2), indicating that CMA may be a good marker demonstrating the glycation of collagen. Taken together, our studies suggest that antibodies and MS analyses have their own advantages, and it is appropriate to use them individually or together.

Although quantification of AGEs by LC-MS takes time and multi-step preparation is still required to analyse many clinical samples, we previously developed a device to estimate AGE level by measuring autofluorescence (AF) on fingertip, since it is one of the tissues with a lower accumulation of melanin, resulting in the stable evaluation of skin AF (3). We are now developing a method for simple and precise analysis of AGEs using the combination of antibodies, AF and LC-MS.

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Invited Speaker 20

Title: Mechanochemistry: Maillard Reaction "Through the Looking Glass"
Authors: Varoujan Yaylayan and Haoran Xing
Affiliations: Department of Food Science and Agricultural Chemistry, McGill University
Background and aims: The Maillard reaction has been studied so far under hydrothermal and pyrolytic reaction conditions, although there are several reports on sonochemical, electrochemical, photochemical and microwave-assisted reactions. Mechanochemistry on the other hand, is a solid phase reaction carried out under low temperature and solvent-free conditions providing a unique insight into the mechanism of the early phase of the reaction.
Materials and methods: The reactions were conducted in stainless-steel grinding jars. The jars were seated in a Retsch Mixer Mill that performs radial oscillations in a horizontal position at a frequency of 30 Hz for 30 min. The composition of the reaction mixtures was analyzed by (LC)-ESI-qTOF-MS/MS, isotope labeling technique, FTIR among others.
Results: With the emergence of mechanochemistry as a fast and efficient solvent-free synthetic methodology, we applied this approach to perform the Maillard reaction of glucose with various amino acids that lead to the selective formation of Schiff bases and their Amadori rearrangement products. Specifically, ball milling of glucose and amino acids with basic side chains leads to the formation of reaction mixtures rich in Schiff bases and amino acids with acidic side chains lead to the formation of reaction mixtures rich in Amadori products. Despite the selectivity towards the formation of Schiff base or Amadori compound, the chemical composition of the ball-milled mixtures was also investigated in depth by high-resolution mass spectrometry. The decarboxylation and C2-C3 sugar chain cleavage reactions were identified as the most important minor transformations during ball milling**.**
The thermal properties of the mechanochemically generated glucose-amino acid mixtures were also studied. Direct video recordings of their melting behavior showed that the browning of the ball-milled mixtures started at lower temperatures and proceeded at a slower pace compared to the non-milled mixtures. Furthermore, these ball-milled mixtures exhibited enhanced reactivity upon subsequent thermal treatments generating more browning and pyrazine-rich volatiles, compared to non-milled samples. To rationalize these observations, we further investigated the mechanochemistry of the Maillard reaction replacing glucose with glycolaldehyde. Based on the analysis of the data, the conversion of Schiff bases into reactive 5-oxazolidinones in these model systems was proposed as one of the reasons for the observed enhanced reactivity of milled samples.
The hypothesis that mechanical energy can also induce glycation of proteins was also tested using lysozyme. The ESI-qTOF-MS analysis of the milled samples has indicated that milling of sugar-protein mixtures in stainless steel jars for 30 min and at a frequency of 30 Hz generated mainly mono-glycated proteins even with the highly reactive ribose. Enzymatic activity measurements have also indicated that milling of lysozyme alone leads to a significant loss in enzymatic activity in contrast to milling in the presence of sugars.
Conclusion: Under ball milling conditions, the Maillard reaction effectively stops at the Schiff base/Amadori stage with only a small percentage of these intermediates undergoing degradation.

Keywords: Mechanochemistry, ball milling, Schiff base, Amadori compound, glycation.

Invited Speaker 21

Title: Organelle stress and metabolic derangement in kidney disease
Authors: Reiko Inagi, PhD.
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Organelle stress, such as mitochondrial or endoplasmic reticulum (ER) damage, is a causal factor for cellular dysfunction, leading to the progression of various diseases. In kidney disease, organelle stress in tubular cells aggravates tubular inflammation and tubulointerstitial fibrosis, and organelle stress in glomerular cells causes severe proteinuria. Notably, such organelle stress is closely associated with metabolic alteration, including glucotoxicity (1) and lipotoxicity (2).

Meanwhile, intensive research, including ours, has revealed how ER stress induces mitochondrial stress: decreased mitochondrial fatty acid metabolism, suggesting the pathogenic link of each organelle stress. Interaction of an organelle with another organelle also plays a critical role in maintaining cellular homeostasis and is called organelle crosstalk (3). Mitochondrial damage causes ER-mediated tubular inflammation via mitochondrial DNA leakage into the cytosol (4). Importantly, organelle crosstalk determines cell fate from the point of view of intracellular metabolism (5). Our recent studies point out that not only the mitochondria-ER axis but also the mitochondria-primary cilia axis is essential for homeostatic mitochondrial metabolism (6, 7).

This presentation will summarize the most recent evidence on organelle stress associated with defective organelle crosstalk and metabolic alteration in kidney disease, especially chronic kidney disease (CKD), including diabetic kidney disease (DKD) related to high glycation status.

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Invited Speaker 22

Methylglyoxal metabolism is a targetable liability of glycolytic metabolism in cancer
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Complex regulatory mechanisms enable cell metabolism to match physiological state. The major pathways cells use to turn nutrients into energy and to synthesize macromolecules have been elucidated; however, there remain many unanswered questions regarding how metabolism supports cancer cell proliferation and thus how best to target metabolism for cancer treatment. It has been known for decades that cancer cells take up glucose at higher rates than most other tissues, as well as increase fermentation regardless of oxygen availability (aerobic glycolysis). Many efforts to talk increased glucose metabolism in cancer have focused on limiting glucose use, however it may be possible to instead exploit increased glucose use by cancer cells. This possibility, as well as the potential benefits of aerobic glycolysis will be discussed.

Invited Speaker 23

Title: Glycation and mental health: Schizophrenia
Authors: Makoto Arai
Affiliations: Schizophrenia Research Project, Department of Psychiatry and Behavioral Sciences, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
Background and aims: In the field of medical science, the abnormal accumulation of dicarbonyl metabolites leads to modification of proteins and the creation of AGEs. As glycation contribute to cell and tissue dysfunction, it is a risk factor for various diseases in the later life, such as diabetes, heart disease, and dementia. However, the developmental effects and health-related impacts of glycation in the early life stages, i.e., from fetal stage to adolescence, and the molecular basis of these effects, are unknown. The onset of mental disorders is often observed during adolescence and can greatly alter the affected person's life.
Targeting a homogeneous population of patients with schizophrenia has been recognized as a promising strategy for extracting biological insights from the manifestation of clinical phenotypes. We first identified patients with schizophrenia having genomic mutations in the glyoxalase gene and found that a subtype of patients with schizophrenia show an increase in plasma pentosidine levels. We also discovered from our population-based adolescent cohort that the developmental trajectory of glycative stress during pregnancy and adolescence could be predictive for later psychotic symptoms. From these findings, we hypothesized that glycation in early life increases the risk of mental disorders.
In this symposium, we hope that new data will provide information for elucidating novel molecular mechanisms within the fields of mental health.
Materials and methods: In this study, we investigated a prospective association between AGEs (fingertip-AGEs) and psychotic symptoms. A total of 277 community-dwelling adolescents aged 13 years without antipsychotic medication were analysed. AGEs were measured in adolescents using a novel, non-invasive technology that can be used quickly. The trajectory of psychotic symptoms in a 12-month follow-up was assessed by experienced psychiatrists using a semi-structured interview.
Results: Among the 277 participants, 13 experienced persistent psychotic symptoms, 65 experienced transient psychotic symptoms, and 199 did not display any psychotic symptoms. Multinomial logistic regression analysis adjusted for age and sex revealed that baseline AGEs might predict the risk of persistent psychotic symptoms (odds ratio = 1.7).
Conclusion: The AGEs potentially predicted the trajectory of psychotic symptoms among drug-naive adolescents, which indicated its involvement in the pathophysiology of early psychosis. The findings of the present study have important clinical implications for the role of AGEs in the pathophysiology of early psychosis and may provide new insight and aid in early intervention to prevent psychosis.

Invited Speaker 24

Title: Design and synthesis of near-infrared fluorescent probe targeting tumour metabolite methylglyoxal for visualization study.

Authors: Chunyong Ding[a], Zhiai Xu[b], and Ao Zhang[a].

Affiliations: [a]Shanghai Jiao Tong University, Minhang District, Shanghai, China and [b]East China Normal University, Putuo, Zhongshan N Rd, Shanghai, China

Glyoxalase 1 (GLO1) is a unique metabolic enzyme closely related to the malignant degree in the evolution of breast cancer, which can detoxify the tumor metabolite methylglyoxal (MGO). The imaging of GLO1 molecular function is expected to realize the visualization of diagnosis, therapeutic effect evaluation and prognosis judgment of breast cancer. However, the visualization research based on the molecular function of GLO1 is very rare. Herein, we developed activatable near-infrared (NIR-I/II) fluorescent probes based on the donor-acceptor-donor type benzothiadiazole fluorescent scaffold to specifically image endogenous MGO that induced by GLO1 inhibition in different malignant degrees of breast cancer cells, and establish the accurate correlations between fluorescence signal, GLO1 expression level, pathological characteristics and therapeutic effects of GLO1 inhibitors, which are beneficial for understanding the malignant nature of breast cancer and the discovery of new biomarkers. Compared to previously reported MGO fluorescent probes, our probes exhibit several distinct advantages, including NIR-I/II emission, high selectivity with MGO detection limit of 18 nM, and 131-fold on-off ratio. The probes could sense GLO1 activity and monitor the therapeutic effect of GLO1 inhibitors by imaging tumorous MGO in a both real-time and in situ manner. Furthermore, they enable the visualization of tumorous MGO induced by GLO1 inhibitors in a mice xenograft model. These studies provide the scientific basis for the early diagnosis of breast cancer, the efficacy evaluation of GLO1 inhibitors and the judgment of prognosis based on the visualization of GLO1 molecular function.

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Invited Speaker 25

Title: Glycation and machine learning – diagnostic algorithms for diabetes, arthritis and autism

Authors: Naila Rabbani[1] and Paul J Thornalley[2]
Affiliations: [1]College of Medicine, Qatar University, Doha, Qatar and [2]Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar

Protein glycation adducts are valuable clinical biomarkers. Glycated proteins in plasma and glycated amino acids in plasma and urine are common analytes. Protein glycation biomarkers are influenced by heritability, aging, decline in metabolic, vascular, renal and skeletal health, and other factors. Development of diagnostic algorithms by artificial intelligence machine learning is enhancing the applications of glycation biomarkers. Glycation biomarkers have recently been applied to: (i) risk prediction of vascular complications of diabetes, (ii) diagnosis of autism; and (iii) diagnosis and classification of early-stage arthritis - specifically osteoarthritis.

Diabetic kidney disease occurs in *ca.* 40% patients with diabetes, with early decline in renal function (EDRF) in 1 in 3 patients. Patients who later develop EDRF have higher fractional excretion (FE) of 6 glycated amino acids - fructosyl-lysine and 5 advanced glycation endproducts (AGEs), compared to patients with stable renal function. No individual FE could classify patients. Application of artificial intelligence machine learning to develop diagnostic algorithms using Extreme Gradient Boosting, patients with and without EDRF could be conclusively diagnose by algorithm with features: A1C, log(ACR), $FE_{N\omega\text{-carboxymethylarginine}}$ (CMA). $FE_{\text{glyoxal-derived hydroimidazolone}}$ (G-H1) and plasma N_ϵ-carboxymethyl-lysine (CML). The positive likelihood ratio was 11.0, indicating strong, often conclusive evidence of EDRF. With further validation, this method may markedly improve risk prediction of EDRF.

Autism is a developmental disorder of childhood. There are long delays for expert referral of children for diagnosis. We found autism was associated with increased plasma protein CML, CMA and oxidative damage marker, dityrosine, and lower 3-deoxyglucosone-derived hydroimidazolone, 3DG-H. Support vector machine-developed diagnostic algorithm combining these analytes classified children with and without autism with diagnostic odds ratio (DOR) of 68. With further validation, this may lead to a simple blood test for diagnosis of autism.

Arthritis is the most common cause of chronic disability worldwide. Early detection of osteoarthritis (OA) may guide lifestyle and other interventions to prevent disability, and early detection of rheumatoid arthritis (RA) with treatment with disease modifying drugs may produce a radical cure. Other inflammatory joint disease (non-RA) is often self-resolving. An algorithm based on plasma glycated, oxidized and nitrated amino acids and hydroxyproline for classification of early-stage arthritis, had DOR = 116 – a powerful test for early-stage arthritis. In a second 3-classification algorithm to classify the type of early-stage arthritis (OA, RA or non-RA), anti-cyclic citrullinated peptide (CCP) antibody status replaced hydroxyproline and had DOR =103 for OA. Plasma glucosepane free adduct was a good early indicator of development of OA in a Guinea pig experimental model, correlating with OA severity and knee cartilage stiffness. Increased plasma glycation free adducts are reporting on increased knee joint proteolysis.

Protein glycation biomarkers are applicable to populations of differing ethnicities, often reporting mechanistic factors close to the phenotype. They are likely to find continued and expanding clinical use, reporting on multiple pathogenic processes.

Keywords: biomarkers; machine learning; algorithms; diabetes; arthritis; autism. Invited Speaker 26

Invited Speaker 26

Title: Glycation based therapeutics: Glo1 inducers and Glo1 inhibitors. COVID-19 repurposing

Authors: Paul J Thornalley[1] and Naila Rabbani[2]
Affiliations: [1]Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar and [2]Department of Basic Medical Science, College of Medicine, Qatar University, Doha, Qatar

Glycation by increased reactive dicarbonyl metabolites, particularly methylglyoxal (MG), in dicarbonyl stress is linked to activation of the unfolded protein response and related inflammatory and apoptotic signalling. This is implicated in development and progression of disease – particularly insulin resistance and development of type 2 diabetes, vascular complications of diabetes and aging. In severe dicarbonyl stress, apoptotic signalling is dominant and contributes to the mechanism of action of clinical anticancer drugs. Therapeutics in development to alleviate and enhance dicarbonyl stress are, respectively, inducers and inhibitors of glyoxalase 1 (Glo1).

Increasing expression and activity of Glo1 is an efficient strategy to counter dicarbonyl stress as it increases catalytic metabolism of Glo1 and often corrects a disease or aging-linked decrease in Glo1. We exploited the regulatory antioxidant response element (ARE) of the GLO1 gene and developed Nrf2 activator Glo1 inducers. Glo1 inducer activity was optimised to the combination of *trans*-resveratrol with hesperetin (tRES-HESP). In clinical trial, tRES-HESP corrected insulin resistance and improved dysglycemia and vascular inflammation in overweight and obese subjects. tRES-HESP corrects dicarbonyl stress in human aortal endothelial cells and periodontal ligament fibroblast in primary culture and improves wound healing and angiogenesis in diabetic mice. An off-target effect was correction of heoxhnase-2 linked glycolytic overload. tRES-HESP is well-suited for evaluation of prevention and reversal of type 2 diabetes and treatment of vascular complications of diabetes.

S-p-Bromobenzylglutathione cyclopentyl diester (BBGD) is the prototype cell permeable prodrug Glo1 inhibitor. Competitive substrate analogue inhibitor of Glo1, S-p-bromobenzyl-glutathione (BBG), is delivered into cells and stabilized to extracellular degradation by diesterification; cellular non-specific esterases from the BBG thereafter. BBGD is used experimentally to induce dicarbonyl stress. It has antitumor and antimalarial activity *in vitro* and has antitumor activity in mice *in vitro*. BBGD may find use in countering Glo1-linked multidrug resistance in cancer chemotherapy – particularly of breast cancer – where high expression of Glo1 is a negative survival factor.

For anti-COVID-19 pandemic response, sequenced-based prediction from the coronavirus SARS-CoV-2 proteome indicated a 4.6-fold enrichment of arginine residues in functional domains, compared to human host, suggesting SARS-CoV-2 is sensitive to inactivation by MG. SARS-CoV-2 is inactivated by *ca.* 28 µM MG in culture medium but this is *ca.* 50 - 200-fold higher than MG concentration in plasma *in vivo*. Glo1 inducer may provide a better approach. In human small vessel alveolar epithelial cells, tRES-HESP decreased plasma membrane-associated transmembrane protease serine 2 (TMPRSS2) and furin – proteases essential for infection and propagation of SARS-CoV-2. It also decreased secretion of monocyte chemoattract protein-1 (MCP-1) and interleukin-8 (IL8) - risk predictors and likely risk factors for severe symptoms and risk of mortality of COVID-19. tRES-HESP also decreased these inflammatory mediators in clinical trial of overweight and obese subjects. Further evaluation is on-going.

Keywords: dicarbonyl stress; methylglyoxal; glyoxalase 1**;** unfolded protein response; inflammation; insulin resistance; diabetic vascular complications; cancer chemotherapy; multidrug resistance; SARS-CoV-2; COVID-19**.**

Qatar Glycation Collaboration 1

Title: Measurement of glyoxalase activities
Authors: Sowndramalingam Sankaralingam
Affiliations: College of Pharmacy, Biomedical and Pharmaceutical Research Unit (BPRU), Qatar University, Doha, Qatar

Methylglyoxal (MG) is a highly reactive metabolite formed from glucose, fatty acids and protein. MG is a precursor for advanced glycation end-products (AGEs). It reacts with lysine and arginine residues in proteins and alters their function. It also causes DNA damage and strand breaks. MG is implicated in diabetes, obesity, cardiovascular diseases and their complications. MG is efficiently detoxified primarily by the glyoxalase (Glo) enzyme pathway that is ubiquitously present in most mammalian cells. Glo1 catalyses the conversion of hemithioacetal (formed from MG and reduced glutathione (GSH)) to S-D-lactoylglutathione (SLG). Subsequently, Glo2 catalyses the hydrolysis of SLG to D-lactate. Glo1 and Glo2 activities are performed on cytosolic fractions of cells or tissues. Homogenization or sonication of tissues or cells is performed in sodium phosphate buffer (10 mM) containing EDTA-free protease and phosphatase inhibitor cocktail, centrifuged @ 20,000 g for 30 min at 4°C and the supernatant cytosolic extract is used for Glo assays. For Glo1 activity measurement, hemithioacetal serves as the substrate which is pre-formed by incubation of MG with GSH for 10 min. Glo1 activity is measured spectrophotometrically using a quartz cuvette by following the initial rate of increase in absorbance at 240 nm (A_{240}) UV range) in the linear range that indicates formation of SLG and calculated using the change in molar absorption coefficient of 2.86 mM/cm. On the other hand, Glo2 activity is determined by monitoring the initial rate of decrease in A_{240} for which the change in molar absorption coefficient is 3.10 mM/cm. In the past, imidazole buffer or high concentrations of magnesium were used in assay buffer and should be avoided. The Glo activity assays can also be measured using other substrates such as glyoxal or hydroxypyruvaldehyde. The microplate assay allows for rapid screening of Glo activities of several bioactive compounds. The reaction is performed as indicated for the spectrophotometry procedure using cuvettes except that UV-transparent 96-well microplates are used. This technique also allows for the use of small volumes of cell lysates and reagents. For calculation of Glo activities, appropriate bandwidth and length of light path must be used. The A_{240} should be monitored every 1 min for 10-20 minutes and this duration and the amount of cell protein used must be optimized. The A_{240} in the linear range must be used for estimation of Glo activity. Thus, the microplate assay would be useful to determine mechanisms of bioactive compounds and changes in Glo activity in cells in response to various drug treatments.

Qatar Glycation Collaboration 2

Title: Genetics of glycation - glycated hemoglobin, GLO1 single nucleotide polymorphisms and copy number variation
Authors: Maryam Al-Motawa[1], Naila Rabbani[2] and Paul J Thornalley[1]
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In physiological systems there is genetic variation of glycation through influence on the level of glycating agents, turnover of target proteins and repair or degradation of the glycated proteins. In healthy subjects, 62% of population variation in glycated hemoglobin HbA_{1c} (A1C) is genetic; remaining variation is environment (23%) and age (14%). Such influences are often characterized in studies of monozygotic and dizygotic twins. Genes influencing A1C are in metabolic pathways involved in glycemic status, red blood cells and others – including fructosamine 3-kinase (FN3K) which catalyses the deglycation of A1C. Genetic influence of A1C is also found in patients with diabetes. Genetic effects explained 74% of population variance in serum advanced glycation endproduct, Nϵ-carboxymethyl-lysine (CML) which could not be fully explained by heritability of fasting glucose and A1C.

For glycation by methylglyoxal (MG), genetic polymorphism in glyoxalase 1 (GLO1) may contribute to variation of MG concentration and related MG-derived AGEs, such as hydroimidazolone MG-H1. Glo1 catalyses the metabolism of MG in the glyoxalase system. Human Glo1 protein is dimeric with identical or similar subunits of 184 amino acids, molecular mass *ca.* 21 kDa encoded by GLO1 gene of 27 kB located on chromosome 6. There are 3 common single nucleotide polymorphisms (SNPs) in human GLO1: ss1049346 and rs1130534 - linked to decreased Glo1 activity and associated diabetic nephropathy and diabetic retinopathy; and rs4746, linked to unchanged Glo1 activity. Nonsynonymous SNP rs2736654 in exon 4 was associated with increased serum levels of soluble receptor of AGEs (sRAGE). rs4746 C419A polymorphism in exon-4 is determined by PCR–restriction fragment length polymorphism. Historically, this was assessed by allozyme analysis, A111E sequence polymorphism, in non-denaturing electrophoresis with one and three dimeric allozymes in homozygotes and heterozygotes, respectively. Forty-seven other low frequency SNPs have been detected in GLO1. Low prevalence duplication of GLO1 (*ca.* 2%) was also detected in the human population. This may be associated with histone demethylation in early-stage embryogenesis and was weakly inducible in severe dicarbonyl stress. The GLO1 gene was linked to anthropometric measurements of obesity by sib-pair analysis in human subjects and to body weight by quantitative trait loci analysis in mice. Other gene polymorphism that may be influential MG concentration are: aldose reductase (AKR1B1), aldolases A, B and C (ALDOA, ALDOB and ALDOC), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and triosephosphate isomerase (TPI) – influencing levels of triosephosphates, and SNPs in transcription factors known to regulate Glo1 expression - E2F4, AP-2α, Nrf2 (gene NFE2L2) and HIF1A.

We recently identified HUWE1 as a candidate ubiquitin ligase catalysing the removal and degradation of protein modified by MG-H1. Interesting, conditional knockout of HUWE1 in pancreatic beta-cells of mice accelerated the age-dependent decline of insulin secretion and glucose homeostasis, which may mimic the effect of chronic dicarbonyl stress in clinical insulin resistance.

In our current work, we are exploring GLO1 and other related genetic polymorphism in the in Qatari population with genomic sequence data from the Qatar Genome Program.

Keywords: genetics; heritability, twin studies; glycated hemoglobin; methylglyoxal; glyoxalase; diabetic complications; ubiquitin ligase.

Qatar Glycation Collaboration 3

Title: Assay of glyoxalase metabolites: methylglyoxal, S-D-lactoylglutathione and D-lactate
Authors: Paul J Thornalley[1] and Naila Rabbani[2]
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The assay of methylglyoxal (MG) is achieved to high sensitivity and specificity by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry (LC-MS/MS) with prior derivatization by 1,2-diaminobenzene (DB) [1]. [13C3]MG is used as internal standard. Analytical recovery is 94 – 98% and interbatch coefficient 3 - 12%, depending on sample type. Control of interferences is essential as MG may be formed during pre-analytic processing from sample matrix, DB and interaction of sample matrix peroxidatic activity with DB. Peroxidase activity of the sample matrix is inhibited by addition of sodium azide during pre-analytic processing. Conditions to avoid in pre-analytic processing are: heating, high pH, prolonged incubation at physiological pH and oxidising conditions. High purity MG and $[^{13}C_3]$MG may be prepared by published methods [1]. Reference estimates of MG concentration in healthy control human and rat plasma are 132 ± 63 nM and 358 ± 139, mouse tissue 0.30 – 3.26 nmol/g wet weight and plant tissue 2.9 – 4.1 nmol/g fresh weight. Typical MG concentrations in human cells in culture are 2 – 4 µM. MG concentrations may increase 2 – 5 fold in diabetes and model hyperglycemia.

S-D-Lactoylglutathione (SLG) is measured by LC-MS/MS where stable isotopic reduced glutathione [*glycine*-$^{13}C_2$,^{15}N]GSH may be used as internal standard [2]. The steady-state concentration of SLG in cells is typically very low, <0.1 pmol/10^6 cells or <0.1% GSH. This may be due to potential impairment of cell function of spontaneous protein lactoylation and formation of N-D-lactoylcysteine by lactoyl transfer with rearrangement to cysteine which is an inhibitor of pyrimidine synthesis at high SLG concentration [3, 4].

D-Lactate is conventionally measured by endpoint enzymatic assay, using bacterial D-lactic dehydrogenase, with fluorimetric detection. This can be readily adapted for high throughput microplate-based measurements. LC-MS/MS methods are also available. An interference is racemization of L-lactate during pre-analytic processing – present at ca. 100 higher concentration than D-lactate in mammalian plasma. Racemization occurs at high pH and bicarbonate buffering during neutralization of acidic deproteinized extracts in pre-analytic processing avoids this [5]. The concentration of D-lactate in healthy human subjects is 9.7 ± 4.3 µM and increased 2 – 3 fold in diabetes [6]. Flux of D-lactate in mammalian cell cultures is a surrogate indicator of flux of MG formation and is a similar low fraction, 0.05 – 0.1% flux of glucose metabolism for all cell types evaluated. Extrapolated to glycolytic cell mass, this indicates that healthy adult human subjects produce ca. 3 mmol MG per day.

Estimates by the above methods are corroborated by mathematical metabolic modelling of the glyoxalase pathway with measurement of steady-state levels of GSH and MG-derived glycation adducts and accounting for protein turnover.

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Keywords: methylglyoxal; S-D-Lactoylglutathione; D-lactate; glyoxalase; dicarbonyl stress; LC-MS/MS; metabolic modelling.

Qatar Glycation Collaboration 4

Title: Quantitation of glycation adducts by stable isotopic dilution analysis LC-MS/MS
Authors: Naila Rabbani[1] and Paul J Thornalley[2]
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Quantitation of glycation adducts, early-stage fructosamines and advanced glycation endproducts (AGEs), may be performed by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry (LC-MS/MS). This is the reference analytical method, providing a high sensitivity, high specificity multiplexed method for concurrent detection of glycation adducts. It may be expanded for simultaneous detection of protein oxidation and nitration adducts, other post-translational modifications such as citrullination by arginine deiminases and formation of N_ε-(γ-glutamyl)lysine crosslink by transglutaminases, and the amino acid metabolome. We have called this method AGEomics. LC-MS/MS has been used to measure glycated amino acids – also called glycation free adducts - in physiological fluids. Similar adduct residues in proteins may be quantified with prior exhaustive enzymatic hydrolysis. Application of this technique to cellular and extracellular proteins give estimates of the steady-state levels of protein modification by glycation, and measurement of the accumulation of glycation adducts in cell culture medium and urinary excretion gives an indication of flux of adduct formation. Measurement of glycation free adducts in plasma and urine provides for estimates of renal clearance or fractional excretion of free adducts; as glycation free adducts pass readily through the glomerular filter, this provides a functional report of glycation free adduct re-uptake and/or active secretion by the kidney.

For methodology, ultrahigh performance liquid chromatography (UPLC) systems are usually used where an in-line fluorescence detection may provide corroborative fluorimetric detection of pentosidine. We have used graphitic column chromatography which avoids use of pre-column derivatization and ion pair reagents. It works well providing column clean-up and re-equilibration is optimized. A high performance tandem mass spectrometer is required to give high dynamic range for detection of both precursor amino acid and low and ultra-low level glycation adduct analytes. Robust and absolute quantitation of glycation adducts has revealed key aspects of glycation adduct physiology: proteins are minimally modified by glycation adducts in vivo – extent of moderation ranging from 0.01 – 10 mol%; glycation free adducts are the major form of excretion by glycation adducts in mammalian metabolism in urine, with normally high renal clearance; methylglyoxal-derived hydroimidazolone MG-H1 is a major AGE in physiological systems; and the AGE pyrraline originates from digested glycated proteins in food and be used to estimate the flux of glycation adducts from endogenous origin and food in clinical studies. It also provides a reference method to corroborate and optimize performance of glycation adduct immunoassays and other assay methodologies.

Diagnostic potential of glycation adducts in clinical studies has been enhanced by combination of estimates of multiple adducts in optimized diagnostic algorithms by machine learning. Algorithms require training and testing in independent datasets of adequate study group size for statistical power. Recent applications have been in early-stage detection of metabolic, vascular, renal and arthritis, metabolic control and risk of developing vascular complication in diabetes, and a blood test for autism.

Keywords: fructosamine; advanced glycation endproducts; LC-MS/MS; AGEomics; biomarkers; machine learning.

Qatar Glycation Collaboration 5

Title: Proteomics of glycation

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Proteomics studies of glycation aim to identify glycated proteins, sequence location of glycation adducts and their abundance in the proteome. Initial approaches involved 2-dimensional electrophoresis with immunoblotting to detect glycation and mass spectrometry of excised spots to identify proteins. Pre-analytic enrichment of glycated proteins has involved boronate affinity chromatography for proteins with fructosamine adducts and immunoaffinity chromatography of proteins with advanced glycation endproducts (AGEs). With the development of nanoflow liquid chromatography-high mass resolution Orbitrap mass spectrometry, it has been possible to detect glycated proteins in cell, tissue and plasma proteins without prior enrichment using a conventional bottom-up approach. A typical workflow involves: (i) preparation of a protein extracts of samples of interest, (ii) reduction and alkylation of sample protein, (iii) limited proteolysis of proteins – usually by trypsin or lys-C and trypsin sequentially; (iv) partial resolution of tryptic peptides by nanoflow reversed phase liquid chromatography, and (v) detection and sequencing of tryptic peptides by high resolution mass spectrometry. Peptides are sequenced by fragmentation by collision induced dissociation (CID), high-energy collisional dissociation (HCD) or electron transfer dissociation (ETD) with detection of characteristic fragment ion series. Glycation proteomic workflows require minor modification to avoid compromise of abundance and location of glycation adducts by avoiding use of high temperatures and high pH during pre-analytic processing.

A consensus criterion for protein identification is detection and sequencing of a minimum of two tryptic peptides unique in sequence, "unique peptides", for the protein of interest. Often with glycation, a tryptic cleavage is missed and the precursor dipeptide is preferably a unique dipeptide for secure identification of the related glycation proteins. A generally available method for quantitation is the label-free method, requiring no additional sample manipulation but rather employs peptide ion responses for quantitation. In physiological samples, unglycated proteins are typically found at 20 – 100 fold higher abundance than their glycated counterpart. Typical mean sequence coverage of proteins detected is *ca.* 20% and so many glycation adducts may be missed. Variable specificity of proteolytic enzymes for peptide sequences also make it difficult to characterize experimentally a peptide glycation motif. Despite these limitations, recent advances in application of glycation proteomics have bene made.

In studies using boronate affinity enrichment of proteins in human plasma and red blood cells, 7749 unique glycated peptides corresponding to 3742 unique glycated proteins were identified. In studies of methylglyoxal (MG)-derived hydroimidazolone MG-H1 residues in human endothelial cytosolic extracts, 1262 proteins were detected with 411 sites of MG-H1 modification detected on 220 proteins. Pathway enrichment analysis showed that MG-modified proteins were enriched in protein folding, protein synthesis, glycolysis and gluconeogenesis. Protein domain targets of MG modification were: TCP-1 chaperonins, phosphoserine and phosphothreonine binding sites of 14-3-3 proteins, GroEL chaperonins, proteasome alpha/beta subunits and conserved sites of aminoacyl-tRNA synthases.

Keywords: fructosamine; methylglyoxal; mass spectrometry; sequencing; pathway enrichment analysis.

Qatar Glycation Collaboration 6

Title: Application of functional genomics in studies of dicarbonyl stress.

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Functional genomics approaches in dicarbonyl stress research have mostly involved overexpression and silencing knockout of genes catalyzing the metabolism of fructosamine and reactive dicarbonyl metabolites and precursors of advanced glycation endproducts (AGEs), glyoxal, methylglyoxal (MG) and 3-deoxyglucosone (3-DG). Glyoxal and MG are mainly metabolized by glyoxalase 1 (Glo1) of the glyoxalase system in most human tissues where MG is the main physiological substrate. Knockdown of Glo1 may induce compensatory increase in aldoketo reductases. 3-DG is mainly metabolized by aldoketo reductases.

For *in vitro* studies of MG-linked dicarbonyl stress, Glo1 may be overexpressed by vector transfection, with empty vector transfection as control, and expression decreased by siRNA silencing. Glo1 overexpression decreased cytotoxicity of antitumor drugs and prevented hyperglycemia-induced increased vascular inflammation and decreased angiogenesis. Silencing of Glo1 induces dicarbonyl stress in normoglycemia and is associated with activation of the unfolded protein response and increased apoptotic, inflammatory and pro-thrombotic signalling.

Functional genomics of dicarbonyl stress *in vivo* has involved overexpression and siRNA silencing of Glo1 in *Caenorhabditis elegans, Drosophila melanogaster* and development of Glo1 overexpressing transgenic mice and Glo1 knockdown mice with expressing Glo1 siRNA, and more recently, Glo1 knockout mice produced by CRISPR-Cas technology. Early attempts in producing Glo1 transgenic mice gave lines of mice with variable increase of Glo1 expression and gene trapping in the Lexicon putative Glo1 knockout mouse retained Glo1 expression due to inadvertent use of embryonic stem cells with Glo1 gene duplication. Overexpression of Glo1 in *C. elegans* increased lifespan and silencing of Glo1 decreased lifespan, linking dicarbonyl stress to aging. Silencing of Glo1 in *Drosophila melanogaster* with genetic mutants associated with Huntingdon's disease have shown an enhancement of neuropathology disorder. Overexpression of Glo1 decreased the development of insulin resistance in high fat diet-fed mouse model of obesity, suggesting dicarbonyl stress promotes the development of insulin resistance and type 2 diabetes. Overexpression of Glo1 decreased development of experimental diabetic nephropathy, retinopathy and peripheral neuropathy; and silencing of Glo1 increased the development of experimental diabetic nephropathy. This suggests dicarbonyl stress has a key role in the development of microvascular complications of diabetes. siRNA silencing of Glo1 in a mouse model of hepatocellular carcinogenesis increased tumor development, suggesting that Go1 is a tumor suppressor protein. Functional genomics tools provide powerful approaches to advance understanding of the pathobiology of glycation. New high throughput analysis method, such as single cell transcriptomics and proteomics will provide an opportunity to further understand dicarbonyl stress at the systems level and the responsive heterogeneity of cell and tissue to dicarbonyl stress.

Keywords: functional genomics; fructosamine; fructosamine 3-kinase; methylglyoxal; glyoxalase; dicarbonyl stress.

Qatar Glycation Collaboration 7

Title: Mathematical modelling of dicarbonyl stress

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Mathematical modelling of improves understanding of glycation processes – providing insights into factors influencing steady-state levels of glycation adducts. Predictions are tested experimentally with model refinement. Mathematical Ordinary Differential Equations (ODEs) describe the relationships between steady-state levels of glycation agents, protein substrates and glycated proteins.

Our group have used glycation models to predict concentrations of methylglyoxal (MG). In the steady-state, the rate of formation of glycated protein formation *in situ*, $r_{Glycation\ adduct}$ = rate of glycated protein degradation, $r_{Glycated\ protein\ degradation}$ (= [glycation adduct]$_{Steady\ state}$ x protein turnover). From chemical kinetics, $r_{Glycation\ adduct}$ = $k_{MG,Protein}$ [MG][Protein]; $k_{MG,Protein}$ was determined by following protein labelling in experiments with [^{14}C]MG. [MG] is the only unknown and can be deduced by measuring MG-H1 content of the target protein. Such models indicate MG concentration in human plasma is predicted to be *ca.* 130 nM and MG content is mouse brain is *ca.* 880 nM. Experimental estimates similar to this have been recorded; estimates markedly different to this likely suffer analytical interference.

Cellular models of the glyoxalase pathway predicted how glyoxalase 1 (Glo1) inhibitors and Glo1 inducers affect cellular MG concentration and related advanced glycation endproduct (AGE) formation. They also indicated that in vascular endothelial cells increased formation of MG and decreased metabolism by Glo1 synergize to increase cellular MG concentration – which was validated experimentally. Glo1 inducer potently corrected dicarbonyl stress by both countering both of these factors. *In vivo*,

The mathematical models we employ are based on ODEs with enzyme kinetic descriptors and solving numerically with the biochemical systems simulator Copasi (http://copasi.org/). We recently incorporated in a large model of hepatocyte glucose metabolism (König *et al*., 2012). The latter model is thoroughly validated with experimental data and includes glycolysis, glyceroneogenesis and glycogen metabolism, as well as hormonal effects of insulin, glucagon and epinephrine. Using this full integrated model, we computed MG concentrations and fluxes through the glyoxalase system in hyperglycemic and hypoglycemic conditions and under impaired hormonal regulation to mimic the diabetic state.

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Qatar Glycation Collaboration 8

Title: Glycation in drug repurposing for COVID-19
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With development of the global pandemic of COVID-19 disease, an immediate therapeutic strategy was development of vaccines to increase host immunity to the SARS-CoV-2. Vaccines may decrease virus transmission and prevent severe disease and mortality. Vaccine development takes time, effectiveness may be compromised by new virus variants and have limited utility for treatment of established severe COVID-19 disease. An additional requirement is for antiviral drugs.

Few antiviral drugs with broad antiviral activity are available. None are effective against COVID-19 to date. Given the long regulatory approval process in drug discovery, repurposing of existing, clinically approved drugs was pursued. Common targets for antiviral drug evaluation include: virus entry, replication, assembly, and release. Herein, we describe the initial methods used for drug repurposing in antiviral discovery.

Initially, a search of drug databases is performed to identify candidate drugs of anti-viral potential. As characteristics of SARS-CoV-2 life cycle were reported, pharmacological targets emerged: inhibition of essential proteases, susceptibility to drugs increasing cellular methylglyoxal, and others. *In silico* informatics analysis may be performed to predict susceptibility of the viral receptor to the candidate drugs.

Next, the cytotoxicity of the selected drug will be determined *in vitro* using cell culture models that support virus replication. For SARS-CoV-2, cell models include: Vero and Vero E6 cells - kidney epithelial cells of an African green monkey, Calu-3 cells – human non-small cell lung cancer cell line, human small alveolar epithelial cells in primary culture, and others. There are then 3 types of common *in vitro* evaluation of the drug dose-response relationship of antiviral activity. Typically, cells are infected with clinically-derived SARS-CoV-2 virus for 1 – 2 h, cells washed and then development of the infection characterized over the following 2 – 3 days.

1. **Virus cytopathicity assay**. Host cells are inoculated with SARS-CoV-2 to induce cytopathicity and the concentration of the drug found to prevent half-maximal cytopathicity determined, median inhibited concentration IC_{50}.

2. **Median tissue culture infectious dose ($TCID_{50}$) assay**. Host cells are cultured with varying dilutions of the viral-containing culture medium or cell extract. After incubation, the proportion of samples with infection – often by plaque-forming assay - for each dilution is recorded and the drug concentration for 50% sample infection, $TCID_{50}$, is deduced.

3. **Viral RNA assay**. Host cells are cultured with varying dilutions of the viral-containing culture medium or cell extract and, after incubation, viral RNA copy number determined by quantitative RT-PCR assay.

An important variable in such evaluations is multiplicity of infection (MOI) - the number of virions that are added per cell during infection. For SARS-CoV-2 studies, this is typically 0.05 – 1.0. Drugs are typically often more effective against lower MOI.

If antiviral activity is found at clinically translatable concentrations without cytotoxicity, drugs may be evaluated in animal models: such as human ACE2 transgenic mice and Syrian hamster. The readiness of these techniques and protocols contributes to the drug screening and development process for effective treatment and prevention of COVID-19.

Keywords: SARS-CoV-2; COVID-19; antiviral; cytopathicity; $TCID_{50}$; viral RNA; multiplicity of infection.

Qatar Glycation Collaboration 9

Title: iPSC application in diabetes and glycation research
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Diabetes mellitus is the most prevailing disease with progressive incidence worldwide. To date, there is no permanent treatment available for diabetes. The establishment of induced pluripotent stem cell (iPSC) technology has allowed for generation of pluripotent stem cells without the need of a human embryo enabling us to avoid ethical concerns. iPSCs have a high capability to differentiate into insulin-producing β cells, which are closer in nature to the *in vivo* β cells than those differentiated from other types of stem cells. Furthermore, iPSCs generated from diabetic patients, provide cells genetically identical to the patient to be used for *in vitro* disease modeling and eventually cell-based therapies. In our lab, we generated several patient-derived iPSC lines representing different forms of diabetes. These patient-specific iPSCs harbor genetic defects associated with diabetes and could be differentiated into diabetes-relevant cells, such as pancreatic β cells and insulin-target cells (liver, fat, and skeletal muscle). Currently, we are using these patient-derived cells to get key information about the mechanism underlying the development of polygenic and monogenic forms of diabetes. These models can also be used for large-scale screens to examine candidate drugs on β cells and insulin-responsive targets. In my lab, the established platform provides an essential tool for understanding the genetic factors of diabetes that can eventually be translated into effective treatment. In this presentation, I will explain how iPSC-based models can be used in diabetes research. Also, I will present our results on making iPSC-derived pancreatic islet organoids to study genetic defects associated with diabetes.

Invited Short Talks 1

Title: Immunogenicity and allergenicity of glycated cows' milk proteins

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Background and aims: Food-derived Maillard reaction products (MRPs) have been linked to the increasing prevalence of diet- and inflammation-related diseases including food allergy. Maillard reaction (MR)-modified proteins show enhanced immunogenicity demonstrated by interaction with receptors present on antigen presenting cells (APCs). The aim of this work was to identify the role of MR and aggregation in the immunogenicity and allergenicity of cow's milk proteins, β-lactoglobulin (BLG), as well as whey protein hydrolysates (WPHs). We hypothesize that the binding of glycated proteins to the receptors present on APC is facilitated by both aggregation and formation of the advanced glycation end products (AGEs), thus enhancing their immunogenicity.

Materials and Methods: The binding and uptake of heated vs glycated BLG and WPHs to receptors (RAGE, CD36, Galectin-3, SR-AI) present on APCs were analyzed by ELISA and direct cell binding assays. Immunogenicity was determined by measuring the secretion of pro-inflammatory cytokines by macrophage-differentiated THP-1 cells, while allergenicity was measured using RBL cells loaded with whey-specific serum IgE from allergic individuals. The role of Nε-carboxymethyl lysine (CML) in receptor binding was analysed by the use of chemically modified β-lactoglobulin.

Results: Our results indicate increased immunogenicity of heated and glycated BLG based on the enhanced binding to and internalisation via Galectin-3 ad scavenger receptors class I and II (CD36 and SR-AI). The binding to Receptor for Advanced Glycation End Products (RAGE) was also observed but did not lead to internalisation. Receptor affinity of BLG was dependent on increased hydrophobicity, exposure of β-sheets and aggregation. Digests of glycated BLG maintained the binding to sRAGE and Gal-3 indicating decreased digestibility induced by glycation. CML-modified BLG showed binding to RAGE, CD36 and Gal-3 in a CML concentration-dependent manner. These results underline the important role of CML in the formation of the ligands for AGE receptors. CML was also detected in high molecular weight fractions (aggregates above ≥100 kDa) isolated from WPHs. These aggregates were characterized by high binding to RAGE, ability to induce the secretion of pro-inflammatory cytokines by THP-1 cells and to degranulate serum IgE coated FcεRI^{+} RBL cells.

Conclusions: Glycation of milk proteins may enhance immunogenicity by triggering innate (via RAGE) as well adaptive immune responses (via Gal-3), and can also increase the allergenicity of these modified milk proteins, although the exact role of glycation during the sensitization process is not clear. These findings reveal a need for better understanding of the influence of MR and agglomeration of milk proteins and their role in sensitization, tolerization and the effector phase of cow's milk allergy.

Invited Short Talks 2

Title: The Structure of Melanoidins formed in the MAILLARD Reaction of Methylglyoxal with L-Alanine or L-Lysine

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Background and aims: The formation of melanoidins in food is still not fully understood. To limit the pathways of the complex reaction, experiments with individual MAILLARD intermediates are commonly used. In this study the role of methylglyoxal (MGO) for colour formation was investigated. MGO is known as a highly reactive Maillard intermediate that is formed in food by retro-aldol reactions of carbohydrates or by various cleavage reactions of α-dicarbonyl compounds such as 1-desoxyglucosone or 3-desoxyglucosone.

Materials and methods: MGO was incubated in absence of an amino acid as well as in presence of L-alanine (Ala) or L-lysine (Lys) in aqueous solution at 100 °C and pH 5 for up to 300 min. The reaction mixtures were analysed in regards of absorbance at 420 nm, concentration of MGO by HPLC-DAD after derivatization with *ortho*-phenylendiamine, molecular weight distribution by SEC, and structure of the colorants by ESI-HR-orbitrap-MS. The mass spectra were submitted to KENDRICK mass analysis and VAN KREVELEN analysis.

Results: The browning reaction of MGO in absence and presence of amino acids results in a fast conversion of the reactant and the formation of colorants with a molecular weight well above 100 kDa. In the HR-MS spectra oligomers of MGO formed by aldol addition and condensation could be found. The varying degree of dehydration was revealed by VAN KREVELEN diagrams of the reaction mixtures. KENDRICK mass analysis showed that typical cleavage, reduction, and Strecker products of MGO, such as formaldehyde, acetaldehyde, acetol, and aminoacetone were involved in the formation of the aldol products as well.

Conclusion: The reaction of an individual MAILLARD intermediate such as MGO results in a variety of products with similar structural features. Most of these products will undergo aldol reactions and statistical copolymers emerge in the reaction mixture. Substructures formed by aldol addition might dehydrate and form conjugated double-bond systems that act as chromophores. Crosslinking of oligomeric chains is possible via aldol reactions of MGO with remaining carbonyl functions or by reactions of both amino functions of Lys. These reaction mechanisms – as well as the tools used to identify them – can be applied to the even more complex reaction of carbohydrates and, in consequence, help to unravel the structures of food melanoidins.

Invited Short Talks 3

Title: Fluidized bed roasting drives Maillard reactions toward A more aromatic cocoa

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Background and aims: Roasting is a relevant step in cocoa nibs processing and it is performed to reduce moisture content below 2%, and to develop desirable aroma throughout Maillard Reactions (MR). MR is also responsible for the production of high molecular weight polymers known as melanoidins. Traditional roasting in drums or ovens is a long process compared with fluidized bed roasting, but there is no any published report about the use of these technique in cocoa nibs. The aim of this research is to compare the decrease sugars and amino acids, and the production volatile organic compounds (VOC) and melanoidins in cocoa nibs roasted in oven vs. roasted in fluidized bed roaster.

Materials and methods: Forastero cocoa nibs sized from 4.0 to 7.0 mm were roasted at 120 and 140°C in oven, referred as Slow Roasting (SR-120 and SR-140) and in fluidized bed roaster, referred as Fast Roasting (FR-120 and FR-140), respectively until they reach 1.0 ± 0.5% moisture content. Unroasted cocoa was used as a control. Fructose, Glucose and Sucrose content, and the concentration of 20 amino acids were measured in the defatted cocoa powders by HPLC and LC-MS-MS methods respectivelly. The VOC, including alcohols, andehydes, fatty acids, esters, ketones, pyrazines were determined by GC-MS. The yield of formation of melanoidins was estimated gravimetrically by extracting high molecular weight (HMW) compounds >20kDa via ultrafiltration. The obtained HMW extract were analyzed by measuring their pH, their absorbances (at 420nm), and by measuring their bound phenolic content by LC-MS/MS.

Results: The decrease in sugars had high preference for fructose, followed by glucose for all roasting conditions studied. Compared to unroasted cocoa, the total free amino acid content decreased significantly after roasting. Roasting technique did not make a significant difference ($P > 0.05$), but roasting temperature did, being 140°C the temperature that reduced higher amount of free amino acids. The total VOC content of both FR-120 and FR-140 significantly increased, mainly because of the abundance of fatty acids, aldehydes, ketones, and pyrazines, compounds well known for their contribution to the mild chocolate aroma. The yield of formation of HMW compounds was significantly higher in SR-120, but not significantly diferent in the rest of the treatments and control. The absorbance and pH of FR-140 were significantly higher compared to the HMW extracts from other samples.

Conclusion: Fluidized bed roasting in cocoa favours the MR pathways addressed to produce aldehydes, ketones, and pyrazines. The higher decrease in amino acids at 140°C could be related to the greater formation of VOC in FR-140 cocoa nibs and to the higher brown intensity of its HMW melanoidins' extract.

Invited Short Talks 4

Title: Higher habitual intake of dietary dicarbonyls is associated with higher concentrations of corresponding plasma dicarbonyls and with skin autofluorescence: the Maastricht Study

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Background and aims: Dicarbonyls are highly reactive compounds and major precursors of advanced glycation endproducts (AGEs). Both dicarbonyls and AGEs are associated with development of age-related diseases. Dicarbonyls are formed endogenously, but also during food processing. To what extent dicarbonyls from the diet contribute to circulating dicarbonyls and accumulation of AGEs in tissues is unknown. Therefore, in this study we examined associations of dietary dicarbonyl intake with plasma dicarbonyl concentrations and skin AGEs accumulation.

Materials and methods: In 2566 individuals of the population based Maastricht Study (age: 60 ± 8 yrs, 50% males, 26% type 2 diabetes), we estimated habitual intake of the dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG), by combining Food Frequency Questionnaires with our dietary dicarbonyl database of MGO, GO, and 3-DG concentrations in >200 commonly-consumed food products, measured by UPLC-MS/MS. Fasting plasma concentrations of MGO, GO, and 3-DG were measured by UPLC-MS/MS. Skin AGEs were measured as skin autofluorescence (SAF), using the AGE Reader. Cross-sectional associations of dietary dicarbonyl intake with their respective (ln-transformed) plasma concentrations and SAF (all standardized) were examined using linear regression models, adjusted for age, sex, glucose metabolism status, kidney function, history of cardiovascular disease, BMI, smoking, alcohol intake, physical activity, lipid modifying, anti-hypertensive, and glucose lowering medication, education, and total energy intake.

Results: Median intake of MGO, GO, and 3-DG was 3.6, 3.5, and 17 mg/day, respectively. Coffee was the main dietary source of MGO, whereas this was bread for GO and 3-DG. In the fully adjusted models, dietary MGO was associated with plasma MGO ($\beta=0.08$, 95%CI [0.02;0.13], $p=0.004$) and with SAF ($\beta=0.12$ [0.07;0.17], $p<0.001$). Dietary GO was associated with plasma GO ($\beta=0.10$ [0.04;0.16], $p=0.001$) but not with SAF. 3-DG was not significantly associated with either its plasma concentration or SAF. These associations did not change after additional adjustment for individual macronutrients and the Dutch Healthy Diet Index.

Conclusion: Higher habitual intake of dietary MGO and GO, but not 3-DG, was associated with higher corresponding plasma concentrations. Higher intake of MGO was also associated with higher SAF. These results suggest dietary absorption of MGO and GO. Biological implications of dietary absorption of MGO and GO need to be determined.

Invited Short Talks 5

Title: Dynamics of hexokinase-2 linked glycolytic overload driving dicarbonyl stress and endothelial cell dysfunction in high glucose concentration *in vitro*.

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Background and aims: Metabolic dysfunction of endothelial cells in hyperglycemia contributes to the development of vascular complications of diabetes. Increased glucose consumption mediating metabolic dysfunction is caused by glucose-induced stabilization of hexokinase-2 (HK2) to proteolysis, producing a wave of increased intermediates in early-stage glycolysis stimulating increased formation of methylglyoxal (MG) and advanced glycation endproducts (AGEs). In this study, we investigated the dependence of glucose-induced stabilization of HK2 to proteolysis on glucose concentration, duration of exposure to high glucose concentration, correlation with increased flux of consumption of glucose and flux of formation of methylglyoxal (MG) driving dicarbonyl stress, rate of return of HK2 to basal levels with switch to normal glucose concentration and response to glyoxalase 1 inducer, *trans*-resveratrol-hesperetin combination (tRES-HESP).

Materials and methods: Human aortal endothelial cells (HAECs) were cultured under an atmosphere of air with 5% CO_2, 100% humidity at 37 °C in human large vessel endothelial cell growth medium with growth supplement and antibiotics according to the manufacturer's instructions; used during passages 4 - 6 to maintain the endothelial phenotype. Cultures had 4.1 - 20 mM glucose for 6 - 72 h, with and without 5 µM tRES-HESP. HK2 protein abundance was assessed by Western blotting, normalized to ß-actin; and glucose consumption and flux of formation of MG (assessed by accumulation of D-lactate) were determined by assay of glucose and D-lactate at the start and end of cultures, normalizing flux to cell number.

Results: HK2 increased progressively with time in HAEC cultures with high glucose concentration, compared to low glucose concentration controls (20 mM vs 4.1 mM glucose), maximizing after 12 h.
HK2 abundance was increased maximally by 37% whereas total glucose consumption was increased by 59% (6.77 ± 0.12 to 10.75 ± 0.21 µmol/day/million cells; P<0.001, n = 3). HK2 abundance increased from 4.1 mM to 20 mM glucose, increasing *ca.* 2.4% per mM glucose. HK2 abundance correlated positively with initial glucose concentration and flux of glucose consumption (r = 0.96 and r = 0.85, respectively; P<0.001; n = 12, Pearson) and with flux of MG formation (r = 0.93; P<0.001, n = 12); hexokinase-1 abundance did not. Increased HK2 abundance returned to basal levels only after 48 h in low glucose concentration. tRES-HESP prevented increased HK2 abundance, increased glucose consumption and metabolic dysfunction of HAECs in high glucose concentration.

Conclusion: We conclude that HK2 abundance increases with increased glucose concentration in the clinical range in diabetes in HAECs, producing glycolytic overload and metabolic dysfunction in high glucose concentration and tRES-HESP prevents this by normalizing HK2 protein. The slow return of high HK2 abundance to basal levels after a period of high glucose concentration exposure suggests dysfunction is carried over from postprandial to fasting phases. tRES-HESP may provide effective treatment of endothelial dysfunction linked to hyperglycemia in diabetes.

Keywords: endothelial dysfunction; hexokinase-2; glycolytic overload; dicarbonyl stress; methylglyoxal.

Invited Short Talks 6

Title: Pyridoxamine reduces glycation and markers of endothelial function in a placebo controlled intervention trial with abdominally obese individuals

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Background and aims: The formation of dicarbonyl compounds and advanced glycation endproducts (AGEs) in obesity is believed to be one of the drivers in the development of metabolic and (micro)vascular disease. Pyridoxamine (PM), a B6 vitamer and a chemical scavenger of reactive dicarbonyl species has been shown to inhibit the formation of AGEs, adipogenesis, inflammation, microvascular dysfunction, and insulin resistance in experimental studies. No clinical studies have been performed on this matter. Therefore, in this randomized placebo-controlled trial with abdominally obese individuals, we investigated the effects of 8 weeks of PM supplementation on glycation markers, insulin sensitivity, micro- and macrovascular function, and plasma biomarkers of inflammation and endothelial dysfunction.
Materials and methods: Apparently healthy abdominally obese individuals (waist circumference ≥102 cm for men, ≤ 88 cm for women) were recruited (n=108) and assigned to an 8-week intervention with either placebo treatment, 25 mg/day PM (low dosage), or 200 mg/day PM (high dosage). During this period, participants were asked not to change their lifestyle, diet, or physical activity routines. We assessed insulin sensitivity, glucose metabolism, insulin-mediated muscle microvascular recruitment and skin microvascular flowmotion. Macrovascular function was assessed by flow-mediated dilation (FMD), and aortic and carotid stiffness measurements. Dicarbonyls and AGEs were measured by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). A panel of plasma inflammation and endothelial function markers was measured by ELISA. Treatment effects were evaluated by one-way ANCOVA with adjustment for the baseline values. The study was registered at ClinicalTrials.gov with identifier number: NCT02954588.
Results: At follow-up, 108 individuals had finished all primary outcome measurements. A significant treatment effect (p<0.0001) was shown for PM metabolites in plasma and urine over the three groups. In the high PM dose group, we found a significant reduction of plasma MGO of 22 nmol/L compared to placebo (p=0.017) and a decrease in protein-bound MG-H1 of 211 nmol/L (p=0.010) compared to placebo. Despite the reduction of MGO and MG-H1, we found no treatment effect on glucose metabolism, insulin sensitivity, FMD, microvascular recruitment, flowmotion, vascular stiffness and plasma markers of inflammation. We found a reduction of sICAM1 in the high PM dose (p=0.049) and of sVCAM1 in both the low (p=0.026) and high PM dose group (p=0.008), as compared to placebo.
Conclusion: This study offers insight in the clinical potential of the nutraceutical PM. PM is safe and metabolically active in apparently healthy individuals. The reduction of MGO, glycation markers and some markers of endothelial dysfunction provides a good foundation for future research.

Invited Short Talks 7

Title: Short term intraperitoneal administration of mammalian cell derived human soluble receptor for advanced glycation end products (sRAGE) prevents type 1 diabetes onset in mice.

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Background and aims: Type 1 diabetes (T1D) is the most common chronic disease manifesting in childhood and has significant social and economic costs. The receptor for advanced glycation end products (RAGE), and its ligands, particularly advanced glycation end products (AGEs) have a role in the pathogenesis of T1D. Indeed, AGEs are known to be involved in pancreatic beta cell damage. The aim of this study was to determine if administration of recombinant human soluble RAGE (sRAGE), could prevent T1D in mice when delivered at two different sites.

Materials and methods: Non-obese diabetic (NODShiLt) mice were administered 100µg sRAGE produced in a mammalian expression system intraperitoneally (IP) or subcutaneously (SC), or placebo from days 50-64 of life, prediabetes. Diabetes incidence was determined at day 225 (n=10-25/group) using the log-rank test. To examine pharmacokinetics, mice were administered 100µg sRAGE IP or SC (n=24/group) with blood collected at various times to 24 hours and measured by ELISA.

Results: By day 225, sRAGE prevented T1D in 62.5% of treated mice (P=0.0081 vs. placebo) but SC sRAGE did not reduce diabetes incidence by day 225. Pharmacokinetic analysis showed a significantly higher concentration of IP sRAGE in serum (C_{max}, 1971.4ng/mL) peaking 1-hour post-injection compared to SC sRAGE, reaching a C_{max} of 62.7ng/mL at 2 hours. sRAGE was detected using His-tag staining and confocal imaging in pancreatic islets including among immune cell infiltrates at 1-hour post IP injection but was not seen after SC injection.

Conclusion: Short-term human sRAGE delivered prediabetes by IP injection was superior to that given SC, preventing T1D in NOD mice, likely in part due to the higher serum concentration and trafficking to pancreatic islets. Further studies will analyse spleen and lymph node immunology and pancreatic damage in response to IP as compared with SC sRAGE.

Invited Short Talks 8

Title: High hydrostatic pressure processing of human milk avoids the formation of Maillard reaction products and preserves oligosaccharides.

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Background and aims: High hydrostatic pressure (HHP) is a heat-free alternative to Holder pasteurization (HoP) for the treatment of human breast milk donated to milk banks and commonly used for the feeding of preterm babies. HHP has recently been shown to preserve several key bioactive components in human milk that are degraded by HoP but, to our knowledge, no data have been published for Maillard reaction products (that may be deleterious for preterm babies) or human milk oligosaccharides (HMOs, which have multiple beneficial health effects).

Materials and methods: Pooled samples of frozen, human milk from the North of France regional human milk bank were either left untreated, sterilized by HHP (350 MPa at 38°C) or processed by HoP (62.5°C for 30 min). We compared the impact of HHP and HoP processing of human milk on furosine (and indirectly lactoluselysine), carboxymethyllysine (CML) and carboxyethyllysine (CEL), three well-established indicators of the Maillard reaction, by liquid chromatography with tandem mass spectrometric detection, and on 22 HMOs using LC with fluorescence detection.

Results: The thermal action inherent to HoP treatment significantly increased the levels of all the Mallard reaction products studied here, while HHP-treated milk was not significantly different to raw milk in this regard. Furosine (μg/mL) in HoP-treated milk was 64% higher than either raw milk or HHP-treated milk, while the lactuloselysine equivalents (expressed as μmol/mol lysine) were calculated to be 59% higher in the HoP-treated milk. Concentrations of CML and CEL were *ca.*40% and 90 % higher, respectively, in the HoP-treated milk compared with the raw or HHP-treated milk. Neither HoP nor HHP processing significantly affected the concentration of the 22 HMOs studied here when compared with raw milk.

Conclusion: Our findings demonstrate that HPP treatment avoids the formation of Maillard reaction products and does not degrade HMOs. When taken together with other data, which indicates that HHP also conserves other nutritionally beneficial components of human milk, our data add weight to the growing body of evidence of its potential advantage over HoP as a sterilization process for use in human milk banks.

Invited Short Talks 9

Title: The effect of a 4-week diet low and high in AGEs on insulin sensitivity and secretion, vascular function, and markers of low-grade inflammation and endothelial dysfunction of abdominally obese individuals – preliminary results from the deAGEing trial

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Background and aims: The biological relevance of dietary AGEs is a matter of debate. Although some studies report an improvement in insulin sensitivity and plasma biomarkers of inflammation and endothelial dysfunction after a diet low in AGEs, results so far are inconsistent due to several methodological limitations. We therefore studied the effects of a specifically-designed 4-week diet low or high in AGEs on insulin sensitivity and secretion, micro- and macrovascular function, and plasma biomarkers of inflammation and endothelial dysfunction using gold standard methods.

Materials and methods: 82 Abdominally obese but otherwise healthy individuals were randomly assigned to an isocaloric and macronutrient-matched 4-week diet low or high in AGEs in a double blind parallel-design randomized controlled trial (deAGEing trial; NCT03866343). Insulin sensitivity and secretion were assessed by a combined hyperinsulinemic-euglycemic and hyperglycemic clamp technique. Microvascular (endothelial) function were assessed by insulin-mediated microvascular recruitment in muscle, skin microvacular vasomotion, and retinal vessel calibers. Macrovascular function were assessed as flow-mediated dilation, and as aortic (carotid-femoral pulse wave velocity) and carotid (Young's Elastic Modulus and Distensibility coefficient) stiffness. AGEs in food, plasma, and urine were measured by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). A panel of plasma biomarkers of inflammation and endothelial dysfunction were measured by sandwich ELISA. Intervention effects were evaluated by one-factor ANCOVA with adjustment for age, sex, and the baseline value of the outcome of interest.

Results: 35 and 38 participants completed the low- and high-AGE intervention, respectively. Based on dietary logs and our UPLC-MS/MS dietary AGE database we calculated energy-adjusted daily intake of dietary AGEs, which were markedly different between the low- and high-AGE intervention, with mg/day CML intake of 2.2 vs. 6.5, mg/day CEL 1.7 vs. 7.1, and mg/day MG-H1 14.0 vs. 42.7 (p<0.001). Although caloric intake was not significantly different between groups, participants in the low-AGE group consumed 4% more energy as carbohydrates at expanse of fat (p<0.001). Despite the marked difference in dietary AGEs, we generally observed no intervention effects on insulin sensitivity, fasting glucose, microvascular function, macrovascular function, and plasma biomarkers of inflammation and endothelial dysfunction.

Conclusion: The results so far strongly suggest that a 4-week diet low or high in AGEs does not influence insulin sensitivity, vascular (endothelial) function, and inflammation. Based on these preliminary data, we cannot recommend short-term restriction of dietary AGEs as a preventative strategy for age-related diseases in healthy adults.

Keywords: dietary advanced glycation endproducts, ultra-performance liquid chromatography tandem mass spectrometry, insulin sensitivity, insulin secretion, vascular function.

Invited Short Talks 10

Title: Drought-related changes in pea root nodule metabolome

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Background and aims: Drought is the major environmental factor reducing world crop productivity. Dehydration triggers oxidative stress, which is manifested by lipid peroxidation and formation of reactive carbonyl compounds (RCCs). RCCs readily react with side chains of amino acid residues in proteins resulting in their carbonylation and formation of advanced glycation/lipoxidation end products (AGEs/ALEs). Their accumulation might contribute in loss off characteristic functions of plant tissues and organs. As drought stress affects nitrogen accumulation in legume-rhizobial symbiosis and compromises yields of legume crops, here we specifically address drought-related changes of carbonyl metabolome in root nodules of pea (*Pisum sativum*) plants.

Materials and methods: Three week-old pea plants cultivated in aerated hydroponic system were transferred to the growth medium supplemented with 10% (w/v) polyethylene glycol (PEG) 8000. One week later, plants were harvested, grinded, and RCCs were extracted from plant material by methanol and derivatized with 7-(diethylamino)-coumarin-3-carbohydrazide (CHH), prior to extraction with methyl tert-butyl ether. The extracts were analysed by RP-HPLC coupled on-line to Orbitrap Elite (Thermo Fisher Scientific) mass spectrometer via a heated electrospray (HESI) source operated in positive ion mode. The individual correction coefficients were applied to original signal intensities to eliminate the contribution of analyte degradation on the quantitative data. The structures of RCCs demonstrating significantly (t-test, $p \leq 0.05$) inter-group differences were characterized by tandem mass spectrometry.

Results: The plant stress response was confirmed by appropriate physiological and biochemical markers. More than 300 RCC-CHH adducts could be effectively detected by LC-MS. This strategy revealed in total 194 highly-abundant derivatized RCCs present in pooled samples. After FDR correction, 22 abundant RCCs were identified as differentially abundant in stress and control groups. It was shown that 19 RCCs decreased there abundance in the stress group, whereas only three of them (for example, 4,5-dioxovaleric acid and glyceraldehyde) were up-regulated under stress conditions.

Conclusion: 4,5-dioxovaleric acid and glyceraldehyde were up-regulated under drought stress conditions.

Keywords: 7-(diethylamino)coumarin-3-carbohydrazide (CHH), drought, pea (*Pisum sativum*), reactive carbonyl compounds (RCCs), root nodules

Invited Short Talks 11

Title: Evaluation of MG antiviral activity against SARS-CoV-2.
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Background and aims: The ongoing coronavirus disease 2019 (COVID-19) pandemic has resulted in a global health emergency. Rapid development and rollout of vaccines for SARS-CoV-2 is in progress to counter the pandemic. The initial optimism for vaccination as an effective and long-term prophylaxis for prevention of severe COVID-19 has been tempered by the emergence of new SARS-CoV-2 variants, many of which (e.g. beta, gamma and delta variants) exhibit reduced vaccine effectiveness. The emergence of SARS-CoV-2 variants emphasizes the need to develop anti-SARS-CoV-2 drugs until a broad spectrum vaccine is developed. Our previous *in silico* analysis suggested vulnerability of SARS-CoV-2 to inactivation by the endogenous reactive metabolite, methylglyoxal (MG). MG may modify arginine residues in the functional domains of viral spike and nucleocapsid proteins, resulting in loss of charge, protein misfolding and inactivation. Bioinformatics analysis revealed a 5-fold enrichment of arginine residues in functional sites of the viral proteome compared to that of the human host, suggesting selective toxicity to SARS-CoV-2 may be achieved by MG exposure. In this study, we evaluated the antiviral activity of MG against wild-type SARS-CoV-2 using *in vitro* assays.
Materials and methods: Wild-type SARS-CoV-2 with titers of multiplicities of infection (MOI) 0.8, 0.2, 0.02 and 0.01 were incubated with 2-fold serial dilutions of MG (500 to 7.8 µM) in infection medium (Dulbecco's Modified Eagle Medium + 2% fetal bovine serum + 100 units/ml penicillin + 100 µg/ml streptomycin) for 6 h. MG-treated and untreated control SARS-CoV-2 was incubated with confluent cultures of Vero cells *in vitro* for 1 h, cultures washed with phosphate-buffered saline and incubated in fresh infection medium at 37°C for 4 - 5 days until 70% of virus control cells displayed cytopathic effect. We also studied the effect of scavenging MG with aminoguanidine (AG; 500 µM) on virucidal activity. The antiviral activity of MG was judged by assessing virus replication using quantitative RT-PCR and median tissue culture infectious dose ($TCID_{50}$) assays. Data analysis was by logistic regression: $\ln(E/E_{max}-E)$ against $\ln([MG]/\mu M)$ where E is cytopathic effect. $TCID_{50}$ (mean ± SD) was deduced by interpolation.
Results: MG inhibited RNA replication and cytopathicity of SARS-CoV-2 *in vitro*, with $TCID_{50}$ increasing with increasing MOI. MG was most potent at MOI 0.02 and 0.01 where $TCID_{50}$ of MG was 49.6 ± 4.7 µM and 28 ± 1 µM, respectively. Similar findings were also found for a shorter incubation period (3 hours) of MG and virus. Scavenging of MG by AG resulted in virus replication levels equivalent to those seen in the virus control with and without AG.
Conclusion: MG has potent inhibitory activity against wild-type SARS-CoV-2 for virus exposure in cell-free system at low MOI. Further experiments are required to elucidate the MG anti-SARS-CoV-2 mechanism of action, including MG modification of viral spike and nucleocapsid proteins. We are currently investigating the antiviral activity of MG against other SARS-CoV-2 variants including alpha- and beta-variants. Drugs increasing cellular concentration of MG to viricidal levels may have anti-COVID-19 activity.

Keywords: SARS-CoV-2; COVID-19; methylglyoxal; antiviral.

Invited Short Talks 12

Title: Anti-inflammatory activity of glyoxalase-1 inducer, *trans*-resveratrol and hesperetin, in human small airway epithelial cell primary cultures support application for prevention of COVID-19.

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Background and aims: There is currently a global pandemic of infection by coronavirus SARS-CoV2 and associated viral pneumonia COVID-19 with mean case fatality ratio of 2% and *ca.* 4 million deaths. Several vaccines have been developed and rapidly deployed to prevent COVID-19 but there remain no effective treatments. Prevention of SARS-CoV2 infection and treatments for COVID-19 would also help counter the pandemic. Pre-clinical and clinical studies suggest an essential role of proteases, plasma membrane-associated transmembrane protease serine 2 (TMPRSS2) and furin, in SARS-CoV-2 fusion with lung alveolar epithelial cells for infection. Angiotensin converting enzyme-2 (ACE2) and glucose regulated protein-78 (GRP78) are considered to be a receptors for cell surface binding of the spike protein of SARS-CoV2; and risk of progression to severe symptoms and risk of mortality in COVID-19 is linked to increased levels of low grade inflammatory mediators, including monocyte chemoattract protein-1 (MCP-1) and interleukin-8 (IL8), which are also likely risk factors for severe, life-threatening disease. Glyoxalase-1 inducer, *trans*-resveratrol and hesperetin combination (tRES-HESP), decreases activation of the unfolded protein response (UPR). It decreased expression of MCP-1, IL8, the receptor for advanced glycation endproducts (RAGE) and cyclo-oxygenase-2 (COX-2) in peripheral blood mononuclear cells (PBMCs) in clinical trial in overweight and obese subjects. SARS-CoV2 employs chaperonin components of the UPR for folding of viral proteins in viral replication. We hypothesized that tRES-HESP may decrease activation status of the UPR and decrease gene expression linked to SARS-CoV2 infection and progression to severe COVID-19.

Materials and methods: Human small airway epithelial cells (SAECs) were purchased from Lonza. SAECs were cultured in Small Airway Growth Media with supplements, according to the supplier's instructions. SAECs (10,000 cells/cm^2) were cultured with and without 5 µM tRES-HESP and the effect of basal gene expression studied after 72 h for mRNA (qRT-PCR) and protein (Western blotting); and significance of effect assessed by Student's t-test, n = 3 or 4.

Results: In SAEC primary cultures, tRES-HESP decreased basal protein levels of TMPRSS2 (- 58%; P<0.001) and furin protease (- 43%; P<0.01) after 72 h. tRES-HESP also decreased mRNA of TMPRSS2 (- 62%, P<0.01), GRP78 (- 67%, P<0.001), COX-2 (-34%, P<0.001) and RAGE mRNA (- 56%, P<.001) and decreased basal secretion of MCP-1 and IL8 (-29% and - 25%, P<0.001).

Conclusion: tRES-HESP decreased basal expression of proteases essential for SARS-CoV2 infection, GRP78 and secretion of inflammatory mediator risk predictors of severe COVID-19. These effects are likely due to activation of Nrf2 by tRES-HESP and decrease of UPR activation by suppression of dicarbonyl stress. These outcomes suggest previous anti-inflammatory response of tRES-HESP in overweight and obese subjects found in PBMCs may also occur concomitantly in the lung. TMPRSS2 is an antioxidant response element-linked gene down regulated by activation of Nrf2. These responses suggest tRES-HESP is suitable for further evaluation for prevention of COVID-19.

Invited Short Talks 13

Title: Update to the N^6-carboxymethyl lysine story
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Background and aims: N^6-Carboxymethyllysine (CML) is one of the most published posttranslational protein modifications within the field of Maillard research in vivo and in foods. However, from today´s perspective it is clear that CML is central part of a whole mechanistic reaction scheme. Starting from the Schiff base formation of glyoxal with the ε-aminofunction of lysine isomerization, cyclization and redox reactions lead to plethora of follow-up products, which are now extended by the class of amidines. In addition, alternative formation pathways are discussed.

Materials and methods: In vitro model incubations of amino acids or proteins were generally performed at 37 or 50 °C in phosphate buffered solutions at pH 7.4 or 6.0. Tissue and food proteins were hydrolyzed after extraction by both acid and enzymatic hydrolyses to calculate the hydrolyses efficiency of acid labile target structures. Also, samples were processed under both reduced and non-reduced conditions to cope for artifact formation and also for hydrogenolysis lability, respectively. All target structures were synthesized as authentic reference materials and fully characterized by spectroscopic means.

Results: The CML reaction cascade with CML, glycolyl lysine (GALA), glyoxylyl lysine, glyoxal lysine amide (GOLA), glyoxal lysine dimer (GOLD) is discussed on a mechanistic basis and extended to the formation of glyoxal lysine amidine (GLA, N^1,N^2-bis-(5-amino-5-carboxypentyl)-2-hydroxy-acetamidine). Alternative formation pathways to glyoxal-lysine reactions are discussed including chemical stability. The significance of the various structures to physiological systems and to the situation during processing of foods is highlighted by selected examples including extra- and intracellular tissues and wheat bread rolls processing.

Conclusion: Although CML is one of the major Maillard protein modifications found in tissues and in foods on a quantitative basis, many other structures coinciding within the same formation pathways should also be considered as relevant because of their possible physicochemical or pathobiological properties.

Invited Short Talks 14

Title: Enzymatic decarboxylation of *N*-ε-Carboxymethyllysine by ornithine decarboxylases reveals underground metabolism as a route for *in vivo* processing of glycated amino acids

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Background and aims: Products of non-enzymatic browning (Maillard reaction, glycation) are ingested with daily food in substantial amounts. Little is known about the metabolism of the unabsorbed compound *N*-ε-Carboxymethyllysine (CML) in humans, especially the role of the intestinal microbiota. In the present work, the metabolization of CML by *E. coli* should be studied.

Materials and methods: CML and potential metabolites were chemically synthesized. As in a previous work on probiotic *E. coli* strains, deletion mutants from the Keio collection were tested for their ability to metabolize CML. The quantification of CML as well as the identification and quantification of metabolites were performed either by RP-HPLC-UV after dansylation or by RP-HPLC-MS/MS without derivatization.

Results: *E. coli* is able to metabolize CML under formation of three different novel metabolites, *N*-carboxymethylcadaverine (CM-CAD), *N*-carboxymethylaminopentanoic acid (CM-APA), and the *N*-carboxymethylpiperideinium ion. CM-CAD was found to be the major metabolite. A mutant lacking the constitutive ornithine decarboxylase (SpeC) was no longer able to convert CML to CM-CAD. Conversely, overproduction of SpeC as well as of its close homolog, the inducible ornithine decarboxylase SpeF, in *E. coli* increased the ability of the bacterium to metabolize CML.

Conclusion: Glycated amino acids are metabolized by *E. coli* mainly by decarboxylation, and the responsible decarboxylase(s) were identified as the constitutive ornithine decarboxylase SpeC as well as the inducible ornithine decarboxylase SpeF. Both enzymes process CML as a non-standard substrate ("underground metabolism"). The novel biogenic amine CM-CAD is the first example of possible intestinal metabolites of glycation compounds whose role in human physiology and gut microbial ecology needs to be explored in the future.

Invited Short Talks 15

Title: Polyphenols as trapping agents of reactive carbonyl species: new strategy to reduce harmful compounds in e-cigarette emissions

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Background and aims: The use of e-cigarettes is a major issue in public health. At temperature of 300°C, high concentration of glycerol, propylene glycol and flavouring agents render the carrier solvent prone to carbonylation, condensation and dehydration reactions typical of the polyalcohol and sugars fragmentation pools of the Maillard reaction.[1] Within this compartmentalized multiphase environment, reactive carbonyl species (RCS) such as aldehydes and ketones are the most potent toxicants in e-cigarette emissions causing cancer, cardiovascular and respiratory diseases.[2] Currently, most strategies for reducing e-cig toxicants are based on the design of the product.[3] Here we show a new method of trapping RCS in e-cig emissions by adding polyphenols in e-liquid formulations.

Materials and methods: The model e-liquid system, composed by propylene glycol, glycerol and water (70:20:10), was used as control and compared to e-liquid formulations enriched with gallic acid (GA), hydroxytyrosol (HT) and epigallocatechin gallate (EGCG) at four concentrations (0.6; 1.25; 2.5 and 5 mM). The aerosol produced by the Subox Mini C device and the Gram Universal Vaping Machine was then trapped using amorphous silica fibres and analysed by liquid chromatography coupled with mass spectrometer for quantitation of polyphenols and by derivatization and LC-UV for carbonyls.[4]

Results: LC-MS data indicated that formaldehyde, acetaldehyde, methylglyoxal (MGO), and other carbonyls decreased up to 99.6%, 100%, 82.2, and 76% in GA formulation (at four concentrations, respectively) and a similar trend was observed for HT and EGCG model systems. We putatively identified mono-, di-methylglyoxal adducts (Gallic Acid + Methylglyoxal $[M-H]^- =241.20$, Gallic Acid +2 Methylglyoxal $[M-H]^- =313.10$ and Epigallocatechin gallate +2 Methylglyoxal $[M-H]^- =567.10$) and Methylglyoxal adduct of 3,4-dihydroxyphenylacetaldehyde, (dihydroxy-phenylacetaldehyde +glyoxal$[M-H]^- =209.10$), an oxidation product of HT. The short-term cytotoxic effects of e-cig aerosols on two lung cellular models (alveolar A549 and bronchial BEAS-2B) was also investigated. Results showed that carbonyl-polyphenol adducts are not cytotoxic, even though carbonyl trapping did not improve cell viability.

Conclusion: In conclusion, this study established the ability of polyphenols to reduce RCS concentration in e-cig emissions leading to the formation of stable adducts and highlighting their substantial contribution to harm reduction. This strategy suggested the potential value of polyphenols in commercial e-liquid formulations.

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Invited Short Talks 16

Title: Multiple Outcome Studies Confirm Predictive Value of AGEs for Diabetic Kidney Disease

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Background and aims: Advanced glycation endproducts (AGEs) have been implicated in the development of kidney disease (DKD) a significant cause of death and disability in diabetes. To test their use in the clinical setting, we evaluated the prognostic utility of a comprehensive multicomponent AGE panel in predicting DKD in landmark long-term diabetes complications outcome studies of Type 1 (T1D) and 2 diabetes (T2D)

Materials and methods: 5 AGE compounds – carboxymethyl and carboxyethyl lysine (CML; CEL), glyoxal, methylglyoxal and 3-deoxyglucosone hydroimidazolone (GH1; MGH1; 3DGH1), in baseline plasma or serum filtrates were analysed by LC-MS/MS from participants with T1D (Natural History of Diabetic Nephropathy and DCCT/EDIC) and T2D (Pima Nephropathy Study, ACCORD and VADT). DKD progression was based on renal function loss (RFL) defined as persistent 30-40% decline in GFR or histological changes by electron microscopy (EM) in renal biopsy samples. Both of these hard endpoints are needed to accurately characterize the kidney phenotype in these clinical outcome studies. Determination of the risk of DKD was based on relationships between AGEs and DKD adjusted for recognized risk factors.

Results: CEL, CML, and MGH1 and less frequently 3DGH were most likely to be independently predictive of DKD and significantly added to usual risk factors when tested during the initial "silent phase" when clinical signs of this irreversible process were absent. The table summarizes the characteristics and predictive AGEs found for each of our 5 cohorts.

Studies Showing AGEs Predict Diabetic Kidney Disease					
Study	Total/ Cases	Diabetes Type	Primary Endpoint	Follow-up (years)	Products with p<0.05
Natural History of Diabetic Nephropathy	103/ 26	1	EM	5	CEL, CML, MGH1
Pima Indian Nephropathy Study	163/ 104	2	40% RFL EM	12	CEL, MGH1 CEL, MGH1, CML
DCCT/EDIC*	459/ 109	1	40% RFL	30	CEL, CML, 3DGH1
ACCORD#	1150/ 172	2	30% RFL	12	GH1, 3DGH1, CEL, CML, AGE-score‡
VADT†	447/ 108	2	30% RFL	7.5	CEL, CML, AGE-score‡

*Diabetes Control and Complications Trial, # Action to Control Cardiovascular Risk in Diabetes, †Veterans Affairs Diabetes Trial, ‡Mean of 5 standardized (1 SD) products

Based on C-statistics (AUC) and net reclassification improvement (NRI), adding selected AGEs to baseline risk factors for DKD significantly improved predictive power. Blood levels of AGEs improved predictive power for DKD independently from traditional risk factors such as age, duration of diabetes, sex, blood pressure, HbA$_{1c}$, race, GFR, urine albumin levels and treatment group if relevant.

Conclusion: In five major clinical trials of defined diabetes populations we have found strong independent associations between AGE burden and significant progression of diabetes-related renal function loss irrespective of diabetes type and ethnic group. Susceptibility appears to be at least partly determined by how an individual regulates

production or breakdown of AGEs and their reactive precursors in the face of elevated blood sugars. Adding AGE measurement to standard clinical parameters improves the prediction of multiple kidney outcomes, indicating its potential as a prognostic biomarker of diabetic kidney disease. Having this predictive information prior to the development of potentially irreversible structural change would allow the institution of therapies to prevent end-stage kidney disease and its many adverse sequelae.

Invited Short Talks 17

Title: The spliceosome is a target for glycation in methylglyoxal-induced apoptosis and is shielded by glyoxalase 1 in multidrug resistance-linked cancer chemotherapy.

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Background and aims: Methylglyoxal (MG) is a reactive metabolite formed mainly as a by-product in anaerobic glycolysis and metabolized by glyoxalase 1 (Glo1) of the glyoxalase system. A wide range of anticancer drugs increase MG to cytotoxic levels by off-target effects which contributes to their cytotoxic antitumor activity. We investigated the mechanism of MG-induced cytotoxicity in cancer chemotherapy by exploring the early-stage proteomic response to MG-induced apoptosis and functional correlates with protein targets of MG glycation in human tumor cell lines and clinical human tumors - exploring links to cancer patient survival.
Materials and methods: We studied human HEK293 cells treated with exogenous MG. The proteomic response to MG-induced cytotoxicity was characterized by identifying the time to maximum MG-derived protein glycation adduct, MG-H1, content of cell protein during cell exposure to the median growth inhibitory concentration of MG (similar to the minimum exposure time to induce commitment to apoptosis) and then identifying proteomic changes and targets of MG modification. High mass resolution Orbitrap mass spectrometry proteomics analysis was performed of cell cytoplasm, nucleus and mitochondrial membrane, and mitochondrial matrix and intermembrane space subcellular proteome fractions. We also examined functional correlates with Glo1 expression in 1040 human tumor cell lines in CCLE database and 7489 tumors and related cancer patient survival in KM plotter dataset.
Results: MG activated the intrinsic pathway of apoptosis in HEK293 cells, indicated by early-stage loss of cytochrome c from mitochondria. Formation of MG-H1 adduct residues of cell protein, maximized after treatment with MG for 6 h. MG modification targeted the spliceosome – particularly arginine-rich domains of serine/arginine rich splicing factors, leading to decreased spliceosomal proteins and decrease of respiratory electron transport and formation of ATP by chemiosmotic coupling proteins of mitochondria. Spliceosomal gene expression correlated positively with Glo1 expression in human tumor cell lines and clinical tumors, suggesting Glo1 is a protective shield of the spliceosome against MG glycation *in vitro* and *in vivo*. In chemotherapy of breast cancer, increasing expression of Glo1 was associated with decreased patient survival, with hazard ratio HR = 1.82 (logrank P <0.001, n = 683) and upper quartile survival of patients with tumors of high Glo1 expression decreased by 64%
Conclusion: We conclude that MG-mediated cytotoxicity contributes to the cancer chemotherapeutic response by targeting the spliceosome, with activation of the intrinsic pathway of apoptosis. High expression of Glo1 contributes to decreased survival in the chemotherapy of breast cancer, in part likely by shielding the spliceosome from MG modification. Adjunct chemotherapy with cell permeable Glo1 inhibitor to re-establish susceptibility of the spliceosome to MG glycation may improve treatment outcomes.

Invited Short Talks 18

Title: Methylglyoxal targets proteins involved in mitotic fidelity

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Background and aims: Sustained hyperglycaemia associated with Type 2 Diabetes (T2D) leads to an increase in the systemic concentration of the reactive metabolite, methylglyoxal (MGO). MGO can react with lysine (Lys) and arginine (Arg) residues on proteins and deoxyguanosine residues on DNA to form a class of modifications called Advanced Glycation Endproducts (AGEs). T2D is also associated with elevated levels of DNA damage, particularly in the form of micronuclei (MNi). These small nuclear bodies originate around chromosomes or chromatid fragments (formed by double stranded DNA breaks) that persist in interphase after failing to be incorporated into the main nucleus following mitotic exit. Importantly, MNi have been shown to be a predictive biomarker for cancer risk. Two recent meta analyses have shown the mean ratio of MNi for Diabetes Mellitus is 1.99 (T2D) and 1.74 (T1D and T2D) when compared to controls. Despite the observation of increased MNi in individuals with T2D, underlying pathophysiological mechanisms remain largely unexplored.
The aims of this study were 1) to determine if MGO can induce MNi; 2) investigate potential underlying mechanisms.
Materials and methods: WIL2-NS B lymphocyte cells were treated with MGO (100 and 500 µM) for 48 h and DNA damage was assessed using the Cytokinesis Block Micronucleus cytome (CBMNcyt) assay and fluorescent *in situ* hybridization (FISH). Site-specific analysis of AGEs was determined in trypsin and ProAlanase digested whole cell extracts using the ThermoFisher Orbitrap Exploris 480 mass spectrometer.
Results: WIL2-NS treated with 100 and 500 µM of MGO experienced a 1.63 and 2.84-fold increase in MNi frequency compared to untreated cells, respectively ($p < 0.05$). FISH analysis of MNi using peptide nucleic acid probes specific for centromeric DNA revealed that the majority (88%) of MGO induced MNi were centromeric positive, suggesting that whole chromosome malsegregation events were the cause of the elevated MNi frequency. Proteomic analysis identified ca.150 and 250 proteins in untreated and treated (500 µM) WIL2-NS, respectively, containing either MG-H (Arg) or carboxyethyl (Lys or Arg) modifications. Furthermore, STRING protein-protein interaction analysis and Gene Ontology using DAVID revealed various proteins involved in cell division as targets for MGO modification. These included HAUS augmin like complex subunit 5 (HAUS5), chromobox 3 (CBX3), chromosome alignment maintaining phosphoprotein 1 (CHAMP1), centriolin (CNTRL) and cytoskeleton associated protein 5 (CKAP5), amongst numerous others. The aforementioned proteins all play critical roles in various mitotic processes such as spindle formation, microtubule elongation/ nucleation, centrosome duplication, chromosome alignment and attachment of microtubule to kinetochore.
Conclusion: In this study, we found that MGO induces chromosome malsegregation events, leading to an increased frequency of aneuploidy in cell progeny. Furthermore, we provide evidence that this novel pathophysiological effect of MGO may be due to modification of proteins involved in the mitotic processes. Together, these results support the hypothesis that pathophysiological accumulation of MGO modifications results in DNA damage and disruption of the mitotic apparatus and thus provides important insights into underlying mechanisms of MNi formation in T2D.

POSTERS

Poster 1

Title: MS/MS diagnostic ions characteristic of Schiff bases and Amadori products as analytical aids in Maillard "omics" research

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Background and aims: The high-resolution mass spectrometry is emerging as a high-throughput analytics platform for the non-targeted analysis of the Maillard reaction mixtures. However, its full potential on unequivocal structural elucidation of the Maillard reaction intermediates through subsequent tandem mass spectrometry (MS/MS) remains largely unexplored. Here we aim to demonstrate that the diagnostic ions obtained from MS/MS fragmentations can be used for the discrimination between glucose-derived Schiff bases and Amadori compounds, the most important isomeric intermediates involved in the early phase of the Maillard reaction, using amino acids (AA) glycine and proline as examples.

Materials and methods: The (LC)-ESI-qTOF-MS/MS systems were operated under both positive and negative modes to obtain protonated, sodiated, and deprotonated ions. These three different types of molecular ions were subsequently fragmented under different collision-induced disassociation energies of 10, 15, and 20 eV to generate unique MS/MS fragmentation patterns.

Results: Analysis of the MS/MS data has indicated that both protonated and deprotonated precursor ions generated useful diagnostic ions for distinguishing Schiff bases and Amadori compounds, however, the deprotonated ions have been identified to generate both selective and informative MS/MS fragmentation patterns. Both protonated and deprotonated Schiff bases produce the diagnostic ion at $[AA+diose\pm H]^{+/-}$ through C2-C3 retro-aldolization. The protonated Amadori compound produced the diagnostic ion at $[AA+CH_2+H]^+$ through α-fission, while the deprotonated Amadori compound produced the diagnostic ion at $[AA+triose-H]^-$ through C3-C4 retro-aldolization reaction. In general, the protonated Schiff bases exhibited reduced stability.

Although the MS/MS of sodiated ions of Schiff bases and Amadori compounds did not generate unique molecular entities, however their common dominant fragments, such as the ions at $[AA+diose+Na]^+$ and $[tetrose+Na]^+$ formed through C2-C3 retro-aldolization, were significantly different in their intensities, and as such could have some diagnostic value. Moreover, we have also demonstrated that the relative intensities of the diagnostic ions can be used to predict the ratio of Schiff bases and Amadori products in the reaction mixture. Finally, we also illustrated the universality of the identified diagnostic ions by extending this technique to other amino acids and to distinguish the Schiff bases and Amadori compounds generated from the ε-amino moiety of lysine and various dipeptides (glycylglycine and carnosine).

Conclusion: The MS/MS fragmentation techniques can be effectively integrated with high-resolution mass spectrometry for unequivocal differentiation of two important early-stage isomeric Maillard reaction intermediates, which can be used as an important tool for the workers in the field of "omics" research on the Maillard reaction.

Keywords: High-resolution mass spectrometry, tandem mass spectrometry (MS/MS), diagnostic ions, Schiff base, Amadori compound.

Poster 2

Title: Novel strategy to distinguish and quantitate glucose- and fructose-derived glycation products

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Background and aims: Fructose, the sweetest naturally occurring sugar, is associated with diseases such as type 2 diabetes (T2D). As the intake of fructose-based products is increasing, e.g., in form of high fructose corn syrup, there is a need to clarify its metabolism and contribution to non-enzymatic glycosylation (glycation). Both glucose and fructose can react with nucleophilic amines to form after rearrangement so-called Amadori (ARP) and Heyns products (HRP), respectively. Nevertheless, fructose and its glycation products are more extensively involved than glucose in glycoxidation favoring the formation of advanced glycation endproducts (AGEs). Despite the progress made in the analysis of glycation, research targeting the specific detection of fructose-derived modifications is limited. Therefore, a sensitive and specific technique, allowing the identification of isomeric ARPs and HRPs, is required.

Materials and methods: A fructosylated lysine derivative was synthesized and used as a building block for solid-phase peptide synthesis. Glycated peptides were analyzed by LC-MS and characterized by NMR. The fragmentation of eight isomeric Amadori and Heyns peptide pairs using collision induced dissociation (CID) was studied. Characteristic reporter ions were selected allowing a differentiation of all isomers by targeted mass spectrometry.

Results: In a previous report, we quantified tryptic peptides representing 30 specific glycation sites in human plasma as potential biomarkers for T2D.[1] Eight of the 30 peptides were successfully synthesized in high purity and yields using fructose- and glucose-derived building blocks.[2] The identity of one Heyns-peptide was confirmed by NMR. Depending on the charge state, tandem mass spectra were dominated by characteristic neutral losses of parent and/or fragment ions resulting from oxonium, pyrylium and furylium ions. Both ARPs and HRPs shared the known losses of up to three water molecules (18 Da, 36 Da, and 54 Da) and a loss of three water plus one formaldehyde molecule (84 Da) revealing modification-specific intensity patterns. Furthermore, all HRPs displayed an additional loss (96 Da), reported as specific for ketohexose-derived compounds. However, an identification of Heyns peptides solely upon this mass loss is misleading, as it was also observed for several Amadori peptides. Thus, we will present a new approach to distinguish isomeric Amadori and Heyns peptides and allowing also their sensitive and reliable quantitation, even if the peptides are not separated by HPLC.

Conclusion: In order to study the glycation by fructose, Heyns peptides were successfully synthesized. Moreover, MS/MS experiments on synthetic Amadori and the corresponding isomeric Heyns peptides revealed characteristic fragmentation behaviors independent of the sequence. While the previously reported Heyns-specific neutral loss of 96 Da was also observed for several Amadori peptides, the current study revealed a different strategy to identify and distinguish fructose- and glucose-modified peptides even when present at different concentrations. Importantly, the new strategy allows the sensitive quantitation of both ARPs and HRPs.

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Keywords: Amadori/Heyns peptides · Fructose · Glycation.

Poster 3

Title: Synthesis and Characterization of Lysinonorleucine (LNL)

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Background and aims: The non-enzymatic reaction of reducing carbohydrates such as glucose or lactose with the side chain functional groups of the amino acids Arginine or Lysine in proteins is called the Maillard reaction, which was first reported in the field of food chemistry more than 100 years ago. The initially formed intermediates, so-called Amadori reaction products, decompose particularly at elevated temperatures to various Maillard reaction products (MRPs). This reaction also progresses slowly primarily from glucose with proteins *in vivo* and an early product called the Amadori product is converted into advanced glycation end-products (AGEs). AGEs are continuously formed under normal circumstances, but more rapidly under a variety of stresses especially oxidative stress and hyperglycemia. Thus, they serve as markers of stress, body health and disease processes, including inflammation, diabetes, cancer and ageing, and act as toxins themselves. Furthermore, the Maillard reaction commonly occurs in food products, while their increased presence may negatively influence the quality of the product by causing undesired flavors and coloring as well as lowered nutritional value.

In order to analyze the extent to which Maillard Reaction Products are present in food after different kinds of treatment, e.g. heat, appropriate analytical methods are of crucial importance. Thus, MRPs and AGEs are valuable markers for product quality and health state and gained broad attention in cosmetics, biochemistry, food, and pharmaceutical applications. Iris Biotech provides a broad portfolio of Maillard reaction products and AGE standards and is constantly working on improved synthesis routes. Herein, we are presenting a new route of synthesis for N-[(5S)-5-amino-5-carboxypentyl)]-L-lysine, trivial name lysinonorleucine (LNL), which was first described and extracted in 1969 from bovine elastin.

Materials and methods: The final product was purified by HPLC and ion exchange to get a foam, which was characterized by NMR, TLC, ESI-MS and net content was determined. The material is supplied as a salt containing varying contents of the acid counterion. The net content of each batch is specified in the respective certificate of analysis.

Results: Herein, we present a new synthetic route towards the total synthesis of LNL. Based on published syntheses, we developed and optimized a manufacturing route suitable for multi-gram scale. The synthesis is performed in five steps *via* reaction of Boc-L-Nle(epsilon-I)-OtBu (prepared in three steps from Boc-Lysine) with Cbz-L-Lys(epsilon-Bzl)-OMe (prepared in two steps from Cbz-Lysine) in an overall yield of 53% *via* purification by HPLC and ion exchange chromatography.

Conclusion: Herein, we present a robust and economic route of synthesis for lysinonorleucine with standard laboratory equipment in high purity. This derivative as well as other MRPs and AGEs provided by Iris Biotech may serve as analytical tools for the analysis of nutritional quality, body health and disease markers.

Keywords: Lysinonorleucine – Maillard Reaction Products (MRPs) – Advanced Glycation End Products (AGEs) – Analytical Standard – LNL

Reference: Lysinonorleucine. A new amino acid from hydrolyzates of elasin; C. Franzblau, B. Faris, R. Papaioannou; *Biochemistry* 1969; 8(7): 2833-2837. https://doi.org/10.1021/bi00835a021.

Poster 4

Title: Urinary levels of biomarkers of oxidative stress, DNA damage, dicarbonyl stress and glycation are increased in diabetic rats; simultaneous quantitative analysis by ultra-performance liquid chromatography tandem mass spectrometry

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Background and aims: Oxidative- and dicarbonyl stress, and non-enzymatic glycation have attracted increasing attention as biomarkers since they have been found to play a major role in the pathogenesis of multiple diseases including diabetes and cardiovascular disease. Numerous techniques have been developed to quantify biomarkers reflecting these putative stress factors in disease. However, most of these (single analyte) techniques show several shortcomings such as low selectivity and specificity. In this study, we developed and validated an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for the simultaneous quantification of methylglyoxal (MGO), N_ε-(Carboxyethyl)lysine (CEL), N_ε-(Carboxymethyl)lysine (CML),methylglyoxal-derived hydroimidazolone (MG-H1), pyrraline (Pyr), malondialdehyde (MDA), 3-nitrotyrosine (3-NT), 8-oxo-2'-deoxyguanosine (8-oxodG), deoxyguanosine (dG), N2 -carboxyethyl-2'-deoxyguanosine (CEdG), and glyoxal-derived lysine dimer (GOLD) in urine, and applied this method to assess the effect of diabetes on urinary concentrations of these biomarkers.

Materials and methods: For the quantification of these biomarkers, 50 µL of urine was mixed with internal standard mix and derivatized with acidified o-phenylenediamine and subsequently separated on a UPLC C18-column using ion-pair solvents. All biomarkers were detected in positive multiple-reaction-monitoring mode, with MGO and MDA as quinoxaline adducts. Urinary levels of all biomarkers were measured in a rat model of diabetes. Diabetes was induced by a single tail vein injection with streptozotocin. After 24 weeks, urine samples were collected from non-diabetic rats (n=9) and from diabetic rats (n=8). Moreover, the effect of glyoxalase-1 (GLO-I) on these markers of stress was investigated using GLO-I transgenic diabetic rats (n=8).

Results: All biomarkers were successfully separated and detected with UPLC-MS/MS with a run-to-run time of 14 minutes. Linearity of all markers was tested in water and urine matrix and showed good correlation ($r2 > 0.99$) with an intra- and inter-assay coefficients of variations (CV, %) of ~5%. The method was applied for the analysis of all biomarkers in urine samples of rats. All biomarkers were successfully quantified and significantly higher in urine samples of diabetic rats as compared to non-diabetic controls (increase of ~3 to ~1000 fold; $p<0.05$). Although overexpression of GLO-I decreased urinary levels of MGO, CEL, MG-H1, MDA, 3-NT, 8-oxodG, dG, CEdG, and GOLD as compared to diabetic rats, this was only significant for MG-H1 ($p<0.05$).

Conclusion: The presented method proved to be suitable for the simultaneous quantification of MGO, CEL, CML, MG-H1, Pyr, MDA, 3-NT, 8-oxodG, dG, CEdG, and GOLD in urine and could act as an important tool for the detection of non-invasive biomarkers of oxidative- and dicarbonyl stress, oxidative damage to nucleic acids and glycation. All these biomarkers are increased in urine of diabetic rats. The present study will stimulate the clinical utility of these biomarkers and further investigations of the effects of oxidative- and dicarbonyl stress, DNA damage and glycation in the initiation and progression of diabetes.

Poster 5

Title: Rapid pretreatment for multi-sample analysis of AGEs

Authors: Sayuri Kato, Ikuho Ban, Yoshitaka Hiraoka, Nana Katsuta, Yuki Tominaga, Mime Nagai, Hikari Sugawa, Ryoji Nagai
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Background and aims: Currently, it is gradually revealed that advanced glycated end-products (AGEs) are involved in diabetes as well as in various diseases such as osteoporosis and schizophrenia. Many clinical samples need to be measured to clarify the relationship between AGEs and diseases. However, multiple preparation steps such as acid hydrolysis and cation exchange column are required before analysis, making it time-consuming, thereby limiting the number that can be handled at a time. In this study, we attempted to develop a prototype of a pretreatment device to reduce human error and increase the throughput.

Materials and methods: Hydrolyzed calf serum was subjected to cation exchange column and the adsorption efficiency of AGEs such as N_ε-(carboxymethyl)lysine (CML), N_ε-(carboxymethyl)arginine (CMA), N_ε-(carboxyethyl)lysine (CEL), and N_δ-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1) and native amino acids, lysine and arginine, was measured by changing the pressure of decompression pump, which were compared with regular handling. Furthermore, the levels of serum AGEs were compared between normal and diabetic rats.

Results: A conventional vacuum manifold with a water aspirator reached a negative pressure of approximately -30 kPa, whereas the pretreatment device with the diaphragm-type pump reached up to -80 kPa. The measurement of serum AGEs by LC-electrospray ionization (ESI)-quadrupole TOF (QTOF) analysis demonstrated no significant difference in area ratios between the conventional method and pretreatment device. The levels of AGEs in mouse serum measured by the pretreatment device were significantly increased by the induction of diabetes.

Conclusion: The adsorption efficiency of AGEs on the cation exchange column did not change by changing the level of decompression, suggesting that the use of a pretreatment device may stabilize AGE analysis and improve the throughput.

Poster 6

Title: Identification of melibiose-modified amino acids in model MAGEs formed under dry state and conventional glycation in solution

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Background and aims: In our previous studies, we have demonstrated the novel unconventional AGE antigen accumulating in several tissues of a human body [Sci. Rep. 2021. doi:10.1038/s41598-021-82585-7]. Its structural *in vitro* obtained analogous adduct (MAGE) forms during glycation of different proteins by melibiose (mel) in anhydrous conditions and is different from the antigen formed in solution from the same substrates. MAGE structure consists a mel moiety in 2 different conformations, however specific amino acids on the carrier protein modified by mel remain to be determined. The aim of this study was to further characterize MAGE formed *in vitro* on myoglobin (MB) and to identify an extend and the specific amino acid residues modified with melibiose.

Materials and methods: The model MAGE product was obtained by glycation of equine myoglobin with melibiose during microwave synthesis in dry state or using conventional glycation conditions in solution. Melibiose-glycated and the unmodified MB carrier protein were prepared by the Filter-Aided Sample Preparation (FASP) method using the LysC enzyme and were subjected to LC-MS/MS analysis. The obtained data were analyzed with the MaxQuant program to identify the mel-modified amino acids.

Results: Our results confirmed that the model MAGE formed *in vitro* on MB under dry conditions consists the melibiose molecules covalently bound to several amino acid residues. The modifications by melibiose were identified at 3 lysine residues, 1 arginine residue and 1 histidine residue of a MB protein. The peptide containing melibiose-modified arginine showed the highest intensity (153760,0 AU). This type of modification was not found on the unmodified MB. There was substantially less mel bound to MB in the product synthesized under conventional glycation conditions, in which mel-modifications were found at 3 histidine residues and 2 lysine residues, with the average peptide intensity of 43,5 AU.

Conclusion: MAGE adduct forms on MB by coupling of mel to lysine, arginine, and histidine more efficiently under dry glycation than in conventional glycation conditions in solution.

Keywords: melibiose, MB glycation, glycation in dry conditions, mass spectrometry.

Poster 7

Title: Understanding structural microheterogeneity effects on peptide glycation

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Background and aims: Metabolic stress, as seen in hyperglycemia, promotes glycation reactions, which are associated with a multitude of diseases. The intricate reaction cascade of glycation is often detrimental causing accumulation of irreversibly formed advanced glycation end products and thus increased morbidity and mortality of patients with diabetes. Many previous studies analyzing the health effects of glycation products and peptides point to their miscellaneous bioactivities and their potential as nutraceuticals and functional food ingredients. However, large-scale non-targeted peptidomics studies on glycation modifications are constraint by the complexity of the reaction cascade and the enormous peptide heterogeneity. The amino acid composition and sequence specific reaction behavior of peptides in glycation is an under-explored dimension for mass spectrometry based Maillard studies.

Materials and methods: To investigate the interplay between the nature of the peptide and the reaction behavior, we used a longitudinal design and measured complex glucose peptide-model systems with ultrahigh-performance liquid chromatography coupled to a high-resolution quadrupole time-of-flight mass spectrometer. Combination of mass spectrometry, bioinformatics and statistics enabled molecular-level investigation of spray-dried whole casein digests in early glycation reactions, and database search serves as a reference for investigation of sensory- and bioactive peptide reaction behavior.

Results: Analyzing time-resolved Amadori product formation, we explored sequence-reactivity interrelations for 264 casein derived peptides. Statistics assisted intensity profiling together with in-depth computational sequence deconvolution resolved microheterogeneity related differences in susceptibility towards the early phase of glycation. This combinatorial approach revealed that peptides with (iso-)leucine adjacent to the N-terminus are highly susceptible to non-enzymatic modification. Moreover, we used *in silico* sequence mapping to show that particularly reactive peptide collectives converge on potentially important sequence patterns. These patterns contain three amino acids, feature highly similar physicochemical properties and appear in several sensory-active and bioactive peptides from diverse origins. Database search of the herein investigated peptides identified multiple matches and highlighted sequence co-occurrence on peptides from independent sources, which suggests system-wide applicability of our results.

Conclusion: Together, we propose that in peptide glycation the identified molecular checkpoints can be used as indication for sequence reactivity. For selection of suitable peptide candidates, we provide (1) a checklist for estimation of their reaction behavior in early glycation according to the N-terminal amino acid, the neighboring sequence position and presence of relevant sequence patterns, and (2) screening for established sensory attributes and bioactivities.

Keywords: Peptides, glycation, amino acid sequence, Amadori products, mass spectrometry.

Poster 8

Title: Quantitative TD-GC-MS for measuring volatile compounds during processing of food - application to baking
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Background and aims: The generation of numerous newly-formed compounds during thermal treatments has strong implications for food quality and safety. Since many quality related compounds are volatile, there is a need for robust methods that can quantify a broad range of volatile markers and are applicable to on-line or near-the-line monitoring.
The aim of the present work was to develop and optimize quantitative TD-GC-MS with in-tube calibration and isotope standard addition. It was applied and validated for determining 10 process-induced compounds sampled on-line during the baking of a model cake.
Materials and methods: The stock solution of the analytes contained 3-methylbutanal, pyrazine, 2-methylpyrazine, 2,5-dimethlyprazine, 2,6-dimethlyprazine, acetic acid, furfural, 5-methlyfurfural, furfuryl alcohol and 5-hydroxymethylfurfural. The stock solution for the internal standards (*d*IS) contained d4-furfural and d4-pyrazine. The working solution of *d*IS was prepared by diluting a proper amount of stock *d*IS solution. Likewise, working solutions of analytes at 5 concentrations were obtained by diluting the stock solution. Calibration solutions containing a (constant) concentration of *d*IS and increasing concentrations of analytes were set at the same analyte concentration levels as the previous solutions. Air Toxics™ sorbent tubes were used to collect the internal atmosphere of the oven during baking and were then desorbed. The desorbed volatile compounds were analyzed by GC-MS. Chromatographic peak areas for each compound were calculated by extracting the quantifier ions from the SIM mode acquisition data. Calibration curves for each compound were built using the ratio of the chromatogram peak areas (analyte vs *d*IS) plotted against the corresponding concentration. Different criteria were assed to develop the method: desorption time of tubes, repeatability of the spiking modes, reliability of the in-tube *d*IS introduction. Linearity, LOD, LOQ and the matrix effect were evaluated for the developed method.
Results: Vapor and liquid spiking were compared for the 10 analytes. Liquid spiking was chosen as it proved to be more repeatable. For vapor sampling, a step of spiking of the *d*IS solution just after trapping the volatile compounds from the gaseous phase was added. The compared responses of *d*IS for the calibration (where analytes and *d*IS were present in the same solution and simultaneously spiked) and for sampling tubes (where analytes were spiked first, then the *d*IS in a second solution) validated the in-tube spiking method for deuterated internal standards after the online sampling step. The TD-GC-MS method displayed good linearity over extended range of concentrations for all studied compounds (R^2: 0.9950 to 0.8880) with low limits of quantification (LOQs) ranging from 0.0141 to 11.5 ng. The matrix effect was negligible for most compounds, except for 5-hydroxymethylfurfural (21.5%). The method was then applied to analysing the compounds generated by a thermal treatment and sampled from vapors during the baking of a model cake.
Conclusion: On-line sampling coupled to TD-GC-MS with isotope standard addition stands out as an accurate and highly appropriate method to monitor a broad range of concentrations of process-induced compounds with different generation and release behaviors during the baking of a food matrix. This method is sufficiently sensitive to be suitable for such reaction dynamics over time.

Keywords: thermal desorption, aroma, on-line sampling, Maillard reaction.

Poster 9

Title: Quantification of Methylglyoxal derived hydroimidazalone in Food Products: Re-evaluation of Acid Hydrolysis

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Background and aims: Methylglyoxal derived hydroimidazolones (MG-H) are major advanced glycation end-products (AGEs) in human proteome. Recently, there is an increasing amount of publications reporting that MG-H1 could be the most abundant AGE formed during food processing. Mostly enzymatic hydrolysis has been applied for the analysis of MG-H isomers however, some researchers apply acid hydrolysis for the quantification of MG-H1 in food products. This study aimed to investigate the applicability of acid hydrolysis for the analysis of MG-H and to investigate the abundance of MG-H in several food products.

Materials and methods: The applicability of acid hydrolysis for the analysis of MG-H was tested by determining the acid stability of each MG-H isomer (MG-H1, MG-H2 and MG-H3) during microwave and conventional acid hydrolysis. A set of standard solution (1 to 1000 ng/mL) was heated in 6 M HCl in the microwave oven (1 min 150°C + 10 min 165°C) and in conventional oven (23 hours 110°C), then were analysed by LC-MS/MS. Calibration curve was prepared for each standard compound. Acid stability of each compound was determined according to equation (1).

$$\text{Acid stability (\%)} = 100 * (\text{slope}_{\text{acid-treated standard}} / \text{slope}_{\text{standard in water}}) \qquad (1)$$

Several food products) were hydrolysed by using 6 M HCl in microwave oven and were analysed by LC-MS/MS. $[M+H]^+$: 229.1295 and m/z: 114.0665 were monitored for compound identification and quantification, respectively.

Results: It was found that after acid-treatment, the slope of the calibration curve for MG-H1 increased more than 100%, indicating that is not degraded during acid hydrolysis. To understand what happens during hydrolysis, MS/MS spectra of each isomer were investigated before and after acid treatment. We have found that higher-energy C-trap dissociation (HCD) fragments of each isomer are very distinctive. We have postulated that fragmentation follows three pathways under the MS/MS analysis. The ratio of the intensities of fragment ion at m/z 114 to m/z 116 are different for each isomer; 1.33 for MG-H1 and 0.75 and 3.54 for MG-H2 and MG-H3, respectively. However, it increased to 2.40 and 2.81 for MG-H1 after microwave and conventional hydrolysis, respectively. This result clearly indicated that MG-H1 is converted to MG-H3 when heated with strong acid. Since m/z 114 ion is used for quantification, the increased slope for the MG-H1 calibration curve after acidic hydrolysis treatment could be well explained. Since it was not possible to separate the isomers chromatographically, it was not possible to quantify the exact concentration of individual isomers. Therefore, the concentrations of MG-Hs in food products were expressed as MG-H3-equivalents, since we have proven the transformation of MG-H1 to MG-H3. Our results could help to understand why different studies show varying concentrations of MG-Hs in food products.

Conclusion: Our study revealed that during acid hydrolysis MG-H1 is converted to MG-H3; therefore quantification should be based on MG-H3 after acid hydrolysis. More research is needed to understand the extent of interconversion of isomers during acid hydrolysis before acid hydrolysis could be considered as a reliable sample preparation technique.

Keywords: Methylglyoxal derived hydroimidazolone, acid hydrolysis, stability, food products.

Poster 10

Title: Acesulfame potassium: an artificial sweetener with anti-AGE property

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Background and aims: The noncovalent interaction of amino groups of proteins and carbonyl group of sugars lead to formation of advanced glycation end products (AGEs). The rate of formation of AGEs increases during the hyperglycaemic conditions. These products are involved in many metabolic and neurodegenerative disorders. In last few decades there has been a several fold increase in the usage of artificial sweeteners in various food products because of their less caloric value and less dependence on sugars to prevent Diabetes. However, there are very few reports on the implications of these sweeteners in the process of Glycation.

Materials and methods: In the present study, the acesulfame potassium (Ace-K), an FDA-approved artificial sweetener, was investigated for its involvement in glycation and aggregation process on the protein (BSA). The glucose-mediated glycation was analysed spectroscopically with fructosamine content by NBT method and carbonyl content by DNPH method. The AGEs and aggregation (Th T) of glycated products were assayed fluorometrically. Glycation-induced-secondary structure alteration of BSA was analysed with Circular Dichroism. Further, DLS, TEM, and SDS-PAGE analysis were performed to analyse the aggregation of proteins.

Results: Ace-K decreased the Amadori products and carbonyl content by 65.33% and 63.38% at 28 days incubation in comparison to glucose-mediated glycation of BSA. The presence of Ace-K also caused only 41.27% AGEs and 42.87% cross β-amyloid structures formation. TEM analysis indicated the size range of 50-300 nm of glycated BSA in presence of Ace-K. The secondary structure of BSA shown to be like native protein in the presence of Ace-K in the glycation system. Similarly, migration patterns of glycated BSA in the presence and absence of Ace-K indicted the antiglycating role of this sweetener.

Conclusion: The decrease in the amount of Amadori products, carbonyl content, and total AGEs, inhibition of glycation-induced aggregation and maintenance of secondary structure of glycated proteins in the presence of Acesulfame potassium indicate the potential antiglycating property of this artificial sweetener.

Keywords: Acesulfame potassium, Aggregation, AGEs, BSA, Glycation, Thioflavin T.

Poster 11

Withdrawn.

Poster 12

Title: *In-situ* generation of methylglyoxal scavenging agents from histidine and carnosine side chains

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Background and aims: Histidine has been shown to be an efficient anti-crosslinking agent along with carnosine. The ability of the initial Schiff base formed between histidine and carbonyl compounds to undergo Pictet-Spengler type reaction and subsequently form volatile pyrido[3,4-d]imidazole derivatives has been studied. However, the nature of non-volatile reaction products when histidine derivatives were reacted with carbohydrate-derived carbonyl compounds such as methylglyoxal has not been identified. Considering the crucial role of these carbonyl moieties *in vivo* and *in vitro,* understanding the chemical pathways through which histidine can scavenge these reactive precursors during the processing of foods may provide promising insight into their application strategies.

Materials and methods: Aqueous methanolic model systems containing histidine or histamine in the presence of glucose, methylglyoxal, or glyoxal were prepared at 1:1 or 1:2 molar ratio with excess 1,2-dicarbonyl, and either heated at 150 °C for 1.5 h or were kept at RT for a week and analyzed using ESI-qTOF-MS/MS and isotope labelling technique. Glucose was replaced with [U-^{13}C$_6$]glucose to identify the glucose carbon atoms incorporated in the products.

Results: Various sugar-generated carbonyl compounds ranging in size from C1 to C6 carbons were captured by histidine or histamine. The majority of the fragments incorporated were either C3 or C2 units originating from glyoxal (C2) or methylglyoxal (C3). The ESI-qTOF-MS/MS analysis indicated that histamine could react with either of the two carbonyl carbons of methylglyoxal utilizing the α-amino group and/or the imidazolium moiety. Furthermore, when histidine was added to 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP) generating model system, it completely suppressed the formation of PhIP due to scavenging of phenylacetaldehyde.

Conclusion: The carbonyl scavenging activity of histamine generated from degradation of histidine or carnosine under the Maillard reaction conditions was studied and results confirmed further the importance of the concept of in-situ generation of carbonyl scavenging agents from amino acid side chains as a promising strategy to mitigate the accumulation of toxic compounds in food products.

Keywords: Histidine, histamine, 1,2-dicarbonyl compounds, [U-^{13}C$_6$]glucose, scavenging activity.

Poster 13

Title: Thermal processing induces glycation of endogenous milk peptides

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Background and aims: Thermal processing of milk promotes the Maillard reaction yielding highly diverse protein-bound glycation products. Besides proteins, which have been studied extensively as targets for glycation, milk also contains a great variety of endogenous peptides with many of them carrying known biological functions. Although native peptides present in raw milk may also be modified by the Maillard reaction affecting their biological functions, this has not been studied so far. For instance, a peptide with anti-inflammatory properties might trigger the receptor for advanced glycation endproducts (RAGE) after glycation thereby inducing a pro-inflammatory response although being anti-inflammatory in its unmodified state.

In order to evaluate the influence of industrial processing of milk on its endogenous peptidome, native unmodified and glycated peptides were identified in unprocessed milk, ultrahigh temperature (UHT) treated milk as well as in first-stage infant formula (IF).

Materials and methods: Endogenous peptides were extracted from milk samples by a chloroform/methanol/water procedure, followed by solid phase extraction and nanoRPC-ESI-MS/MS analysis. As for endogenous peptides no specific enzyme is selected for the database searches, a complex identification strategy was applied to reduce the number of false positive identifications. Thus, for the confident identification of peptides, only peptides proposed by two independent software were considered. Moreover, acquired tandem mass spectra of proposed glycated peptides were confirmed manually.

Results: Overall 801 unmodified and 175 modified endogenous peptides were identified in the analysed milk samples. Interestingly, unmodified peptides originated mainly from α_S- and β-caseins, whereas modified peptides were mainly derived from both α_S-caseins. Only five modified β-casein peptides were identified. Additionally, a smaller number of low abundant milk proteins were associated with glycated endogenous peptides. Thermal processing induced a significant increase in the number of modified peptides. Compared to unprocessed raw milk the number of modified peptides increased two-fold in UHT and even four-fold in IF. The majority of modified peptides carried a lactosylated lysine residue, following a very similar trend as reported at the protein level applying a bottom-up proteomics strategy.

Conclusion: Heat treatment of milk leads not only to the formation of protein-bound glycation products, but also modifies its native peptidome inducing mainly lactosylation. In particular, α_S-casein derived peptides are prone to lactosylation with the numbers of modified peptides increasing in accordance with the harshness of applied processing conditions. Further studies should evaluate how glycation of endogenous milk peptides affects their known biological functions.

Keywords: Glycation, Milk, Endogenous peptides, Mass spectrometry.

Poster 14

Title: Effect of leavening agents on Maillard reaction products in the crust of traditional French bread.

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Background and aims: Bread is widely consumed throughout the world, and is considered as a staple food in many countries. In France, bread is the main source of carbohydrates in the diet (45 kg/year/person). Among all types of bread, yeast and sourdough breads are the most popular in France. They are both considered by French people as "healthy" and essential to maintain a balanced diet and their sensory qualities are crucial for their approval by consumers.

During baking, the Maillard reaction (MR) produces neo-formed compounds (MRPs) that contribute to the development of the aroma, taste and color of the bread. In the crust, key odorants are mainly generated by the Strecker degradation, an intermediate step in the Maillard reaction. The Strecker degradation converts α-amino acids into structurally related aldehydes and mainly depends on the availability of free α-amino acids in the dough.

Melanoidins, another type of MRPs, generated during the last stages of the Maillard reaction, are responsible for the color of the crust. These pigments are suggested to have health benefits (anti-inflammatory and bifidogenic effects). Their formation mechanism remains partly unknown and, so far, three theories on melanoidins synthesis have been described. This study is aimed at evaluating the effect of leavening agents on the formation of Strecker aldehydes and melanoidins in the crust of yeast and sourdough breads.

Materials and methods: Yeast bread (YB) and sourdough bread (SB) were prepared according to an artisanal process. In the dough, fermentable sugar contents were determined by HPLC-ELSD and protein fractions were analysed by SDS PAGE. In the crust, volatile compounds were semi-quantified by GC-MS and water-soluble melanoidins were analysed by a HPLC system coupled to a fluorescence detector.

Results: The MR precursors were first measured in the dough. SDS PAGE analysis showed that gluten proteins were more degraded during SB fermentation. This result could be explained by the activation of flour proteases under acidic conditions. As for fermentable sugars, they were more consumed by yeast in YB than by lactic acid bacteria in SB after 26h of fermentation. Thus, before baking, the Maillard reaction precursors were predominant in the dough of SB compared to YB.

In the crust, key odorants generated from the Maillard reaction, especially Strecker aldehydes, were much more concentrated in SB than in YB. For instance, relative concentrations of 2-methyl-butanal and 3-methyl-butanal were ten times higher in SB than in YB. This result could be explained by the degradation of gluten proteins during SB fermentation releasing free amino-acids, the main reactants of the Strecker degradation.

Conversely, the level of melanoidins produced in the crust was significantly higher in YB than in SB for the same baking time and dough hydration. Thus, the melanoidization pathway seemed to be privileged in YB.

Conclusion: Both types of leavening agents had a significant effect on the Maillard reaction pathways. The Strecker degradation could direct the Maillard reaction towards aroma generation rather than melanoidins formation, thus affecting the sensory quality of the crust. Further experiments will be conducted to evaluate the links between these MRPs and their potential health benefits for consumers.

Key words: French bread, Fermentation, Strecker degradation, Melanoidins.

Poster 15

Title: Anti-glycation stress effect of black beans (Glycine max 'Kuromame')
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Background and aims: Glycation is a non-enzymatic reaction between reducing sugars and amino group proteins and forms advanced glycation end products (AGEs). AGEs are accumulated in diverse tissues and organs. Along with aging, AGEs induce inflammations, pigmentations and deuteration of physiological functions, and is involved in the onset and progression of life style related diseases. This study verified the inhibitory actions of black soybean extract on glycation.

Materials and methods: As samples, 1 soybean (Glycine max (L.) Merr.) variety and 8 black soybean (Glycine max 'Kuromame') varieties, and 6 isoflavones (3 types of aglycone: daidzein, genistein, glycitein and 3 types of glycosides: daidzin, genistin, and glycitin) were used. The bean samples, obtained in markets, are crushed using a food processor and then extracted with hot water at 80 °C for 1 hour to obtain an extract. As additional specimens, extracts were prepared after dividing the beans into seed coats and cotyledons. Each extract is added to human serum albumin and glucose, then incubated at 60 °C for 40 hours, and the fluorescence value of excitation wavelength 370 nm / detection wavelength 440 nm is measured, followed by calculating the inhibition activity on fluorescence AGE formation (%).The antioxidant activity was evaluated by calculating Trolox equivalent/L based on the DPPH radical scavenging activity method. The contents of various isoflavones in the extract were measured by reverse phase HPLC.

Results: Inhibitory actions on glycation was observed in all samples of soybean and black bean. Among black bean extracts, Kurosengoku showed the maximum inhibitory action with 85.3 ± 2.5% (mean ± standard deviation). Comparing the seed coat and the cotyledon, the former showed a higher inhibitory activity than the later in all black soybean samples, and the maximum value was 99.68 ± 0.28% in the seed coat and 58.46 ± 1.05% in the cotyledon. The isoflavone content was higher in the seed coat than in the cotyledon. Antioxidant activity was observed in all varieties of black beans. Among them, Kurosengoku showed the maximum activity, 286.14 µmol / L-Trolox equivalent / µL at solid content concentration 7.5 mg / mL.

Conclusion: It was suggested that Black bean is a functional food with both anti-glycation and anti-oxidation actions.

Poster 16

Title: Glycation Stress Suppressing Actions of Soup Stock "Dashi"
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Background and aims: Accumulation of AGEs (advanced glycation end product) due to glycative stress causes aging and illness. Methods for reducing glycative stress can be divided, according to the mechanism, into ameriolation of postprandial hyperglycemia, inhibition of glycation reaction, and promotion of decomposition / excretion of AGEs. In this study, we investigated the inhibitory actions on glycation by soup stock "dashi", which is used for various dishes in Japanese cuisine.

Materials and methods: Three types of commercially available samples were used: dried bonito (karebushi, arabushi) and niboshi, from which 0.5%, 2.5%, 7.5% and 20% dashi were prepared by extraction according to the method described on the package. Using a human serum albumin glucose glycation model, the inhibitory action of each sample was verified using the amount of fluorescent AGEs, CML, pentosidine, and intermediates (3DG, glyoxal, methylglyoxal) as indicators. Regarding karebushi, the extract was fractionally purified by gel filtration chromatography, and the inhibitory actions were verified for each fraction.

Results: The inhibitory action on formation of fluorescent AGEs was observed in karebushi and niboshi, while actions on CML and pentosidine were observed in all three samples. No action on 3DG was observed in these samples. The actions on glyoxal formation was observed only in karebushi, while the action on methylglyoxal was noted in karebushi and niboshi. Comparing the fractions of karebushi by gel filtration chromatography. The molecular weight of the substance, contained in the fraction with the greatest inhibitory action on pentosidine formation, was estimated to be 433.

Conclusion: The glycation inhibitory effect was observed in dashi samples, and it was speculated that the pathway that suppresses the formation of AGEs differs depending on the type of dashi. Whereas, it was suggested that dashi may promote the formation of 3DG. A similar phenomenon has been observed in whey, and it is presumed that the component that suppresses the AGEs formation in whey is the lactobacillus decomposition product of milk protein. The component that suppresses the AGE formation, like whey, may be a mold decomposition product of fish protein. Dashi has the action of suppressing the glycation reaction, and may contribute to afford new functionality to Japanese cuisine.

Poster 17

Title: *In vitro* fortification of N^ε-carboxymethyl-lysine in bovine serum albumin model systems.

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Background and aims: Different strategies are used to investigate the biological effects of dietary advanced glycation end-products (AGEs), among which N^ε-carboxymethyl-lysine (CML) is often tested using samples consisting of free or protein-bound CML. The latter is usually prepared by incubating a protein with glyoxylic acid (GA) and $NaBH_3CN$. But this process also forms toxic HCN which may not be fully eliminated after dialysis, prompting us to investigate alternative ways of generating CML-fortified protein.

Materials and methods: The first experiment compared the efficiency of 3 reducing agents, $NaBH_4$, $NaNH_3CN$ and $NaBH(OAc)_3$, when added to a mixture of bovine serum albumin (BSA) and GA. A second compared the amount of CML formed in BSA using different concentrations of ribose and glyoxal without a reducing agent. Each was prepared in a phosphate buffer (0.2M, pH 8.0) and incubated at 37°C for different periods. After incubation each sample was dialysed, and analysed by western blot and LC-MS/MS after acid hydrolysis. A proteomic characterization then followed on the most promising samples.

Results: Compared to the classical reducing agent $NaBH_3CN$, the two other agents showed no high degree of lysine modifying into CML in BSA: 37% for $NaNH_3CN$ vs 3.5 & 1.6% for $NaBH_4$ & $NaBH(OAc)_3$, respectively. After 4 days at 37°C and without reduction, the incubation of BSA in the presence of ribose alone or ribose + glyoxal, induced a total loss of 35 to 57% of lysine with different lysine : ribose : glyoxal molar ratios. The percentages of CML formed on lysine were between 1 and 2%. The formation of AGEs other than CML was demonstrated not only by the high loss of lysine, but also by the increased fluorescence intensity and the formation of BSA polymers.

The CML fortification of BSA with increasing concentrations of glyoxal alone, under the same conditions as above, formed CML in proportion to the glyoxal concentration: 0.5, 1.0, 4.5, 9.8 & 18% of lysine with 20, 40, 100, 200 and 300 mM glyoxal, respectively. The BSA polymers also formed in proportion to the glyoxal concentration. The BSA glycated with glyoxal at 300mM was further characterized. In addition to the 18% of lysine transformed into CML, 15% more of the lysine and 55% of the arginine were chemically modified. Proteomic analyses identified GOLD (a lysine-lysine dimer) and N^ε-carboxymethyl-arginine in this preparation.

Conclusion: The glycation of BSA with 300mM glyoxal alone is a suitable alternative to GA with $NaBH_3CN$ as a reducing agent, and yields a model protein free of HCN with 18% CML compared with about 30% using GA + $NaBH_3CN$**.** It must be noted that all tested methods, including using glyoxylic acid, led to the formation of AGEs other than CML. A model protein selectively fortified only with CML remains to be discovered.

Poster 18

Title: Reactions of dicarbonyl compounds during simulated digestion of proteins
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Background and aims: Substantial amounts of dicarbonyl compounds, formed during food processing, are ingested with our daily diet. Physiological and toxicological consequences resulting from this intake are currently under debate. Due to their high reactivity, dicarbonyl compounds may form advanced Maillard reaction compounds during digestion. Corresponding reactions with dietary amino acids and proteins in the intestine offer the potential of a "scavenging effect" for dicarbonyl compounds. As additional part of a study presented by Treibmann et al., we were focussing in the current study on reactions (i) of the less reactive but quantitatively more relevant dicarbonyl compounds 3-deoxyglucosone and 3-deoxygalactosone and (ii) with proteins instead of free amino acids.
Materials and methods: Methylglyoxal, 3-deoxyglucosone, 3-deoxygalactosone, amino compounds and proteins were implemented in the study. Simulated digestion experiments consisted of a gastric and an intestinal stage. The quantitation of dicarbonyl compounds was performed with HPLC-UV of chinoxaline derivatives. Further glycation compounds were analysed with LC-MS/MS by using the specific isotopologues.
Results: As a subject of time and the stage of digestion, a decrease of dicarbonyl compounds was observed for all the dicarbonyl compounds of interest. The formation of the respective reaction products was observed simultaneously depending on the reactivity of the involved amino and dicarbonyl compounds.
Conclusion: The data suggest that digestive glycation compounds are also formed in systems containing 3-deoxyglucosone, 3-deoxygalactosone and protein that resemble food more closely. Hence, a proportion of the dicarbonyl compounds in food is reacting with amino compounds and proteins in the bolus and are thus "scavenged" and not available for reactions with endogenous proteins. Glycation compounds formed during digestion may undergo similar metabolic transit reactions as dietary glycation compounds.

Keywords: Dicarbonyl compounds, simulated digestion, glycation.

Poster 19

Title: Formation of polymeric structures originating from D-galacturonic acid intermediate products

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Background and aims: Previous investigations showed a high browning potential during caramelization of sugar acids in comparison to reducing carbohydrates. For example, the heat treatment of aqueous model-systems of D-galacturonic acid exhibits a ten times higher absorbance at 430 nm compared to that of D-galactose. The main difference between these two carbohydrate systems is the ability of D-galacturonic acid to decarboxylate. The major degradation reaction postulated for the release of carbon doxide leads to α-ketoglutaraldehyde, being responsible for the formation of several chromophoric substances e.g., 2,3-dihydroxybezaldehyde. The aim of this study was to investigate the chemical structure and formation mechanisms of the polymeric structures responsible for the strong color formation from D-galacturonic acid under heat treatment.

Materials and methods: Aqueous solutions of D-galacturonic acid and various degradation products of D-galacturonic acid such as norfuraneol, furfural, and 5-formyl-2-furoic acid were heated in combination with 2,3-dihydroxybenzaldehyde at temperatures of 130 °C for 120 min. The solutions were diluted, and HR-ESI-MS measurements were performed. The mass spectra were processed to gain structure informations with the help of VAN KREVELEN and KENDRICK diagrams.

Results: All model systems showed a significant absorbance at 430 nm after heat treatment and a variety of products resulting in many pseudomolecular ions in the HR-ESI-MS spectra. To simplify the analysis of the complex mass spectra and to identify the structures of the colorants, KENDRICK diagrams normalized to relevant reactants were plotted. Norfuraneol in combination with 2,3-dihydroxybenzaldehyde shows a step-by-step increase of weight starting from a single molecule norfuraneol that reacts in an aldol addition and condensation reaction with 2,3-dihydroxybenzaldehyde. Further additions of norfuraneol and 2,3-dihydroxybenzaldehyde to this molecule could be observed. These aldol addition and condensation products could also be found in model-systems of norfuraneol and 5-formyl-2-furoic acid. A comparison of the detected *m/z* with the HR-ESI-MS spectra of the D-galacturonic acid model system showed that the formed structures are also found within the sugar acid model system.

Conclusion: The reactions of individual D-galacturonic acid degradation products lead to a variety of oligomers consisting of partly conjugated π-system and thus, impact the color formation of D-galacturonic acid**.** Especially the combination of norfuraneol and 2,3-dihydroxybenzaldehyde that yields reaction products containing aromatic substructures gives rise to very potent colorants. The formation of polyphenolic structures such as 2,3-dihydroxybenzaldehyde is unique for uronic acids and thus, may be the reason for the intense color formation in comparison to reducing sugars such as D-galactose.

Poster 20

Title: Mitigation of acrylamide formation in industrial potato crisp manufacturing.

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Background and aims: An ongoing research project (Measurement and Control of Acrylamide (Acr) in Production Processes, MCAP2) involving academic and industrial partners is investigating strategies for the mitigation of Acr formation during potato crisps manufacturing on an industrial scale. The project has applied post-harvest interventions to optimise the process conditions on an industrial scale to identify conditions that lead to reduced Acr levels in potato crisps.
The impact of different washing regimes on potato slice sugar concentration were studied within a crisp manufacturing factory, to optimise sugar removal.
The variation in acr levels that arises in the manufacture of crisps has also been investigated. Historical industrial processing data was analysed to identify manufacturing conditions that lead to high Acr formation during crisps production.
Materials and methods: Glucose, fructose, sucrose and asparagine were measured using the Konelab Arena 30 Biochemical analyser (Thermo Fisher, Cortaboeuf, France). Partial Least Squares Discriminant Analysis (PLS-DA) identified predictors of high acr formation.
Results: Current hot-wash operational residence time of 3.5 minutes at 70˚C gives a sugar reduction of 27.5%, which could be increased to 48.5% by increasing the residence time to 5 minutes**.** Hot-wash temperatures of 40-60 ˚C were found to increase glucose and fructose content, this can be explained by the activity of the enzyme Invertase which converts sucrose to glucose and fructose which has a maximum activity at 10-50 ˚C. Sugar removal from the cold-wash was found more effective than expected with an average reduction of 21%, dependent on initial sugar content. PLS-DA analysis with a probability threshold of 0.75 can identify around half the potatoes with high Acr levels, however 13% of potatoes are incorrectly predicted as high, resulting in unnecessarily costs to mitigate Acr formation. When the threshold is increased to 0.95 around 30% of high Acr values are identified, and the misclassification is reduced to 7%.
Conclusion: The outcome of the washing regimes study led to a new strategy which has the potential to reduce Acr levels, energy use and costs, without impacting consumer experience: a "double cold-wash" has been implemented for loads with sugars <45 mg/L, while for higher sugar loads (>45 mg/L) a 70˚C hot-wash with medium flow rate is applied to increase sugar removal from potato slices. Analysis of data from the manufacturing line using PLS-DA identified and explained a third of high Acr values whilst maintaining a low level of false predictions. The dominance of fructose and asparagine concentrations as descriptors were identified, in alignment with prior literature indications. These findings are an important tool for process operators to adjust process conditions to reduce Acr.

Poster 21

Title: Effects of lupine and chickpea flour on the formation of acrylamide in biscuits

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Background and aims: Acrylamide (AA) is a toxic compound probably carcinogenic to humans, formed mainly by the interaction of asparagine (asn) with reducing sugars as part of the Maillard reaction.

Several studies have investigated the effect of different flours on AA formation in bakery products; being this ingredient the main source of asn, identified as a limiting precursor of this toxic compound. The asn content was not always proportional to the AA levels in the biscuits, indicating that other flour properties also have an impact on its formation. It was suggested that chickpea proteins may reduce AA because they can bind to carbonyls and thus reduce their availability for the reaction with reducing sugars. On the other hand, high fibre content of legume can promote the AA formation by reducing the a_w of the product and thus increasing the Maillard reaction rate. In order to clarify if the differences in AA content are only based on the asn or also related to other matrix properties, the aim of this research was to investigate the effects of wheat and legume flours in biscuits on AA formation by standardizing the initial asn content.

Materials and methods: The biscuit doughs were prepared following the AOAC method using wheat (W), chickpea (C) and lupine (L) flours and adding extra asn to achieve the same concentration (70 mg/kg) in all formulations. The control sample was formulated with 100% wheat flour (W100), the others were prepared by replacing W flour with 20%, 40% and 60% L (L20, L40, L60) or C (C20, C40, C60) flours. All biscuits were baked in an electric oven at 175 °C for 5, 7 and 9 min. The flours were analysed for asn, protein, fibre, ash contents and for a_w, moisture, pH, particle size. The biscuit samples were analysed for AA, asn and sugars contents, as well as for the main quality parameters (a_w, moisture, pH, colour, texture).

Results: The W100 sample showed an increase in AA during baking, reaching 582 μg/kg after 9 min. No significant differences were identified between W100 and L20 samples, whereas L40 and L60 showed higher AA levels, that could be due to the higher fiber content of L flour (40.8%) compared to W one (2.8%). A positive result was obtained with C flour in AA reduction when used at 20% and 40%, confirming the effect of chickpea proteins. However, when C flour was added at 60%, an AA increase was detected, probably because at this high percentage the effect of its fiber content (11.8%) prevails. Generally, AA levels was not always correlated to the amounts of asn and sucrose found in biscuits during baking, supporting the hypothesis that other flour proprieties may influence the formation of AA**.**

Conclusion: The standardization of initial asn content in the different biscuit formulations has been an effective approach to assess the flours effect on AA formation. L flour was not effective in reducing AA in biscuits at all percentages tested. On the other hand, C flour was a promising strategy for the control of AA when used at the lowest proportions here tested. Moreover, the use of low portion of the C flour not substantially changed the main quality characteristics of the final biscuits. However, further research is needed also to evaluate the effect on their sensory characteristics.

Keywords: Acrylamide, Maillard reaction, biscuit, legume, asparagine.

Poster 22

Title: Kinetics of caramelization and Maillard reactions in model cakes enriched in glucose or glucose+leucine during baking
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Background and aims: A multitude of newly-formed compounds generated during the thermal processing of food can have either positive (aroma, color) or negative (health or nutrition-related) impacts on final quality.
However, establishing the link between composition, reactivity and quality determinants is not a simple task because of the interdependency of physical and chemical parameters and the complexity of real food ingredients. Studying complex transformations in a food matrix under strictly controlled physical, structural and chemical conditions is therefore paramount value.
This study aimed to discuss kinetic concentration data for 12 markers (precursors, α-dicarbonyl intermediates and furanic compounds) measured during the baking of model cakes containing glucose with or without leucine as reactants in order to unravel reaction pathways and verify the hypotheses based on many decades of results obtained in simple liquid systems (far from real foods) or in real products (albeit with a limited understanding of specific reaction pathways).
Materials and methods: An inert model imitative of a sponge cake was developed in order to master the nature and the quantity of reaction precursors. The model cake was added with targeted precursors to activate thermal reactions in a controlled way (caramelization in model *G* - model containing glucose; both caramelization and Maillard reactions in model *G+L* - - model containing glucose and leucine). *G* and *G+L* models were submitted to controlled process conditions (140°C, 170°C or 200°C baking temperatures; high and low convection levels). Monosaccharides and furanic compounds were extracted and quantified by UHPLC--CAD and UHPLC-DAD, respectively. The concentrations of the free amino group from leucine were measured by basic titration using the Sörensen method. α-dicarbonyl intermediates were extracted and quantified by UHPLC-MS. The level of browning was determined by measuring the absorbance of extracted water-soluble brown pigments.
Results: The consumption of glucose was higher and more rapid with increasing temperature and in the presence of leucine. With both the *G* and *G+L* formulas, fructose was measured during the baking time, with amounts and trends that depended on the oven temperature. This finding confirmed that fructose can form easily from glucose in both the caramelization and Maillard reaction pathways in model cakes. The kinetics of α-dicarbonyl intermediates were bell shaped, typical of the simultaneous formation and degradation of intermediate products and significantly accelerated by temperature. All of these compounds were found in relatively small quantities (in the order of $\mu mol.g_{DM}^{-1}$, when the initial concentrations in glucose and leucine were in the order of $mmol.g_{DM}^{-1}$). In both model cakes, the kinetics of furfural and 5-hydroxymethylfurfural formation were very similar, but 5-hydroxymethylfurfural was found in much larger quantities than furfural. The browning was also more pronounced and rapid in the presence of leucine at all the temperatures tested. A precursor specific reaction scheme was confronted to the experimental data in order to unravel reaction mechanisms.
Conclusion: Working with two identical food models that contain either glucose or glucose and leucine as reactants made it possible to unravel the caramelization pattern and the activation of specific pathways by the Maillard reaction.

Keywords: deoxyosone, reaction pathways, heat transfer, kinetics.

Poster 23

Title: Higher habitual intake of dietary dicarbonyls is associated with higher concentrations of corresponding plasma dicarbonyls and with skin autofluorescence: the Maastricht Study

Authors: Kim Maasen, Simone JPM Eussen, Jean LJM Scheijen, Carla JH van der Kallen, Pieter C Dagnelie, Antoon Opperhuizen, Coen DA Stehouwer, Marleen MJ van Greevenbroek and Casper G Schalkwijk

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Background and aims: Dicarbonyls are highly reactive compounds and major precursors of advanced glycation endproducts (AGEs). Both dicarbonyls and AGEs are associated with development of age-related diseases. Dicarbonyls are formed endogenously, but also during food processing. To what extent dicarbonyls from the diet contribute to circulating dicarbonyls and accumulation of AGEs in tissues is unknown. Therefore, in this study we examined associations of dietary dicarbonyl intake with plasma dicarbonyl concentrations and skin AGEs accumulation.

Materials and methods: In 2566 individuals of the population based Maastricht Study (age: 60 ± 8 yrs, 50% males, 26% type 2 diabetes), we estimated habitual intake of the dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG), by combining Food Frequency Questionnaires with our dietary dicarbonyl database of MGO, GO, and 3-DG concentrations in >200 commonly-consumed food products, measured by UPLC-MS/MS. Fasting plasma concentrations of MGO, GO, and 3-DG were measured by UPLC-MS/MS. Skin AGEs were measured as skin autofluorescence (SAF), using the AGE Reader. Cross-sectional associations of dietary dicarbonyl intake with their respective (ln-transformed) plasma concentrations and SAF (all standardized) were examined using linear regression models, adjusted for age, sex, glucose metabolism status, kidney function, history of cardiovascular disease, BMI, smoking, alcohol intake, physical activity, lipid modifying, anti-hypertensive, and glucose lowering medication, education, and total energy intake.

Results: Median intake of MGO, GO, and 3-DG was 3.6, 3.5, and 17 mg/day, respectively. Coffee was the main dietary source of MGO, whereas this was bread for GO and 3-DG. In the fully adjusted models, dietary MGO was associated with plasma MGO (β=0.08, 95%CI [0.02;0.13], p=0.004) and with SAF (β=0.12 [0.07;0.17], p<0.001). Dietary GO was associated with plasma GO (β=0.10 [0.04;0.16], p=0.001) but not with SAF. 3-DG was not significantly associated with either its plasma concentration or SAF. These associations did not change after additional adjustment for individual macronutrients and the Dutch Healthy Diet Index.

Conclusion: Higher habitual intake of dietary MGO and GO, but not 3-DG, was associated with higher corresponding plasma concentrations. Higher intake of MGO was also associated with higher SAF. These results suggest dietary absorption of MGO and GO. Biological implications of dietary absorption of MGO and GO need to be determined.

Poster 24

Withdrawn.

Poster 25

Title: Antiglycation effects of roasted brewer's spent grains

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Background and aims: Intake of α-dicarbonyl compounds (DCs), as methylglyoxal (MGO) and glyoxal (GO) increases the concentration of circulating advanced glycation end products (AGEs) and contribute to the development of chronic diseases.

Melanoidins, high-molecular weight brown polymers formed in the late stages of the Maillard reaction, are a bioactive source of branched polymers containing amino-carbonyl reductones and condensed polyphenols with antioxidant and prebiotic capacities.

In this study, high-molecular weight melanoidins generated by roasting of brewer's spent grains (HMW-BSGM) were tested for their dicarbonyls trapping capacity, as a result of the spatial arrangement of polyphenols with antiglycation activity.

Materials and methods:

Roasting optimization Roasting time and temperature were optimized using response surface methodology, maximizing the bound polyphenol content and antioxidant activity of roasted brewer's spent grains (RBSG). Variations in polyphenols concentration was assessed spectrophotometrically.

Polyphenols characterization HMW-BSGM were purified by dialysis and then were freeze-dried. The HMW-BSGM were subjected to alkaline and acidic hydrolyses to release bound polyphenols. Polyphenols characterization of hydrolysates was achieved through liquid chromatography- tandem mass spectrometry.

Direct DCs trapping capacity Direct DCs trapping capacity was evaluated by LC-UV-Vis after incubating the HMW-BSGM (0.1-4 mg/mL) or individual phenolic standards with either MGO or GO under simulated physiological conditions for up to 7 days.

Antiglycative assay In-vitro glycation assay with bovine serum albumin induced by glucose was carried out to evaluate the antiglycative capacity of the HMW-BSGM extract and that of the individual phenolic standards after a 21-day incubation at 37°C.

Results:

Roasting optimization Roasting at 185 °C for 30 min generated the highest bound polyphenols content (2.55 mg gallic acid equivalent/ g of dry matter (DM)) while preserving an appreciable antioxidant activity of the RBSG (21.20 mg Trolox equivalent/ g of DM).

Polyphenols characterization Ferulic acid (FA) (54.06 ± 8.73 mg/100g of DM) was the most abundant polyphenol followed by 4-hydroxybenzoic acid (4-HBA) (9.05 ± 0.72 mg/100g of DM) and p-coumaric acid (CA) (3.37 ± 0.59 mg/100g of DM) in HMW-BSGM.

Direct DCs trapping capacity HMW-BSGM extract (4 mg/mL) trapped 60% of GO and 95 % of MGO after 48 hours incubation. The HMW-BSGM extract (2 mg/mL) exerted a higher trapping ability toward GO and MGO (95.86 ± 4.15 and 93.11 ± 5.33 %, respectively) compared to free FA (8.87 ± 1.76 and 12.95 ± 1.65%), 4-HBA and CA with respective inhibitions of 9.29 ± 0.98% and 24.58 ± 2.82% toward GO with no trapping of MGO after 7-day incubation.

Antiglycative assay The extract exerted a significant inhibitory activity on the formation of fluorescent AGEs (IC_{50} = 0.60 ± 0.09 mg/mL) comparable to those of FA (IC_{50} = 0.44 ± 0.06 mg/mL) and CA (IC_{50} = 0.45 ± 0.09 mg/mL).

Conclusion: Thermal treatment can tune the spatial arrangement of functional molecules in vegetable byproducts. We demonstrated that polyphenols bound to HMWM-BSG overperform their respective free polyphenols in trapping dicarbonyls and in preventing fluorescent compounds formation. HMWM-BSG can be a valuable source of functional molecules with the possibility to further modulate their antiglycation activity through thermal treatment.

Poster 26

Title: Reactive carbonyl species in differently sweetened soft drinks

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Background and aims: In the production of soft drinks, the use of different kinds of sugar sweeteners, typically sucrose and fructose-glucose syrups, is affected by tradition, availability of raw materials, economy, and technological background and facilities. The substitution of sucrose by fructose-containing syrups (such as HFCS 55) in food production is frequently discussed with regard to the differences in sweetness, metabolism, and satiety profile. However, some other aspects such as the effect of different sugars on the redox status and the levels of sugar-derived reactive carbonyl species (RCS) are also worthy of notice. Most RCS such as 3-deoxyglucosulose (3-DG) and methylglyoxal (MGO) contain an α-dicarbonyl moiety and contribute to oxidative and carbonyl stress in the body. They are formed both endogenously and during the processing and storage of food, especially those rich in sugar. In this work, we compare the stability and transformation rates of sugars and the development of RCS during storage of differently sweetened cola-type soft drinks and various sugar sweeteners including syrups.

Materials and methods: The α-dicarbonyl compounds were derivatized with *o*-phenylenediamine and analyzed using HPLC on phenyl-hexyl RP stationary phase and diode array detection. 5-Hydroxymethylfuran-2-carbaldehyde (HMF) was analyzed with the same HPLC system. An HPLC method with refractive index detector and a stationary phase with an amino functionality was used for the determination of sugars.

Results: Unlike the soft drinks sweetened with HFCS 55, storage of soft drinks with sucrose leads to a significant change in the sugar composition due to inversion. This reaction leads to a change in the intensity and perception of sweet taste. Moreover, fructose is more reactive and a better RCS precursor than glucose and sucrose. Therefore, the true concentration of fructose is a key risk factor for the formation of RCS in soft drinks. The amount of 3-DG in fresh soft drinks with HFCS 55 is mostly given by their concentration in a starting sugar concentrate with acidulants and also by manufacturing conditions. During first weeks of storage of, the level of 3-DG in these soft drinks is still increasing, but, after several months, their levels start to decrease. Most MGO and HMF are formed as late as in stored soft drinks and their levels are still growing during long-term storage. In fresh sucrose-sweetened soft drinks, the content of 3-DG is negligible (around 1 mg/l) and increases during storage. When expired, the soft drinks with sucrose contain still about 10-20 times less 3-DG than those with HFCS 55. Different kinetics was found also in the corresponding syrups (concentrates).

Conclusion: Soft drinks with sucrose represent much lower risk of dietary exposure of α-dicarbonyl compounds and HMF than those sweetened with HFCS 55.

Keywords: Soft drinks; HFCS 55; 3-Deoxyglucosulose; Methylglyoxal; HMF.

Poster 27

Title: Anti-glycative effect and total phenolic content of rice water of different *Japonica* and *Indica* varieties

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Background and aims: Rice is one of the most widely consumed staple food for a large part of the world's population, mainly in Asia. However, rice is also an important beautifying agent. Traditionally, women from Japan, China, and some Southeast Asian countries have used rice water to beautify their face, skin, and hair. In this study, the inhibitory effect on the formation of AGEs by rice water was investigated. Rice water is a common ingredient used traditionally as a beautifying agent but lacks proper scientific research. It is natural, economical and simple, and could be easily included in skincare products.

Materials and methods: 14 *Japonica* and *Indica* rice varieties were used to produce rice water by three traditional methods. Rice was boiled in milliQ at 100ºC, centrifuged and filtered to obtain type 1 rice water. Rice was oven-dried at 160ºC, boiled in milliQ at 100ºC, centrifuged and filtered to obtain type 2 rice water. Rice was soaked in milliQ at room temperature for 6 hours, centrifuged and filtered to obtain type 3 rice water. The anti-glycative effect was evaluated by measuring the inhibition of fluorescent AGEs by the rice water. The solid concentration of samples was adjusted to 1mg/ml. Human serum albumin (HSA) and glucose were incubated with each sample at 40ºC for 60 hours, and the AGE-derived fluorescence was measured (excitation 370nm/emission 440nm). The total phenolic content (TPC) was also checked as it has been reported that TPC was responsible for the anti-glycation potential of rice. TPC was determined according to the Folin–Ciocalteau procedure. The reaction mixture was mixed with sodium carbonate followed by 50% Folin–Ciocalteau reagent. The mixtures were well mixed and incubated at 30 ºC for 30 minutes and at room temperature for another 30 minutes. Absorbance was measured at 660 nm. TPC was expressed as catechin equivalent (µM catechin eq).

Results: All 42 rice water samples inhibited the formation of fluorescent AGEs. Sample number 1 showed the highest inhibition on the formation of fluorescent AGEs among type 1 rice water samples (66.4 ± 0.4 %) and type 2 rice water samples (66.6 ± 0.7 %). Sample number 12 showed the highest inhibition among type 3 rice water samples (69.6 ± 0.8 %). All rice water samples contained phenolic compounds. Sample number 1 showed the highest TPC among type 1 (94.7 ± 6.4 µM catechin eq), type 2 (124.8 ± 6.6 µM catechin eq), and type 3 (176.2 ± 0.4 µM catechin eq). A strong positive correlation between TPC and inhibition of fluorescent AGEs was observed in type 1 (r = 0.906) and type 2 (r = 0.918). A moderately strong positive correlation was in type 3 (r = 0.765).

Conclusion: This study is the first report on a correlation between the inhibition of AGEs formation and the total phenolic content (TPC) in rice water of various pigmented and non-pigmented *Japonica* and *Indica* varieties. This study shows that rice water, specifically type 3 rice water has a strong inhibitory efficacy against fluorescent AGE formation. This study also shows that rice water produced using pigmented rice varieties have a higher inhibitory effect against fluorescent AGE formation, and that there is a strong positive correlation between the inhibitory effect and the total phenolic content of rice water.

Keywords: rice water, pigmented rice, glycative stress, advanced glycation end products (AGEs), total phenolic content (TPC).

Poster 28

Title: Exploring the Potential of All-aqueous Emulsions for Accomplishing the Maillard Reaction

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Background and aims: Microreactors based on biphasic liquid systems, in particular oil-water emulsion droplets have gained a great interest as they confer several advantages for running chemical, microbial and enzymatic reactions. However, environmental and consumers concerns associated with the use of organic solvents and synthetic low-molecular weight surfactants in reaction products encourage scientists/industry to develop alternative reaction media. Fully aqueous liquid biphasic systems, i.e. water-in-water (W/W) emulsion droplets are considered as green, inexpensive, and rather sustainable options for accomplishing a diversity of reactions. Comparable to oil-water emulsion-based media, W/W emulsions compartmentalize reactants in discrete phases. The spatial distribution of reactants can be crucial when dealing with yield and the concentration of the end-products of the Maillard reaction (MR). Hence, our aim is to use W/W emulsion as miniaturized reactors to run and investigate the formation of Amadori compounds and volatile and non-volatile glycation compounds.

Materials and methods: Mixing the non-ionic polymer polyethylene glycol (PEG, 8 kDa) and the salt sodium sulfate yielded fully aqueous emulsions. The emulsion consisting of PEG (50%, wt/wt) and salt(15%, wt/wt) was dyed with fluorescently-labelled dextran and then imaged by an epi-fluorescence microscope. The influence of PEG concentration (i.e. 30%, 40% and 50% wt/wt) on the partition coefficient and recovery (partitioning) yield of the MR precursors, i.e. either hydrophilic or hydrophobic amino acids and glucose at either of the phases of the emulsions was studied. The concentrations of amino acids and glucose over the MR course were quantified by the o-phthaldialdehyde method and an enzymatic method (glucose oxidase and peroxidase), respectively. The emulsions (containing tryptophan and glucose) and controls were heated in water bath and analysed by a spectrophotometer.

Results: Epi-fluorescence microscopy imaging indicated that the continuous and dispersed phases were PEG-rich and salt-rich, respectively. The partition coefficient of tryptophan in the rather hydrophobic (i.e. the PEG-rich) phase increased from 2.91 to 6.17 when the PEG concentration was increased from 30% to 50% (wt/wt). On the contrary, lower partition coefficients were obtained for the alanine and asparagine, as well as, glucose in the rather hydrophobic phase with increasing PEG concentration. After thermal treatment, spectrophotometric measurement suggested that emulsion structure enhanced the formation of MR products.

Conclusion: The partitioning of amino acids and sugars between the phases of all-aqueous emulsions is governed by their hydrophobicity, which can be used to control the compartmentalization of the MR precursors in the emulsion. In this scenario, tryptophan, asparagine, and glucose can be candidates for studying the MR pathways in salt-in-PEG emulsions during thermal treatment or accelerated storage. Mass spectrometric techniques will explain the formation of Amadori compounds in W/W emulsions as biphasic liquid reaction media and the competence of such reaction media to tune the formation of volatile and non-volatiles molecules.

Poster 29

Title: Establishing of an *in vitro* model system to study the mechanism of protein glycation

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Background and aims: One of the key factors influencing the complexity of the Maillard reaction, both *in vivo* and *in vitro*, is the huge range of possible glycation agents, which differ significantly both in their glycation potential and in the specific mechanisms of formation of advanced glycation end products (AGEs). Although the differences in the glycation potential of monosaccharides are well understood, the mechanisms underlying them are not well studied.

Materials and methods: Our model system employed *D*-glucose, *D*-fructose and *L*-ascorbic acid incubated with human serum albumin (HSA) *in vitro*. Thereby, a platform combining three analytical methods was proposed: a proteomic approach considering site specificity in the context of AGE formation and stability, carbohydrate and α-dicarbonyl analysis. Sugars and their α-dicarbonyl intermediates were analysed in parallel with protein glycation patterns (using hydroimidazolone modifications of arginine residues as an example) applying bottom-up proteomics (LC-IT-MS, GC-EI-MS) and computational chemistry (MOE, 2019.0, GNU Image Manipulation Program Version 2.8.16).

Results: The presence of HSA in the incubation mixtures had different effects on the kinetics of carbohydrate degradation. The time curves of glucose and ascorbic acid did not change, whereas fructose degraded much faster in the presence of HSA. The dicarbonyl content derived from glucose and ascorbate gradually decreased during the experiment due to the formation of Amadori compounds formed from glucose, oxidative degradation of ascorbic acid and the reaction of GO and MGO formed with protein side chains. In contrast, α-dicarbonyls formed from fructose showed a higher relative abundance throughout the experiment. This suggestion is supported by similarities with GO and MGO kinetics in the presence of unmodified HSA. Glycation of HSA with sugars revealed 9 glyoxal- and 14 methylglyoxal-derived modification sites, respectively. Their dynamics were sugar-specific and depended on α-dicarbonyl concentrations, the kinetics of their formation and the presence of stabilizing residues near the glycation sites.

Conclusion: In the context of food chemistry and nutrition, it is critical to understand the factors underlying the glycation potential of food sugars. This will enable the development of food products that are safe to eat and have a low pro-glycaemic effect and hence contribute less to inflammation-related diseases. The proposed *in vitro* model system and the integrated analytical approach allow the glycation potential of individual food sugars to be assessed, providing access to all participants in the Maillard reaction.

Keywords: Advanced glycation end products (AGEs); Blood monosaccharides; GC–MS; Glycation; Glyoxal (GO); LC–MS; Methylglyoxal (MGO)

Poster 30

Title: Anaerobic Degradation of Dietary Advanced Glycation End-Products by Human Intestinal Bacteria

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Background and aims: Modifications of lysine and arginine residues contribute to the amount of dietary advanced glycation end-products (d-AGEs) reaching the colon. Microbial populations can ferment modified protein sources, but the effects of food processing neo-formed compounds on host health is still unknown. In this respect, the molecular characterization of the microbial d-AGEs metabolites can be crucial in deciphering the interconnection between glycation compounds and health.

Materials and methods: Fecal microbiota from donors exposed to processed foods were transferred in growth media containing CML, pyrraline and methylglyoxal-hydroimidazolone isomers (MG-H1). Successive transfers were used to identify and isolate species able to metabolize d-AGEs; enrichments were performed in anaerobic bicarbonate-buffered mineral salt medium with trace elements and vitamins. Bacterial community analysis was achieved through DNA extraction from the pellet. Upon purification, PCR products generated a clone library of full-length 16S rRNA gene sequences that were aligned with the multiple sequence aligner SINA and merged with the Silva SSU database. Phylogenetic trees were constructed in the ARB software package by the same algorithm. Aqueous supernatants were used to identify metabolization products of the three d-AGEs through zwitterionic HILIC and PFP reversed phase - high resolution tandem mass spectrometry (HRMS). Metabolites identification, time course profile and degradation of the three d-AGEs was performed in Compound Discoverer environment through an implemented workflow tree consisting in spectra selection, retention time alignment, isotope pattern matching, expected and unknown compounds detection, background subtraction and predicted fragments ions.

Results: Anaerobic bacteria enrichments from donors exposed to processed foods used 77 and 100% of CML, and up to 99% of MG-H1, while in the case of pyrraline the percentage of utilization decreased down to 56%. In the case of CML, *Oscillibacter* and *Cloacibacillus evryensis* increased in the two donors after the second transfer, highlighting that the bacteria from these taxa could be candidates for anaerobic CML degradation. A pure culture of *Cloacibacillus evryensis* led to a tentative identification of CML metabolites: carboxymethylated biogenic amines and carboxylic acids were identified as CML degradation products. In the case of pyrraline up to five compounds were differentially formed: decarboxylation and deamidation occurred on the alpha side moiety, while sulfoxidation of pyrrole residues was the preferred putative biotransformation. In the case of MG-H1, steric hindrance of imidazole side chain rendered microbial enzymatic activity unfavoured and, as for pyrraline, most of the modifications occurred in position alpha.

Conclusion: d-AGEs selectively increase the absolute abundance of specific microbial populations that consequently affect metabolization of d-AGEs into an intricate array of compounds with potential effects on bio-functionality of human gut.

Poster 31

Title: Drought-related changes in the nutritional properties of pea (*Pisum sativum* L.) seeds in the context of protein glycation

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Background and aims: In plants, advanced glycation end products (AGEs) can be accumulated to significantly high levels under stress and ageing. Indeed, drought results in dramatic decrease of water potential of plant tissues triggering overproduction of reactive oxygen and nitrogen species. To minimize stress effects plants can adjust the contents of amino acids, polyamines and sugars. However, the accumulation of reducing sugars may enhance monosaccharide autoxidation and generation of α-dicarbonyl precursors of AGEs. Although water deficit is already known to stimulate protein glycation in leaves, its effect on glycation profiles in seeds is still unknown. Moreover, the effect of drought on biological activities of seed protein in mammalian systems is still unstudied with respect to glycation. Therefore, here we address the effects of a short-term drought on the patterns of seed protein-bound AGEs and accompanying alterations in pro-inflammatory properties of seed protein in the context of seed metabolome dynamics.

Materials and methods: Pea plants were subjected to drought simulated as polyethylene glycol-induced osmotic stress at the stage of seed filling. After harvesting, seed metabolites were extracted and analyzed with GC-MS and LC-MS approaches. The total protein of pea seeds was isolated and subjected to exhaustive enzymatic hydrolysis. Resulted protein hydrolysates were analyzed by the method developed for known dietary AGEs based on LC-MS/MS and tested for pro-inflammatory effects in a model of human neuroblastoma cell line SH-SY5Y.

Results: A short-term drought resulted in the significant decrease of physiological parameters. However, biochemical markers demonstrated the minor intensity of stress. Meanwhile, seed germination test does not demonstrate significant changes suggesting that the damage extent to the seeds was minor.
Metabolome analysis revealed the suppression of primary seed metabolism, although the secondary metabolome was not affected. This was accompanied by suppression of NF-κB activation in human neuroblastoma cells after the treatment with protein hydrolysates, isolated from the mature seeds of drought-treated plants. However, analysis of known dietary AGEs did not reveal changes in glycation adducts content. Most likely, the prospective anti-inflammatory effect of short-term drought is related to antioxidant effect of unknown secondary metabolite protein adducts, or down-regulation of unknown plant-specific AGEs due to suppression of energy metabolism during seed filling.

Conclusion: The observed changes in the biological activity can be explained by drought-related alterations in the patterns of primary metabolites. Given the contents of the marker AGEs were not affected by drought, decrease in formation of some unknown glycation products can be proposed.

Keywords: protein glycation; drought; inflammation; osmotic stress; seed metabolism

Poster 32

Title: Glycation events in food impacts the tumour microenvironment to promote prostate cancer growth

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Background and aims: The study premise is that the glycoxidation, oxidation or lipoxidation of free amino, lysyl, arginyl and carboxyl groups provided by the excessive consumption of proteins, fats, and sugars leads to the increased formation of advanced glycation end product (AGEs) to create a microenvironment conducive for tumor growth. In addition, the high heats and pressures associated with modern cooking and food manufacturing processes, promote glycoxidative, oxidative and lipoxidative stresses to rapidly accelerate AGE content in food, the rate of which is dependent in part upon its protein, fat, and sugar content. Studies show that glycation of biological molecules causes genetic infidelity, protein dysfunction and aberrant stress response, all cancer associated processes. Meta-analyses studies also show that higher AGE exposure through diet positively correlates with increased tumor growth. The aim of this research was to define a direct cause and effect relationship between food glycation and tumor growth to demonstrate for the first time its pro-tumorigenic potential.

Materials and methods: The heat driven induction of glycoxidative, oxidative and lipoxidative stresses was used to drive AGE formation in experimental mouse chow. AGE specific diets were then fed to mice and the effects on prostate tumor progression assessed. A limitation to the use of heat driven induction to drive glycation events is that it will also alter other heat-labile nutrients that may be responsible for any observed oncogenic effects. Therefore, the ability of direct *in vivo, ex vivo* and *in vitro* treatment with AGE specific peptide was assessed in order to reproduce the protumorigenic effects observed upon AGE consumption.

Results: It is herein reported chronic AGE consumption has significant oncogenic potential *in vivo*, stimulating both prostate tumor growth and metastatic potential. The identification of the receptor for AGE (RAGE) in the stroma as the key substrate and effector of AGE tumorigenic function provided key mechanistic insight. AGE mediated activation of RAGE conferred an activated phenotype on stromal cells to create a pro-tumorigenic microenvironment conducive for prostate tumor growth and progression. Critically dietary AGE mediated effects observed upon AGE consumption were successfully reproduced using in vivo, ex vivo and in vitro molecular models when treated with exogenous AGE peptide.

Conclusion: These data provide significant rational to assign food associated glycation as a protumorigenic consequence of modern dietary patterns. They serve to focus further studies on the oncogenic potential of modern dietary habits known to increase AGE bioavailability. Reaction products resulting from the glycation of reactive groups contained in food may reflect the complex molecular underpinnings associated with consuming the variety, combination and amounts of foods associated with modern dietary habits.

Poster 33

Title: Obesity rather than insulin resistance per se is associated with high serum D-lactate levels in adolescents: correlation with chylomicron remnants

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Background and aims: Methylglyoxal (MG) is a toxic molecule that mediates damage produced by glycation, a key pathogenic factor in diabetes mellitus. We have previously shown that the MG catabolic product, D-lactate, is higher in obese adolescents (1) and that apoB48 is higher in adolescents with insulin resistance (IR) (2). We tested the hypothesis that increased D-lactate is associated with ApoB48 (remnant) dyslipoproteinemia and IR in adolescents

Materials and methods: This cross-sectional study in anew cohort included euglycemic adolescents between 15 and 19 years old, classified in 4 groups according to BMI and HOMA-IR: lean adolescents without (MHL) alterations (n = 20), lean adolescents with metabolic alterations (MUL, n=20) metabolically healthy obese (MHO) n = 20 and metabolically unhealthy obese adolescents (MUO) n = 24. D-lactate was measured with an enzymatic kinetic method optimized in our lab (3).

Results: D-lactate levels did not differ between MHL and MUL participants (0.29 +/- 0.15 vs 0.29 +/- 0.13 mmol/L), they show a trend to be higher in MHO (0.38 +/- 0.15 mmol/L) and are significantly higher (0.46 +/- 0.15 mmol/L) in MUO vs MUL or MHL (p=0.003). D-lactate correlated with BMI (Rho=0.39, p=0.0002); HOMA-IR (Rho=0.33, p=0.002) and apoB48 (Rho=0.33, p=0.002).

Conclusion: MUO displayed 58% higher levels of D-lactate than lean adolescents. D-lactate correlates with BMI, HOMA-IR and with chylomicron remnants. We suggest that IR does not suffice to produce increased MG metabolism (MHL and MUL have the same levels) but potentiates the effect of obesity. Increased D-lactate levels show dysregulation of glycolysis leading to MG production, in association with poor chylomicron catabolism. The intimate link remains to be explored.

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Poster 34

Title: Methylglyoxal (MGO)-driven protein glycation provokes lung vascular dysfunction

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Background and aims: Pulmonary hypertension (PH) is a fatal disease characterized by functional and structural changes in the pulmonary vasculature. The persistent remodeling of the pulmonary arteries results in the reduction of the vessel lumen, contributing to increased pulmonary arterial resistance, which eventually leads to right heart failure and death. Metabolic dysfunction has emerged as one of the major areas of research in the pathobiology of PH. It is becoming increasingly recognized, that PH patients exhibit a metabolic shift resembling the one observed in cancer patients. Our recent clinical study shows that this metabolic shift is a common consequence of PH, also linked to physical activity. We have also reported that this shift is manifest in disturbances of the amino-acid metabolism and, in addition, others have reported altered glucose homeostasis and dysregulated fatty acid metabolism in PH.

This project aims to discover the relevance of MGO-induced dicarbonyl stress in lung vascular dysfunction.

Materials and methods: Lung tissues were obtained from idiopathic pulmonary arterial hypertension (IPAH) patients who underwent lung transplantation. Nonimplanted donor lungs that had been collected, but not used (e.g., size limitations) for transplantation served as controls. MG-H1 accumulation, lipid accumulation and GLO1-activity were investigated on these samples. Isolated human pulmonary arterial cells were obtained from human lung tissues in order to investigate their metabolic status under normal conditions and upon disease or lipid overload.

Results: GLO1 is present in the pulmonary vasculature, mainly in the endothelium, however its activity is decreased in PH lungs. This is also mirrored by elevated MG-H1 levels in PH. An elevation in circulating free fatty acids might be connected to the dysregulated glyoxalase system in PH.

Conclusion: Our preliminary results suggest that in pulmonary arterial hypertension the altered metabolism triggers methylglyoxal (MGO)-driven dicarbonyl stress, which facilitates pulmonary arterial dysfunction, thereby perpetuating PH-related metabolic alterations.

Poster 35

Title: Serum levels of the soluble Receptor for Advanced Glycation Endproducts are prospectively associated with Pulmonary Arterial Hypertension in Systemic Sclerosis

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Background and aims: Interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH) are the leading causes of death in Systemic Sclerosis (SSc). Markers for early detection of these pulmonary complications are urgently needed. The receptor for advanced glycation endproducts (RAGE) is highly expressed in lung tissue. RAGE is involved in cell-matrix adhesion, proliferation and migration of alveolar epithelial cells, and remodeling of pulmonary vasculature. Therefore, we aimed to investigate soluble RAGE (sRAGE) in its association with current and future incidence of SSc-related pulmonary complications.

Materials and methods: In a case-control study, sRAGE levels in 20 patients with SSc (median age 51 [IQR 44-58] years, 13 female, disease duration since first non-Raynaud's Phenomenon 2 [1-8] years, 50% lung involvement, 40% gastrointestinal involvement) were compared with 20 age- and sex-matched healthy controls (age 52 [45-62] years, 14 female). Subsequently, sRAGE was measured in an independent retrospective cohort of 188 patients with SSc (64 years [55-72], 145 female, 60% ACA positive, 89% limited SSc, 83% sclerodactyly, 53% history of pitting scars or digital ulcers, 74% telangiectasia, 36% calcinosis cutis, 68% gastrointestinal involvement) who were followed for the long-term occurrence of pulmonary events and mortality. Levels of sRAGE were measured by an enzyme-linked immunosorbent assay in serum.

Results: Serum sRAGE levels were significantly higher in patients with SSc compared with controls. In the second cohort, levels of sRAGE were significantly lower (median 735.0 pg/ml [IQR 525.5-1988.5], p=0.001) in SSc-ILD (n=41) and higher in SSc-PAH (n=12; 4099.0 pg/ml [936.3-6365.3], p=0.011) compared to patients without pulmonary involvement at baseline (n=124; 1444.5 pg/ml [966.8-2276.0]). sRAGE levels in patients who had ILD as well as PAH at baseline (n=11) did not differ significantly from those without pulmonary involvement. Regression analyses revealed that sRAGE levels were positively associated with the presence of SSc-PAH (p<0.001), ACA serology (p<0.001), and sclerodactyly (p=0.017), independent of age, gender, ILD, COPD, use of vasodilators, or immunosuppression. Lung involvement developed in 13% (4% PAH after median time of 57 [35-83] months and 9% ILD after median time of 56 [33-82] months) of the patients without baseline lung involvement. sRAGE levels > 4th quartile were associated with the incidence of PAH (log-rank p=0.01) and PAH-related mortality (p=0.001). However, low sRAGE levels were not associated with ILD occurrence (p=0.713) or ILD-specific mortality.

Conclusion: This is the first study to demonstrate that serum sRAGE levels are increased in patients with SSc, with significantly lower levels in SSc-ILD and higher levels in SSc-PAH. The association with PAH was independent of potential confounders. Importantly, high sRAGE levels in patients without baseline lung involvement were prospectively associated with the incidence of PAH and PAH-related mortality in SSc, potentially indicating its role as a predictor for SSc-PAH and a putative future therapeutic target for early interventions.

Poster 36

Title: Release of High-Mobility Group Box-1 after an Raynaud's attack potentially leads to fibroblast activation and interferon-γ Induced Protein-10 production in Systemic Sclerosis

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Background and aims: Raynaud's Phenomenon (RP) leading to repetitive ischaemia and reperfusion (IR) stress, is the first recognisable sign of systemic sclerosis (SSc). Although RP has been linked to SSc aetiology, direct observations substantiating this are scarce. High-mobility group box-1 (HMGB1) is a nuclear factor released by necrotic cells, of which serum levels may rise quickly after IR injury in other diseases. IR injury promotes interferon (IFN) inducible genes, which have been implicated in SSc pathophysiology. Since HMGB1 can signal through the receptor for advanced glycation endproducts (RAGE), we investigated whether an RP attack promotes release of HMGB1, leading to fibroblast activation and upregulation of IFN-inducible genes in a translational study.

Materials and methods: A cold challenge was performed to simulate an RP attack in patients with SSc (N=10, age 56.2 ± 11.5 years), primary RP (N=10, 47.8 ± 16.2), and healthy controls (N=10, 23.9 ± 2.7). We measured levels of HMGB1 and IFN gamma-induced Protein-10 (IP-10) before, 10 and 30 min after cold challenge in blood drawn from the ipsilateral forearm. Digital perfusion was assessed by photoplethysmography. For studying in vitro effects of HMGB1, healthy human dermal fibroblasts were stimulated with HMGB1 or TGF-β1 as control. Inflammatory (Interleukin-6 [IL-6] genes, profibrotic (type 1 collagen [Col1], α-smooth muscle actin [α-SMA]), connective tissue growth factor [CTGF]) genes, and IFN-inducible genes (IFN α-induced 44L [IFI144L], Myxovirus resistance protein 1 [Mx1], Lymphocyte antigen 6 complex, locus E [LY6E], and IP-10) were measured by RT-PCR. RAGE signalling was determined by preincubation with its inhibitor FPS-ZM1. Differentiation to myofibroblasts was assessed by staining of α-SMA. In an independent cohort, sera were obtained from 20 patients with SSc (median age 51 [IQR 44-58] years, 13 female) and 20 age- and sex-matched healthy controls (52 [45-62], 14) to determine HMGB1 and IP-10 levels.

Results: During cold challenge, finger perfusion was reduced in SSc and recovered slower than in primary RP and healthy controls. HMGB1 increased significantly 30 min after cold challenge in SSc compared to healthy controls, but not to primary RP. IP-10 remained stable. In vitro stimulation of fibroblasts with HMGB1 resulted in 20-fold increase in mRNA expression of IP-10, while IL-6 increased 3-fold. Col1, α-SMA, IFI144L, Mx1, and LY6E did not change. IP-10 expression was inhibited by 50% by preincubation with FPS-ZM1. TGF-β1 stimulation promoted IL-6 and CTGF, without effects on IFN gene expression. HMGB1 stimulation induced myofibroblast differentiation and formation of α-SMA fibers. Both HMGB1 and IP-10 were significantly higher in patients with SSc compared to healthy controls.

Conclusion: In this translational study, we show for the first time that an RP attack in patients with SSc leads to release of HMGB1. In vitro, HMGB1 induces IFN regulated gene expression in fibroblasts, especially interferon-γ inducible IP-10, at least in part in a RAGE dependent manner. This potentially links HMGB1 release following an RP attack to IFN inducible proteins as putative sequential steps leading to disease progression in SSc.

Poster 37

Title: Detection of OP-lysine in serum of ApoE-deficient mice by LC-MS/MS

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Background and aims: GA-pyridine (GA-p) is generated from the reaction between glycolaldehyde and lysine (Lys) (Nagai et al., J. Biol. Chem. 2002), whereas glyceraldehyde-derived pyridinium (GLAP) is generated from glyceraldehyde (Glyc) and Lys (Usui et al., Biofactors 2004). Furthermore, Argirov et al. (J. Biol. Chem. 2004) reported that OP-lysine is generated from GA and Glyc in the presence of Lys and accumulates in human lens proteins. Horvat et al. (Carbohydr Res. 2010) also reported that 3-hydroxypyridinium (3-HP), which has the same structure as OP-lysine, is produced from glucuronic acid and Lys. These AGE structures possess pyridinium ling and 3-HP structures. In the present study, we investigated whether OP-lysine is produced only from glucuronic acid or from other carbonyl compounds too.

Materials and methods: Carbohydrates, such as glucose and its metabolite glyoxal, were incubated with Lys with or without DTPA (1 mM) at 37 °C for 3 or 7 d, and the products were measured by LC-ESI-QTOF. Serum OP-lysine levels were compared between normal and ApoE-deficient mice (N=6). OP-lysine was also measured in human carotid endarterectomy (CEA) samples (N=3).

Results: The retention time and mass-to-charge ratio (m/z) of precursor and fragment ions matching with OP-lysine were detected in ribose-lysine. The yields of OP-lysine from the reaction of Lys with ribose were equivalent to the reaction of Lys with GA in the presence of Glyc. OP-lysine was detected in the serum of normal mice, which was increased in ApoE-deficient mice. OP-lysine was also detected in the CEA samples.

Conclusion: The structures of GA-p and GLAP contain the same structure of OP-lysine in their molecules, and the yields of OP-lysine from ribose were equivalent to GA with Glyc, and higher than GO. Since OP-lysine was detected in arteriosclerotic mice as well as in the human artery in the arteriosclerotic region, ribose may be involved in the development of arteriosclerosis.

Keyword: OP-lysine, ribose, 3-HP, GA, Glyc**,**

Poster 38

Title: In vitro glycation of human serum albumin as a model of diabetes

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Background and aims: The death toll of diabetes and its associated diseases corresponds to around 1.3 million people per year, which is the eighth most common cause of death. In case of type 2 diabetes (T2D), half of all new cases remain undiagnosed for years due to lack of reliable screening tests resulting in vascular, tissue, and organ damage that could be prevented or at least delayed by earlier diagnosis. The hyperglycemic condition in diabetes triggers protein glycation. Thus, glycated plasma proteins were found to be good biomarkers for an early sensitive diagnosis of T2D and for monitoring the progression of the disease. Especially the interest in human serum albumin (HSA) as a biomarker has steadily increased, as it is the most abundant plasma protein accounting for approximately half of the plasma proteins content. Due to the half-life of HSA (14-21 days), glycated albumin reflects the average blood glucose level for the preceding two to four weeks providing a good marker for short-term interventions in glycemic control. Consequently, the main objective of this study was to understand the mechanisms of HSA glycation and to evaluate the glycation degrees at different time points.

Materials and methods: HSA was glycated *in vitro* with glucose at 37 °C for 1, 2, 4, and 7 days. The samples were ultrafiltrated (10 kDa-cut-off) and the total glycation level was determined by the colorimetric fructosamine assay (nitroblue tetrazolium assay (NBT)). Glycation sites were identified by nanoRP-HPLC-ESI-MS/MS after tryptic digestion and solid phase extraction (SPE) using database searches considering glycation and several AGEs at lysine and arginine residues. Furthermore, one specific glycation site was quantified by parallel reaction monitoring (PRM) using an isotope-labelled peptide as internal standard.

Results: Based on the fructosamie assay, the total glycation degree increased over time from initially 1% (starting material) to about 20% after seven days. In addition, several sites for glycation and AGE modifications could be identified, especially in the samples of the later time points. The glycation occurred mainly at lysine residues, although a few arginine residues were also modified. Using an isotope-labelled standard glycated peptide, the glycation degree of lysine-414 increased from initially 3 pmol/mg HSA to 136 pmol/mg HSA after seven days.

Conclusion: HSA has already been identified as a biomarker for glycemic control due to its high abundance in blood plasma and its half-life time of 14 to 21 day. Here, the *in vitro* glycation was used to monitor the effect of high glucose concentration on the entire molecule. In addition, the effect of the increased glucose concentration on individual glycation sites was investigated to better evaluate their potential as biomarkers for T2D.

Keywords: Diabetes, Human serum albumin, *in vitro* glycation, LC/MS, Fructosamine assay.

Poster 39

Title: Plasma Advanced Glycation Endproducts (AGEs) and the Subsequent Risk of Microvascular Complications in Type 1 Diabetes: the DCCT/EDIC Study
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Background and aims: We investigated the impact of glycemic control on plasma protein-bound AGEs and their association with future microvascular disease and neuropathy at one *vs.* three time points in the DCCT/EDIC.
Materials and methods: Eleven AGEs were measured by LC/MS/MS in banked plasma samples from 466 participants at three time points (TPs): DCCT years 4 (TP1) and 8 (TP2), and EDIC years 5-6 (TP3). Cross-sectional associations were assessed by correlation coefficients and subsequent risk of complications by Cox proportional hazards models.
Results: Glucose-derived glycation products fructose-lysine (FL), glucosepane (GSPN) and carboxymethyl-lysine (CML) decreased with intensive glycemic control at both TP1 and TP2 ($P < 0.0001$), but were similar at TP3 and correlated with mean HbA1c. Their values at TP1 were associated with the subsequent risk of several microvascular outcomes, but these associations did not remain significant after adjustment for spot HbA1c, except methionine sulfoxide (MetSOX) which remained associated with diabetic nephropathy. Using all 3 TPs, glucose-derived AGEs were associated with subsequent risk of proliferative diabetic retinopathy (PDR, $P < 0.0001$), clinically significant macular edema (CSME, $P < 0.0008$) and confirmed clinical neuropathy (CCN, $P < 0.005$). Adjusted for age, sex, BMI, diabetes duration and mean HbA1c, the associations remained significant for PDR ($P < 0.021$) and CCN ($P \leq 0.005$, except CML, NS), CML with CSME ($P = 0.033$), and MetSOX with CAN ($P = 0.031$).
Conclusion: Spot AGEs are not superior to spot HbA1c for risk prediction, but glucose-derived pAGEs at three TPs and MetSOX remain robustly associated with progression of microvascular complications in Type 1 diabetes despite adjustment for HbA1c and other factors.

Poster 40

Title: Glyoxalase 1 – a multidrug resistance factor in cancer chemotherapy.

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Background and aims: Methylglyoxal (MG) is a reactive metabolite formed mainly as a by-product in anaerobic glycolysis, metabolized by glyoxalase 1 (Glo1) of the glyoxalase system. It has been suggested that Glo1 is a mediator of multidrug resistance (MDR) by countering increase of MG to cytotoxic levels by off-target effects of anticancer drugs as part of their mechanism of action. Herein, we investigated the effect of overexpression of Glo1 on the cytotoxic, antiproliferative activity of a multiple classes of antitumor drugs.
Materials and methods: A stable transfectant HEK293 cell line with 4 - 5 fold increased expression of Glo1 activity was produced by transfection with pIRES2-GLO1-EGFP plasmid (Glo1+ vector); and a stable transfectant control produced using pIRES2-EGFP plasmid (empty vector). HEK293 cells stably transfected with empty and Glo1+ vectors were incubated for 2 days with and without clinical anticancer drugs and also the experimental cell permeable Glo1 inhibitor, S-p-bromobenzylglutathione cyclopentyl diester. The effect on cell growth was assessed by viable cell number counts, using the Trypan blue exclusion method and median growth inhibitory concentrations GC_{50} deduced (n = 18). For BBGD, cultures were also performed under an atmosphere of 3% oxygen as a model of hypoxia.
Results: We found overexpression of Glo1 in HEK293 cells decreased the antiproliferative activity of most anticancer drugs. In order of increasing effect, MDR (fold increase in GC_{50}) was: vincristine, 1.3-fold; etoposide, 2-fold; mechlorethamine and methotrexate, 7-fold; paclitaxel, 8-fold; mitomycin C, 15-fold; and doxorubicin, 16-fold. We measured the cellular concentration of MG in HEK293 cells after treatment for 3 h with drugs for which Glo1 overexpression produced resistance. Cellular MG concentration increases were: 2-fold (mitomycin C), 3-fold (mechlorethamine and etoposide), 5-fold (doxorubicin, paclitaxel and BBGD) and 8-fold (methotrexate). This was often linked to drug-induced increase in glucose metabolism and flux of formation of MG. In an atmosphere of 20% oxygen, BBGD had GC_{50} of 5.12 ± 0.33 μM. In 3% oxygen atmosphere, the flux of glucose consumption and formation of MG were increased 2-fold and the expression of Glo1 decreased 69%. The combined effect of increased MG formation and decreased Glo1 expression was associated with a concomitant increase in potency of the anti-proliferative activity of BBGD of *ca.* 60-fold; GC_{50} was 0.085 ± 0.010 μM, Co-treatment of anticancer drugs with the GC_{50} concentration of BBGD increased cytotoxic antiproliferative activity.
Conclusion: We conclude that Glo1 is a likely mediator of multidrug resistance in cancer chemotherapy applicable to multiple classes of antitumor drug: alkylating agents, topoisomerase inhibitors, anti-tubulins and anti-metabolites. This is linked to drug-induced increase of cellular MG concentration to cytotoxic levels by off-target effects on glycolysis. High expression of Glo1 may contribute to poor patient survival in cancer chemotherapy. Adjunct chemotherapy with Glo1 inhibitor may improve treatment outcomes.

Key words: cancer chemotherapy, glyoxalase 1; methylglyoxal; multidrug resistance.

Poster 41

Title: Highly glycated albumin is more genotoxic than minimally modified albumin in WIL2-NS cells assessed using the cytokinesis-block micronucleus cytome assay.

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Background and aims: Non-enzymatic glycation leading to the production of AGEs impacts both protein structure and function in biological systems. Previous studies investigating AGE-induced cellular dysfunction *in vitro* using glucose-albumin (Glu-BSA) model systems have incubated at higher than physiological temperature to reduce preparation time. They have shown that highly glycated albumin prepared at 60 °C for 6 weeks induces genotoxicity in WIL2-NS cells measured by the cytokinesis-block micronucleus cytome (CBMNcyt) assay. It is likely that highly glycated albumin may not be physiologically relevant as normal body temperature is 37 °C and the degree of glycation may exceed the extent of modification *in vivo*. Therefore, we hypothesised that highly glycated albumin displays a distinct chemical, genotoxic and cytotoxic profile compared to minimally glycated albumin.

Materials and methods: The N^{ϵ}-carboxymethyllysine (CML) level in the Glu-BSA model systems incubated either at 60 °C or 37 °C for 6 weeks was quantified by liquid chromatography tandem mass spectrometry. The extent of protein modification was determined by the assessment of fructosamine level, total fluorescence, protein oxidation and aggregation of each of the model systems. The genotoxic and cytotoxic effects of highly glycated albumin (Glu-BSA incubated at 60 °C) versus minimally glycated albumin (Glu-BSA incubated at 37 °C) was assessed in WIL2-NS cells using the CBMNcyt assay after 3, 6, and 9 days of exposure. We also investigated the effect of the purification method by comparing the genotoxic effect of both Glu-BSA model systems prepared via trichloroacetic acid (TCA) precipitation and ultrafiltration (UF).

Results: The N^{ϵ}-carboxymethyllysine (CML) level was higher for the Glu-BSA model system incubated at 60 °C (28.13 ± 1.19 nmol CML/mg protein) compared to Glu-BSA incubated at 37 °C (2.84 ± 0.42 nmol CML/mg protein). The genotoxicity observed over 9-days was more pronounced in WIL2-NS cells treated with highly glycated albumin purified by TCA precipitation compared to minimally glycated albumin purified by UF. The number of micronuclei indicative of chromosome mal-segregation and/or loss was increased up to 4-fold after 9 days of treatment with highly glycated albumin compared to 2-fold after 9 days of treatment with minimally glycated albumin.

Conclusion: The present study highlights the importance of the extent of glycation and the purification technique when generating AGEs *in vitro* via Glu-BSA model systems. The increase in genotoxicity observed using Glu-BSA model systems incubated at 60 °C compared to 37 °C, may be due to structural changes of the glycated protein occurring at higher temperatures. Employing *in vitro* AGE model systems that are physiologically relevant will enable future studies to better reflect *in vivo* situations of albumin glycation conditions. Furthermore, it will allow for more appropriate and meaningful pre-clinical models that enable the translation of therapeutics aimed at mitigating the pathological consequences of AGEs.

Keywords: genotoxicity, cytotoxicity, glycation, micronucleus.

Poster 42

Title: Determination of early- and advanced-stage glycation biomarkers in nail clippings using isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS).
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Background and aims: Advanced Glycation End products (AGEs) are implicated in the aetiology of diabetes-related complications, but while glycated haemoglobin (HbA1c) exhibits a positive association with some diabetic complications, these relationships are not causal. Haemoglobin is a relatively short-lived protein in the body (half-life 6-8 wks), so HbA1c represents endogenous, early-stage glycation reflective of a subject's glycaemic status. This measure is also not without its confounding factors and requires a blood sample.
Nails are >80% keratin and fingernails take 3-5 months to grow from the germinal matrix to the free edge. Glycation biomarkers are incorporated during differentiation of proteins in the germinal matrix, but also from the circulation via the underlying, highly vascularized nail bed – they may also form here, proteins in the nail bed being glycated over the 3+ months of the growth period. Both early- and advanced-stage glycation markers are detectable in nails
Fingernails are relatively stable compared with biological fluids and can be easily and non-invasively collected by untrained personnel (including subjects themselves). They thus have potential economic benefits and may be useful to increase and widen participation in cohorts, or improve adherence to recommended clinical follow-up. We developed an isotope-dilution liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) for **the** analysis of both early- and advanced-stage glycation markers in nails as a potential surrogate matrix reflecting glycation of distal tissues.
Materials and methods: Fingernails were hydrolyzed with 6M hydrochloric acid and analyzed for furosine, CML, CEL, and the amino acids lysine, arginine and phenylalanine, by isotope-dilution LC-MS/MS on a Waters Quattro Premier instrument. Chromatography was performed on a Hypercarb column (100 x 2.1mm, Thermo) using a Waters Acquity UPLC pumping a binary mixture of aqueous 10mM nonafluorpentanoic acid and acteonitrile. The LC-MS/MS method itself was rigorously characterized. We used this method to study the levels of glycation biomarkers among healthy individuals and their fluctuations of over time, examining the effect of different sample storage times and temperatures, and various sample pre-treatment approaches. We then compared healthy subjects' nails with those from diabetic patients.
Results: Linearity for all molecules was greater than $r^2 = 0.995$ while limits of detection (LOD) and quantification (LOQ) were, respectively: 0.05 and 0.1 ug/mL for furosine, 0.005 and 0.01 ug/mL for CML and CEL, and 0.5 and 1 ug/mL for the amino acids. Excellent reproducibility of between X and Y % was achieved (depending on the molecule), and the method proved to be very robust. Measured levels of amino acids were consistent with literature reports and theoretical values. Further, more detailed methodological data will be presented, as well as preliminary results comparing healthy subjects with diabetic patients which found significantly higher levels of both early- and advanced-stage glycation biomarkers in nails from the latter.
Conclusion: We have developed a sensitive, specific and robust LC-MS/MS method for the analysis of both early- and advanced-stage glycation biomarkers in nails which can discriminate between healthy and diabetic subjects.

Keywords: Nails, Glycation biomarkers, Diabetes, LC-MS/MS.

Poster 43

Title: Risk prediction of early decline in renal function in diabetic kidney disease with an algorithm including fractional excretion of glycated amino acids.

Authors: Alberto de a Fuente,[1] Naila Rabbani[2] and Paul J Thornalley[1]
Affiliations: [1]Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar; and [2]Department of Basic Medical Science, College of Medicine, QU Health, Qatar University, Doha, Qatar.

Background and aims: Diabetic kidney disease occurs in *ca.* 40% patients with diabetes. Approximately 1 in 5 patients with type 1 diabetes mellitus (T1DM) and 1 in 3 patients with type 2 diabetes mellitus (T2DM) develop early decline in renal function (EDRF), requiring renal dialysis after 5 - 20 years. Currently, at the time of normoalbuminuria or new onset microalbuminuria (incipient diabetic nephropathy), it is uncertain which patients are at risk of EDRF. With Joslin Kidney Study investigators, we found patients with T1DM who later developed EDRF (Decliners) have higher fractional excretion (FE) of 6 glycated amino acids - fructosyl-lysine and 5 advanced glycation endproducts (AGEs), compared to patients with stable renal function (Non-decliners). However, FE of any single glycated amino acid could not classify Decliners or Non-decliners. The aim of this study was to apply artificial intelligence machine learning to develop diagnostic algorithms to classify Decliners and Non-decliners by optimum combination of levels of glycated and oxidized amino acids in plasma and urine, related FEs and conventional clinical chemistry variables.

Materials and methods: Patients with T1DM with stable renal function (n = 63) and EDRF (n = 22) were recruited for this study. Data on levels of 14 glycated and oxidized amino acids in plasma, urine, related FEs, glycated hemoglobin A1C, log(urinary albumin creatinine ratio, ACR), age, gender and duration of diabetes at the time of new onset microalbuminuria were included as features in algorithm development. Algorithms were trained and tested on 90%/10% data split, repeated 1000 times, using the Extreme Gradient Boosting method.

Results: The algorithm gave an optimal classification of Decliners and Non-decliners. Optimum with features: A1C, log[ACR], FE(N_ω-carboxymethylarginine, CMA), FE(glyoxal-derived hydroimidazolone, G-H1) and plasma concentration of N_ε-carboxymethyl-lysine (CML) free adduct; For The diagnostic performance for risk prediction of future EDRF was (mean ± SD): sensitivity 74 ± 9%, specificity 91 ± 45 and accuracy 87 ± 4%. The positive likelihood ratio LR+ was 11.0, indicating that this method gives strong, often conclusive evidence of future EDRF in patients with T1DM. In contrast, algorithms with A1C and logACR only as features gave LR+ 2.6, providing small evidence of risk of future EDRF.

Conclusion: With conclude that FEs of glycated amino acids are novel risk predictors of EDRF, likely linked to reporting of early-decline of cationic amino acid transporter function in the renal tubular epithelium. Genetic polymorphism of these amino acid transporters has been linked to rapid decline in renal function in genome-wide association studies. Measurement of only 3 glycated amino acids, CMA, G-H1 and CML, produced an algorithm with optimum risk prediction of EDRF. With further validation, including in patients with T2DM and with chronic kidney disease without diabetes, this method may markedly improve clinical risk prediction of EDRF.

Key words: diabetic kidney disease; early decline in renal function (EDRF); glycated amino acids; fractional excretion; machine learning; risk predictors.

Poster 44

Title: Linking the functionality of glyoxalase 1 to the changes in its structure

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Background and aims: 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.

Methods and Results: Consistent with our *in silico* modelling, we confirm that selective mutation at T107 of GLO1 changes the structure of GLO1, as demonstrated by Small Angle X-ray Scattering (SAXS) and the macromolecular and microfocus beamlines at the Australian Synchrotron (ANSTO, Melbourne, VIC, Australia). In particular, a phospho-mimetic mutant T107D-GLO1, shows an increase in protein dimensions when compared to wild-type GLO1. Ni-NTA affinity purified T107D-GLO1 and T107E-GLO1 also demonstrate little or no catalytic-activity compared to wild-type GLO1. Other mutants including T107A and T107P also show reduced activity, while T107V retains the activity of wild-type GLO1, but cannot be phosphorylated/de-activated.

Conclusion: These findings support the critical nature of residue at position 107 in maintaining GLO1 structure and function and may have implications for age-related decline in GLO1 activity and ageing *per se*.

Key words: Glyoxalase 1; GLO1; Post-translational modification; Phosphorylation; Structure

Poster 45

Title: Dicarbonyl stress increases expression of thioredoxin interacting protein and CHOP linked to the NLRP3 Inflammasome in human aortal endothelial cell *in vitro*.

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Background and aims: Metabolic dysfunction of endothelial cells in hyperglycemia contributes to the development of vascular complications of diabetes. Increased glucose consumption by glucose-induced stabilization of hexokinase-2 to proteolysis produces a wave of increased intermediates in early-stage glycolysis with increased formation of methylglyoxal (MG), MG glycated protein and activation of the unfolded protein response (UPR). MG is metabolized by glyoxalase 1 (Glo1) of the glyoxalase system. We investigated the effect of increased MG imposed by high glucose concentration, Glo1 siRNA silencing and Glo1 inhibitor, S-p-bromobenzylglutathione cyclopentyl diester (BBGD), on expression of thioredoxin-interacting protein (TXNIP) and CHOP of the NLRP3 inflammasome.

Materials and methods: Human aortal endothelial cells (HAECs) were cultured under an atmosphere of air with 5% CO_2, 100% humidity at 37 ^{o}C in human large blood vessel endothelial cell growth medium with growth supplements and antibiotics according to the manufacturer's instructions; used during passages 4 - 6 to maintain the endothelial phenotype. Cultures had low glucose (4.1 mM, LG) and high glucose (20 mM, HG) concentration incubated for 24 h, 48 h and 72 h, with and without prior treatment with 50 nM Glo1 siRNA (90% Glo1 knockdown, Glo1KD) or 2 μM BBGD.

Results: Incubation of HAECs with high glucose concentration increased TXNIP mRNA by ca. 2-fold (P<0.01) at 48 h and 72 h. Increase of dicarbonyl stress by silencing of Glo1 elevated TXNIP by *ca.* 10-fold in both low and high glucose concentration cultures at 48 h and 72 h (Fig. 1)
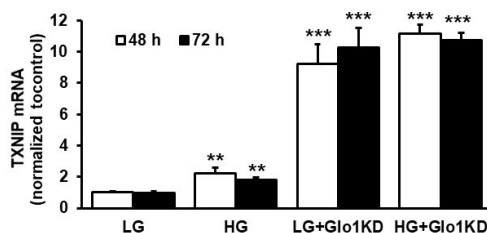
Fig. 1. Effect of high glucose concentration and glyoxalase 1 knockdown on expression of TXNIP in HAECs *in vitro*. Significance: ** & ***, P<0.01 & P<0.001 w.r.t. LG control; *t-test* (n = 3).

Incubation of HAECs with high glucose concentration with and without BBGD for 24 h produced a similar but weaker effect. When the UPR fails to alleviate endoplasmic reticulum stress, apoptosis occurs mainly via CHOP. High glucose concentration increased CHOP mRNA by 45 ± 17% and increased in low and high glucose concentration with Glo1 knockdown by 50 ± 15%, and 127 ± 1%, respectively. Again, incubation of HAECs with high glucose concentration with and without BBGD for 24 h produced similar effects.

Conclusion: We conclude that dicarbonyl stress induced in model hyperglycemia, Glo1 silencing and Glo1 inhibition in HAECs increased expression and TXNIP and CHOP. This likely reflects activation of the UPR where activation of inositol requiring enzyme-1α stabilizes TXNIP mRNA from degradation and XBP1 increases expression of CHOP. Both TXNIP and CHOP are associated with activation of the NLRP3 inflammasome.

Keywords: methylglyoxal; glyoxalase; endothelial dysfunction; thioredoxin interacting protein; NLRP3 Inflammasome; CHOP.

Poster 46

Title: Methylglyoxal impairs endothelial barrier function and vascular reactivity

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Background and aims: Diabetes is currently one of the largest health problems in the world and is a major risk factor for vascular complications. There is now considerable scientific evidence that increased formation of the most reactive dicarbonyl compound methylglyoxal (MGO), by hyperglycaemia, enhances the development of vascular complications. Despite accumulating evidence that oxidative stress may be the major link between MGO and vascular dysfunction, the exact underlying mechanisms, remains to be elucidated. The endothelium, through the secretion of vasodilators and vasoconstrictors, serves as a critical mediator of vascular homeostasis. Endothelium dysfunction can be considered as a shift in the function of the endothelium towards increased permeability and vasoconstriction. We aim to investigate whether MGO is a driver for impaired endothelial (barrier) function and sustained alterations in vascular contractility.

Materials and methods: Chorionic arteries of healthy human term placentas were mounted in a cannulated pressure myograph. To test maximal vascular contractility (expressed as 100%), arteries were first extraluminally pre-contracted by KCl (62 mM). Subsequently, after complete relaxation with HEPES buffer, arteries were intraluminally exposed to KCl (62 mM) to confirm intact endothelial integrity. In healthy arteries, intraluminal KCl is not able to cross the endothelial layer, and thus is not able to induce vascular contraction. Arteries were then intraluminally exposed to 100 µM MGO or only buffer (control) for 2 h and subsequent to KCl (62 mM). Changes between the first and the second intraluminal KCl-induced vascular contraction were used to test the effect of MGO on endothelial barrier function. In an additional experiment, the same type of arteries was mounted into a wire myograph organ bath. Sustained alterations in vascular reactivity induced by MGO against the contractile compound thromboxane A2 (U46619) were tested. To test this, the contraction induced by U46619 (30 nM) before and after exposure to 100 µM MGO was determined.

Results: In chorionic arteries, intraluminal MGO resulted in a significant increase of KCl-induced vascular contraction ($+34\pm3\%$, $p=0.005$, $n=6$), compared to the control. Furthermore, when a mitochondrial-targeted antioxidant MitoQ (1 µM) was present during the 2 h vessel exposure to MGO, the MGO-induced endothelial leakage for KCl could be protected almost completely ($-29\pm6\%$, $p=0.002$, $n=6$). Furthermore, pre-exposure of chorionic arteries to MGO for 2 h significantly increased the vascular response to U46619 ($18 \pm 8\%$, $p=0.003$, $n=8$), compared to the control.

Conclusion: Our experiment showed that MGO induces vascular endothelial (barrier) dysfunction. Furthermore, it also substantiates the significance of mitochondrial-originated oxidative stress in the development of MGO-induced endothelial (barrier) dysfunction. Our second experiment indicated that besides the acute effects of MGO on endothelial dysfunction, it also results in sustained alteration in vascular reactivity, which is in line with the increased risk for cardiovascular diseases in patients with diabetes.

Poster 47

Title: Supplementation of methylglyoxal in drinking water does not affect the cerebral microvasculature and cognitive function in non-diabetic mice

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Background and aims: Diabetes is associated with cerebral small vessel disease (cSVD) and cognitive decline yet the underlying mechanism is poorly understood. Methylglyoxal (MGO), a by-product of glycolysis and a major precursor in the formation of advanced glycation end products (AGEs), is increased in individuals with diabetes and is associated with microvascular dysfunction. We previously showed that MGO and MGO-derived AGEs are increased in brain tissue of diabetic rats. The aim of this study was to investigate whether increased levels of exogenous derived circulating MGO can cause cerebral microvascular dysfunction and cognitive impairment in non-diabetic mice.

Materials and methods: 2-3 months old male C57Bl/6J mice were treated with MGO (50mmol/l, drinking water) or not (control) for 3 months (n=17 per group). Cognitive function was tested before treatment and at 6 and 13 weeks of treatment. Working memory, anxiety, short-term and long-term spatial learning and memory were tested using the Y-maze task, elevated zero maze task, object location task and Barnes maze task, respectively. After sacrifice, MGO and AGEs in plasma and brain were measured by UHPLC-MS/MS. Plasma inflammatory markers were assessed by ELISA. Cortical microvessels were isolated and used for further analysis.

Results: Plasma MGO was increased 2-fold (*p<0.0001)* and free plasma MGO derived hydroimidazolone-1 (MG-H1) and Nε-(1-carboxyethyl)lysine (CEL) were increased 1.2-fold (*p=0.01*) and 1.7-fold (*p=0.01*), respectively in the MGO group, while other AGEs were unchanged. In brains of MGO-treated animals, there was a 1.4-fold and a 1.1-fold increase in free MG-H1 (*p=0.02*) and CEL (*p=0.001*) in comparison to controls. In both plasma and brain, there were no differences observed in protein bound AGEs between MGO treated and control group. Behaviour and cognitive function remained unchanged in the MGO group vs controls. MGO did not change the expression of the plasma inflammatory markers IFN-γ, IL-10, IL-1β, IL-6, TNF-α and CXCL1. In isolated cortical microvessels, expression of inflammatory markers vascular cellular adhesion molecule 1, intercellular adhesion molecule 1, sirtuin 1 and AGE receptor, were unchanged.

Conclusion: Plasma MGO and MGO-derived AGEs were increased by supplementation of MGO in drinking water to a level comparable to that in diabetes. Although this was accompanied by increased levels of free MGO-derived AGEs in the brain, this was not associated with microvascular inflammation in the cortical microvessels and cognitive impairment. This indicates that circulating MGO by itself does not lead to microvascular inflammation nor cognitive decline and that the endogenous formation of MGO in diabetes, rather than circulating MGO, may be of importance for cerebral microvascular dysfunction and cognitive impairment.

Keywords: Methylglyoxal; Cognition; Brain; Microcirculation; Inflammation.

Poster 48

Title: ANFIS classifier for early diagnosis of autistic spectrum disorder based on plasma amino acid metabolome biomarkers.

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Background and aims: Autism spectrum disorder (ASD) is a developmental disorder of high prevalence in children and global public health concern. One in 54 children in the USA has ASD. The diagnosis of ASD is based on clinical behavioural and observational tests with long waiting times for expert referral and outcomes. Discovery and development of robust biomarkers for diagnosis and progression of severity of ASD may improve access to diagnosis and facilitate early-stage treatments. Mechanistic biomarker discovery will also likely reveal new causative factors and treatments. We previously identified changes in plasma protein glycation and oxidation adduct residues in ASD: increased Nε-carboxymethyl-lysine (CML), Nε-carboxymethyl-arginine (CMA) and dityrosine (DT) and decreased 3-deoxyglucosone-derived hydroimidazolone (3DG-H) which, combined in an algorithm, gave diagnostic classification of accuracy 88**%** (Anwar et al., *Molecular Autism* 9, 1-16, 2018).

The aim of this study is to improve the diagnosis utility of protein damage biomarkers in plasma for ASD by using an adaptive neuro fuzzy inference system (ANFIS) mathematical model. ANFIS has parameters optimized by a learning algorithm obtained from neural networks. We base the algorithm of plasma concentrations of amino acids and glycated and oxidised amino acids. These may be readily quantified in plasma ultrafiltrate with minimum pre-analytic sample processing.

Materials and methods: Plasma analyte data were from our previous study of 38 children with ASD (29 male, 9 female; age 7.6 ± 2.0 years) and 31 age-matched healthy controls (23 males, 8 females; 8.6 ± 2.0 years). Amino acids were quantified by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry. An ANFIS network was created using the Fuzzy Logic toolbox in MATLAB. An initial ANFIS is generated using a grid partitioning of the inputs and output. Two generalized Gaussian membership functions were specified for each input variable. The maximum number of training epochs was set to 50. The dataset of plasma concentrations of amino acids – including glycated and oxidized amino acids - is normalized and principal component analysis applied to reduce the number of features to two components. A 5-fold cross validation is applied on these two features components; with a 60:40% dataset split for training and testing. Based on the training error for each fold, the ANFIS is improved by Sequential Feature Selection. Five algorithms were produced. Mean and 95% confidence intervals (CI) for classification parameters are given.

Results: The ANFIS giving the highest accuracy of classification of ASD and children with normal development had plasma amino acid analytes features: arg, met, ser, trp, CML, CEL, FL, MetSO, 3DG-H. The accuracy was 96.6% (CI 94.5 – 98.6%), sensitivity 85.7% (CI 83.6 – 87.8%), specificity 99.0% (CI 97.9 – 100%) and positive likelihood ratio LR+ 85.7 – suggesting strong, often conclusive evidence of diagnosis of ASD.

Conclusion: The application of ANFIS machine learning markedly improved the classification characteristics achieved from amino acid metabolome analytes. With further validation, this method may profoundly facilitate access and timeliness of diagnosis of ASD in children.

Keywords: autism spectrum disorder; machine learning; glycated amino acids; diagnosis.

Poster 49

Title: Machine-learning-based prediction models for early-stage diagnosis of knee osteoarthritis using protein oxidation, nitration and glycation biomarkers.

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Background and aims: Musculoskeletal disease is the most common cause of chronic disability worldwide. Impaired musculoskeletal health accounts for *ca.* 2% of total global disability adjusted life years, of which osteoarthritis (OA) is 1.1% and rheumatoid arthritis (RA) 0.3%. OA is progressive joint disease characterized by joint inflammation and a reparative bone response; the latter is gradually overwhelmed by progression of joint degeneration and insidious chronic pain and impairment of joint mobility develop. It affects *ca.* 100 million people globally and is the primary diagnosis in a majority of 2.9 million joint replacements – including 1.1 million knee joints. If OA and RA could be identified in the early stages, available treatments for RA could be initiated earlier and treatments for early-stage OA developed such that pain and disability of advanced disease could be prevented. Diagnosis of early-stage OA and RA and risk of progression to severe musculoskeletal disease is an unmet clinical need. Moreover, it is fundamental to improve prediction models and subgrouping of patients. Current statistical modelling approaches cannot process a sufficient amount of information. This research work proposes a machine learning-driven diagnosis technique aiming at predicting the development of arthritis at early stage using protein biomarkers.

Materials and methods: Patient recruited for this study were as previously described (Ahmed *et al*., Arthritis Research & Therapy 18: 250, 2016): 46 patients with early-stage OA, 45 patients with early-stage RA, 42 patients with inflammatory arthritis other than RA, non-RA and 53 healthy controls. Glycated, oxidized and nitrated amino acids were quantified by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry. Plasma hydroxyproline and anti-(cyclic citrullinated peptide (CCP) antibody status was also determined. These analytes were used in training and testing of the machine learning algorithms. The trained algorithm enables classification of healthy individuals and type of early-stage arthritic disease.

Results: The computational test results indicate that the proposed solution achieves competitive prediction performance with the following values of sensitivities/specificities: 87.5%/95.8% good skeletal health, 85.7%/96% early-stage OA, 81.8%/100% early-stage RA, and 100%/92.3% non-RA.

Conclusion: Oxidized, nitrated, and glycated amino acids in combination with hydroxyproline and anti-CCP antibody status have been proven to be reliable predictors of early-stage arthritis. A machine learning model trained on these data from patients and healthy control predicted the development of arthritis with high confidence.

Keywords: osteoarthritis; rheumatoid arthritis; machine learning; glycated amino acids; oxidized amino acids; diagnosis.

Poster 50

Title: Visceral adipose tissue seems to increase dicarbonyl stress in women with overweight.

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Background and aims: A higher visceral adipose tissue (VAT) area has been associated with higher methylglyoxal (MGO) urinary levels. Therefore, VAT accumulation could increase dicarbonyl stress. To prove this hypothesis, the aim of this study was to evaluate the levels of Glyoxalase-1 (Glo-1) (MGO detoxifying enzyme), methylglyoxal-derived 1-hydroimidazolone (MG-H1) (an advanced glycation end product derived from MGO), and hypoxia-inducible factor 1α (HIF1α) (a marker associated with VAT accumulation with effects in Glo-1), and to compare these markers in adults with overweight and different VAT area.

Materials and methods: A cross-sectional study was conducted in 75 subjects 20 to 45 years old with overweight (56% were male). Clinical and anthropometrical variables were evaluated, and the body composition was assessed by bioimpedance analysis (InBody S10). The lipid profile and fasting glucose were quantified by standardized methods, and insulin, Glo-1, MG-H1, and HIF1α were quantified in serum by ELISA assays. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using Matthews's equation. Subjects were classified into two study groups according to their VAT area: group 1 (G1) VAT <100 cm^2 and group (G2) VAT ≥100 cm^2. Comparisons between groups for all the variables were performed using the Student t test or Mann-Whitney U test. In addition, Pearson's correlation analyses were used to evaluate associations between VAT area, with Glo-1, MG-H1, and HIF1α. A p-value ≤0.05 was considered statistically significant for all the analyses.

Results: Forty-one subjects were classified in G1 (58% were male), and 34 subjects were classified in G2 (53% were male). Metabolic markers were similar in both groups even after sex-specific analysis. In the group with a VAT area ≥100 cm^2, the levels of MG-H1 were higher when compared to G1 (32.22 ± 5.64 *vs* 28.92 ± 7.66 ng/ml, p-value=0.035), Glo-1 and HIF1α were similar in both groups. However, in the sex-specific analysis, women in the G2 had higher MGH1, Glo-1, and HIF1α levels when compared to the G1 women, Table 1. Additionally, in women the VAT area showed significant positive correlations with MGH1, HIF1α, and Glo-1 (r=0.55, r=0.48, r=0.38 respectively). Furthermore, MG-H1 showed significant positive correlations with HIF1α (r=0.41) and Glo-1 (r=0.34, p-value=0.05**).**

Table 1. Comparative analysis of Glo-1, MG-H1 and HIF1α levels stratified by sex.

	Women			Men		
	Group 1 n=17	Group 2 n=16	p-value	Group 1 n=24	Group 2 n=18	p-value
Glo-1 (ng/ml)	88.3 ± 17.94	101.5 ± 13.18	0.02*	98.3 ± 17.72	98.5 ± 13.33	0.97
HIF1α (ng/ml)	3.8 ± 0.55	4.3 ± 0.65	0.03*	4.0 ± 0.62	3.8 ± 0.57	0.28
MG-H1 (ng/ml)	25.5 ± 7.94	33.0 ± 6.86	0.01*	31.3 ± 6.61	31.5 ± 4.38	0.91

Data presented as means ± SD. Comparisons were determined using Student t test. * represent a p-value ≤0.05.

Conclusion: Higher levels of MG-H1 were found in subjects with an increased VAT area. Furthermore, women with higher VAT accumulation had increased Glo-1, MG-H1, and HIF1α levels, also VAT was associated with MG-H1, Glo-1, and HIF1α. Therefore, VAT accumulation could increase dicarbonyl stress in women with overweight**.**

Keywords: Dicarbonyl stress, visceral adipose tissue, glyoxalase-1, hypoxia-inducible factor 1α, methylglyoxal-derived 1-hydroimidazolone.

Poster 51

Title: Natural Products as a Source of Inspiration for Novel Inhibitors of Advanced Glycation Endproducts (AGEs)
Authors: Stefaniya Velichkova
Affiliations: University of Antwerpen

Background and aims: Protein glycation, a post-translational modification found in biological systems, is often associated with a core defect in glucose metabolism. In particular, advanced glycation endproducts (AGEs) are complex heterogeneous sugar-derived protein modifications implicated in the progression of pathological conditions like atherosclerosis, diabetic complications, skin diseases, rheumatism, hypertension, and neurodegenerative diseases. Undoubtedly, there is the need to expand the knowledge about anti-glycation agents that can offer a therapeutic approach in preventing and treating health issues of high social and economic importance. Although various compounds have been under consideration, only for a few data from clinical trials are available, and there is a lack of approved and registered anti-glycation agents. Next to the search for novel synthetic AGEs inhibitors, more and more efforts of scientists are focusing on anti-glycation compounds from natural origin. The purpose of the current poster is to provide an overview of the state of scientific knowledge in the field of natural products from plant origin (e.g., extracts and pure compounds) as inhibitors of AGEs formation in the past nearly three decades.

Materials and methods: The publication frequency in the research of natural products as potential inhibitors of AGEs formation was monitored over the period 1990-2019. The database used was Web of Science, and the search terms were: "natural products" and "AGEs", which reviled a total count of 4.251 publications.

Results: The plant-derived AGEs inhibitors represent attractive novel therapeutic agents. Together with already existing synthetic drugs, they can join forces in the treatment and prevention of health issues of major importance. The latter includes diabetes, neurodegenerative disorders, and aging. It is of great importance to identify anti-glycation substances and to examine their mode of action. The poster summarized the reports in the past three decades for plant-derived natural products with anti-glycation activity. *The pure compounds were presented according to the established classification of plant secondary metabolites.* In general, the promising anti-glycation activity of the extracts is in a tight correlation with their total phenolic content. However, many nonphenolic compounds such as terpenoids, flavonoids, alkaloids prevented the non-enzymatic glycosylation. The vast number of plant-derived pure compounds showed AGEs inhibition through several mechanisms of action such as: trapping dicarbonyl intermediates, hyperglycemic activity, decrease expression of RAGE, potent free radical scavenging activity. Additionally, some of them possessed other pharmacological properties like anti-inflammatory activity or reducing insulin resistance. Therefore, the synergistic effect can improve the overall glycemic control through several mechanisms of action.

Conclusion: On the contrary to synthetic agents, the full potential of plant products has been still unrevealed and requires further comprehensive analysis to expand the antiglycation phytomolecules. The use of validated analytical methods is a crucial aspect to identify the potent and promising AGEs inhibitors from natural origin. Consequently, this can determine the outcome for developing medications using plant products, conducting clinical trials, and eventually, having new therapeutic agents reaching the market.

Poster 52

Title: AGE consumption and cognitive capacity in elderly
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Background and aims: During the aging process, several metabolic and behavioral changes occur, such as increased plasma glucose and worsening of cognitive functions. These changes can be accelerated due to the adoption of the Western lifestyle that includes inappropriate habits such as smoking, physical inactivity, high consumption of ultra-processed foods and high in fat and sugars. One of the implications of this reality is the increased the advanced glycation end products (AGE) formation and consumption. This study aimed to estimate the AGE consumption in elderly people and to relate them with their cognitive capacity.

Materials and methods: Thirty-one elderly people (on average 70 years old) of both sexes were recruited and performed an anthropometric evaluation (measured body mass, height and abdominal circumference), social-demographic questionnaire and cognitive evaluation (Montreal Cognitive Assessment - MoCA). To estimate the intake of AGEs, the food reminder of three different days was applied, which made it possible to estimate the dietary intake of the participants. The consumption of AGEs was estimated from a database containing 549 food with respective AGEs values (Uribarri J et al 2010). The project was approved by local ethical committee (CAAE 30592019.4.0000.0089).

Results: The subjects consumed 22654±12325 AGE (KU/day), an amount of AGE above the advised amount. The BMI was 25.5±3.6 (mean ± SD).The cognitive assessment score was 22.2±2.3 (mean ± SD). As expected, we observed higher performance in the attention task the higher the education level of the elderly [r=0.460; p=0.009]. Interestingly, when correlating the attention task with AGE consumption, we observed that higher performance in the attention task correlated with lower AGE consumption [r=0.-431; p=0.015].

Conclusion: The high AGE consumption may interfere with the cognitive ability in elderly, as evidenced by the performance in the attention task. Thus, some changes in diet or in food preparation may contribute to the preservation of cognitive aspects in the elderly.

Poster 53

Title: *Staphylococcus aureus* biofilm formation is promoted by glycated keratin

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Background and aims: Non-enzymatic glycation leads to the accumulation of advanced glycation endproducts (AGEs) in body tissues, contributing to the pathogenesis of aging and related disorders. Glycative Stress can increase susceptibility to infection by reducing immune function and potentially by altering host pathogen interactions. While it has been reported that glycation of the bladder epithelium plays a role in increased susceptibility to urinary tract infection, the role of glycative stress in other infectious disease systems is unknown. Diabetes mellitus and skin disorders such as atopic dermatitis and psoriasis, which are characterized by increased glycative stress, are often associated with skin lesions that are colonized by *Staphylococcus aureus* and its biofilms, exacerbating symptoms and slowing healing. Diabetes is also associated with increased carriage and abundance of *S. aureus* on the skin. Therefore, we hypothesized that glycative stress may play a role *S. aureus* related dysbiosis on the skin. Here, we examined the capability of glycated proteins produced from glucose and keratin to trigger increased biofilm formation in *S. aureus in vitro.*

Materials and methods: Glycated keratin solution was produced by incubating glucose and keratin in phosphate buffer (50 mM phosphate buffer (pH 7.4), 0.60 mg/mL keratin, and 40 mM glucose) at 60°C for 10 days, followed by centrifugal ultrafiltration to remove unreacted glucose. *S. aureus* strain ATCC12600 was cultivated in tryptic soy broth with supplemented glycated keratin under static conditions at 37°C for up to 48 hours, and biofilm formation was measured photometrically by absorbance at 587nm after staining with 0.1% crystal violet solution.

Results: Addition of 1.0 mg/mL of glycated keratin to *S. aureus* static cultures induced a 3.9-fold increase in biofilm formation (n = 8, p <0.001). Glycated keratin increased biofilm formation in a dose dependent manner, with absorbance approaching a maximum absorbance of roughly 3.0 above the initial value at dosages over 2.0 mg/mL (ED50 at 0.6 mg/mL). The higher molecular weight fraction (> 10 kDa) invoked a greater response than the low molecular weight fraction (3—10kDa) at the same dosage (0.36 vs 0.61 respectively, p < 0.001).

Conclusion: Exposure to glycated keratin *in vitro* significantly increased biofilm formation in *S. aureus*. Elevated glycative stress and accumulation of AGEs in the skin may contribute to increased pathogenicity of skin microbes like *S. aureus*, leading to dysbiosis and greater susceptibility to infection. Efforts to reduce glycative stress in those suffering from skin conditions associated with *S. aureus* dysbiosis may help to reduce the risk of pathogenic colonization.

Keywords: *Staphylococcus aureus*, glycative stress, biofilm, advanced glycation endproducts.

Poster 54

Title: Urinary glucosepane as a marker of glycemic control in non-diabetic subjects – insight from the HATFF study.

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Background and aims: Glycated hemoglobin A1C is the primary clinical biomarker of glycemic control. The steady-state level of A1C *in vivo* is influenced by deglycation by fructosamine 3-kinase and blood glucose concentration during the 120 days prior to sampling. A1C is, therefore, relatively unresponsive to improvements in glycemic control in short-term intervention studies to improve dysglycemia – particularly for the low level changes expected for intervention studies in non-diabetic overweight and obese subjects. Glycated (fructosamine-modified) albumin provides a report on glucose control over a shorter period, 14 - 20 days, prior to blood sampling but suffers interference from change in albumin transcapillary escape rate, spontaneous deglycation and only reports on glycation in the vascular and interstitial fluid compartments. In studies of stability of glycation adducts for epidemiological and early decline in metabolic health, we found the stable, N_ϵ-fructosyl-lysine (FL)-derived advanced glycation endproduct (AGE), glucosepane (GSP), was stable during sample collection and pre-analytic processing and GSP urinary free adduct was not correlated with pyrraline, suggesting limited absorption of GSP from the diet. GSP is also a major glycation-derived protein crosslink. Urinary GSP is, therefore, expected to report on total body glucose glycation and reflect steady-state levels of protein glycation crosslinking. The aim of this study was to explore the responsiveness of urinary GSP to improvement of dysglycemia with intervention by glyoxalase 1 inducer, *trans*-resveratrol and hesperetin (tRES-HESP) in the healthy ageing through functional food (HATFF) study.

Materials and methods: The HATFF study was a double blind, placebo-controlled crossover study in non-diabetic overweight and obese subjects (18 overweight and 11 obese) evaluating the effect of tRES-HESP (90 mg tRES, 120 mg HESP) given daily by oral capsule before breakfast, for 8 weeks with 6 weeks washout before crossover. The study was approved by National Research Ethics Service Committee West Midlands - Coventry & Warwickshire, U.K. (project number 13/WM/0368) and registered on the Clinicaltrials.gov (identifier: NCT02095873). Urinary GSP and N_ϵ-fructosyl-lysine were assayed by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry.

Results: We found that urinary GSP was decreased in obese subjects post-supplement with tRES-HESP compared to placebo in the HATFF study; urinary GSP (nmol/mg creatinine, mean ± SEM, n = 11) 3.23 ± 0.21 vs 3.69 ± 0.28 (-12%, P = 0.027, *paired t-test*); *cf.* decrease of endogenous methylglyoxal-derived AGE, MG-H1 free adduct, with tRES-HESP treatment of -14% (P<0.01). There was no change in A1C and urinary FL post-supplement with tRES-HESP compared to placebo in the HATFF study, likely due to long A1C half-life and varied contribution of dietary FL to these biomarkers, respectively.

Conclusion: We conclude that urinary GSP may be a valuable epidemiological and interventional biomarker of glycemic control and indicator of whole body protein glycation crosslinking.

Keywords: glycemic control; biomarkers; glycated hemoglobin A1C; glycated albumin; glucosepane; glyoxalase 1 inducer.

Poster 55

Title: Role of glyoxalase 1 in adipogenesis and lipolysis in 3T3-L1 cell culture model of adipogenic differentiation

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Background and aims: Dicarbonyl stress characterized by an increase in methylglyoxal (MG) or a decrease in glyoxalase 1 (Glo1) or both is associated with obesity in both humans and in animal models. Moreover, MG has been shown to promote lipid accumulation and enhance markers of adipogenesis in 3T3-L1 differentiated adipocytes. However, whether Glo1 plays a causative role in adipogenesis is not known. We also wanted to investigate the effect of Glo1 inducers on lipolysis in mature adipocytes.

Materials and methods: Mouse fibroblast cell line (3T3-L1) were cultured in DMEM containing 4.5g/L glucose supplemented with 10% FBS. Differentiation was induced using 1 µM dexamethasone, 0.5 mM methylisobutylxanthine and 0.860 µM insulin for 2 days and was then maintained for 6 days with DMEM media and insulin. After 2, 4 or 6 days cell lysate was collected for further analysis. In some experiments, a separate group of cells were treated with S-p-bromobenzyl glutathione cyclopentyl diester (BBGC) to inhibit Glo1 enzyme activity. Glo1 activity was estimated using an Abcam assay kit by monitoring change in absorption at 240 nm to detect formation of S-D-lactoylglutathione. Protein expression was assessed using Western blotting. Lipid accumulation was assessed using Oil Red O stain. Experiments were repeated at least 3 times.

Results: Differentiation of adipocytes was associated with a significant increase in expression of fatty acid synthase (FAS), a marker of mature lipid laden adipocyte and lipid accumulation (Oil Red O stain) after 2, 4 and 6 days. On the other hand, adipocyte differentiation was associated with a decrease in Glo1 activity. Compared to day 2, Glo1 activity (107.7 ± 7.1 vs. 79.5 ± 3.0 nmol/mg/min) was decreased ($P<0.05$) by ~26% after day 6. Further more, Glo1 inhibition with BBGC (2.5 uM) was associated with a ~27% decrease in Glo1 activity and ~40-50% increase in FAS expression. In addition, in 6 day differentiated adipocytes, Glo1 inducers (*trans*-resveratrol and hesperetin, tRES-HES, 5 uM) significantly increased Glo1 activity (110 ± 4 vs. 85 ± 3, $P<0.05$) and ATGL and PGC1-alpha expression that are indicative of lipolysis after 24 hours and reduced lipid accumulation as measured by Oil Red O stain.

Conclusions: Inhibition of Glo1 activity results in enhanced adipogenesis. Activation of Glo1 using tRES-HES promotes lipolysis.

Keywords: Glyoxalase 1, adipogenesis, lipolysis, trans-resveratrol, hesperetin.

Poster 56

Title: Ultra-high temperature (UHT) treatment and prolonged storage of liquid infant formula induces protein modifications, gut dysfunction and inflammation in preterm pigs

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Background and aims: Ultra-high temperature (UHT)-treated infant formula (IF) is increasingly being used for hospitalized infants when human milk is unavailable. UHT treatment eliminates pathogens and extends shelf life, but heating and storage may negatively affect the quality by reducing bioactivity and increasing the formation of Maillard reaction products (MRPs). We hypothesized that stored UHT-treated IF negatively affects gut health in sensitive newborns.

Materials and methods: Using preterm pigs as a model for sensitive newborn infants, we fed liquid IFs subjected to indirect UHT (UHT), UHT with storage at 40°C for 60 days (SUHT) or just pasteurization (PAST). Diet bioactivity and MRP levels were determined together with markers of gut maturation and health. Accumulation of advanced glycation endproducts (AGEs) in the gut tissues collected from piglets after a 5-day-feeding trial was investigated by LC-MS/MS.

Results: Relative to PAST, the UHT-IFs contained reduced levels of bioactive proteins (IgG, lactoferrin). Storage increased MRP levels (up to 13-fold) and non-reducible protein aggregates (SUHT vs. UHT). Furthermore, SUHT had lower antimicrobial capacity (versus *E. faecalis, S. epidermidis*) than PAST. Following five days of feeding, pigs fed SUHT had more diarrhea than pigs fed PAST and more signs of intestinal inflammation (necrotizing enterocolitis) than PAST and UHT pigs. UHT and particularly SUHT pigs showed lower intestinal villus heights and higher crypt depths and an increase in MPO-positive cells (monocytes and neutrophils), relative to PAST. Additionally, digestive enzyme activities (lactase, aminopeptidase N) were reduced in SUHT vs. PAST pigs, with intermediate values in pigs fed UHT. In SUHT pigs, this was accompanied by gut accumulation of MRPs (furosine and AGEs, including N-ε-carboxymethyllysine (CML)) as well as the protein-cross-links lysinoalanine (LAL) and lanthionine (LAN) and RAGE-mediated inflammatory responses involved upregulation of genes involved in acute inflammatory responses and cell turnover (e.g. *C3, TNFA, TNFAIP3, IL6, MCP1, CD62L, CASP3, PCNA, OLFM4, TGFB1)*.

Conclusion: UHT treatment followed by prolonged storage of IF reduced protein bioactivity and increased MRP and protein cross-link accumulation. This was associated with impaired gut maturation and function in preterm pigs in the first days of life. UHT-treatment and storage may negatively affect the quality of liquid IFs and may thereby negatively affect organ development in newborn infants, particularly those that are very diet-sensitive or immature.

Keywords: liquid infant formula, advanced glycation endproducts, gut health, preterm pigs.

Poster 57

Title: The postprandial methylglyoxal formation during an oral glucose tolerance test is derived from exogenous glucose

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Background and aims: Methylglyoxal (MGO), a reactive dicarbonyl compound and a major precursor in the formation of advanced glycation endproducts, is mainly formed as a byproduct of glucose during glycolysis. Excess formation and accumulation formation and accumulation of MGO are linked to diabetes and its vascular complications. We have previously shown that plasma MGO concentrations rapidly increased both during an oral glucose tolerance test (OGTT) or a mixed meal test, with a higher increase in individuals with type 2 diabetes. Spikes of MGO are believed to be a major contributor for vascular complications. However, the exact source of MGO is unknown. The aim of the study was therefore to investigate whether postprandial MGO formation directly originates from exogenous glucose.

Materials and methods: We performed a stable isotope labeled OGTT in 12 healthy males (age 25 yrs (range 21-30 yrs), BMI 22.5 kg/m^2 (range 19.2-24.7 kg/m^2). The treatment was a solution of 50 g glucose of which 2% is universally labeled D(+)13C glucose and 98% unlabelled 12C glucose. Blood samples were taken every 15 min for first two hours and every half an hour for the next four hours after the intake of glucose. Concentrations of MGO and glucose in plasma during OGTT (6 h) were measured at eleven time-points with ultra-performance liquid chromatography-tandem mass spectrometry. 13C MGO data were corrected for enrichment (*50) and for a difference in mass spectrometry response factor between 13C MGO and 12C MGO.

Results: During the first 180 min of the OGTT, plasma 12C glucose levels increased rapidly and reached a peak at 30 min, after which levels started to decline with the lowest level at 180 min post-load. After 180 min, plasma concentration of 12C glucose showed a slight increase. The plasma 13C glucose curve showed the same pattern as for 12C glucose, but the increase after 180 min was not observed, probably reflecting formation of 12C glucose in the second phase as a compensation for insulin-mediated glucose lowering below baseline levels. During the first 180 min of the OGTT, plasma 12C MGO and 13C MGO followed the same pattern with a rapid increase with a peak at 45 min, after which levels started to decline, with the lowest level at 180 min post-load. After 180 min post-load, 12C MGO, but not 13C MGO, showed a clear increase. We calculated that newly formed MGO in the early phase of the OGTT is completely derived from exogenous glucose, while the increase of MGO after 180 min is not.

Conclusion: These data show that the rapidly increase in levels of plasma MGO during an OGTT arise from exogenous glucose, while the increase of MGO after 180 min originates from an endogenous substrate. Where exactly MGO is produced in the postprandial phase is subject of further research.

†Authors GD and MA were employees of Unilever R&D at the time this research was designed and conducted and have since retired from Unilever. MA changed her professional affiliation.

Poster 58

Title: Ingestion, digestion and physiological impact of exogenous N^{ε}-carboxymethyl-lysine: dietary protein *versus* protein supplement

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Background and aims: The biological effects of dietary advanced glycation end-products (AGEs), including N^{ε}-carboxymethyl-lysine (CML), **are linked to mechanisms identified by *in vitro* and/or *in vivo* studies**: problem of digestibility of glycated proteins, generation of neo-allergens and induction of inflammatory signals following interaction of AGEs with receptors, depending on the specificity of the latter for free or peptide-bound AGEs. These studies have been supplemented by epidemiological data and clinical trials demonstrating the benefits of dietary AGE restriction. To our knowledge, few studies have differentiated the effect of exogenous AGEs on health depending on the protein source: dietary protein or protein supplement. Most of the murine experimental studies have been carried out with animal food supplemented with glycated or non-glycated bovine serum albumin (BSA). The use of protein supplement may underestimate the biological effects of exogenous glycation. Using the nematode *Caenorhabditis elegans*, we compared the ingestion and digestion of 2 exogenous AGEs: dietary protein-bound CML and protein supplement-bound CML. We analyzed the biological effects of these 2 glycated proteins in this *in vivo* model.

Materials and methods: To generate CML, the main food of the worms (bacteria) and bovine serum albumin (BSA) were glycated with glyoxylic acid in the presence of a reducing agent, $NaBH_3CN$. Non-glycated BSA and control bacteria were exposed to $NaBH_3CN$ alone. After incubation of the worms in the control and CML-rich diets, the relative amount of CML epitopes in the worms was quantified by dot-blot. CML-bound peptides were analyzed by western blot. Different markers of cell homeostasis and CML elimination were evaluated by RT-qPCR. Microscopic analyzes of *C. elegans* development were performed.

Results: Worms ingested and digested both glycated proteins: dietary protein-bound CML and protein supplement-bound CML. However, higher amounts of CML were detected in worms incubated with dietary protein-bound CML. This diet modulated the expression of genes coding for proteins involved in i) antioxidant response, ii) cytoplasmic and mitochondrial unfolded protein responses, iii) luminal and lysosomal protein digestion and iv) scavenging response. Protein supplement-bound CML also induced such responses but with higher concentrations of CML in the culture medium. When the worms were incubated with dietary protein-bound CML, their reproduction was significantly impaired. This characteristic was not observed after incubation of the worms with glycated protein supplement.

Conclusion: Our results confirm the biological effects of exogenous CML *in vivo*. The severity of these effects depends on the source of exogenous CML: dietary proteins or protein supplement.

Poster 59

Title: Control of endogenous glucose-induced N^ε-carboxymethyl-lysine production and its impact on the lifespan of *Caenorhabditis elegans*
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Background and aims: The link between hyperglycemia and endogenous glycation has long been established. There are several hypotheses regarding the mechanisms inducing endogenous glycation. Using the nematode *Caenorhabditis elegans*, Schlotterer *et al.* have shown that methylglyoxal (MGO) produced during glycolysis induces the production of MGO-derived hydroimidazolone (MG-H1). This AGE induces mitochondrial dysfunction and reduces the worms' longevity (Diabetes. 2009. doi: 10.2337/db09-0567). More recently, our preliminary work showed that N^ε-carboxymethyl-lysine (CML) is also produced when *C. elegans* is grown in a glucose-rich medium, suggesting that a mechanism independent of MGO production is involved. To highlight this mechanism, we first analysed the impact of different environmental factors (dietary nutrients and microbiota) on the induction of endogenous CML by glucose.

Materials and methods: Worms were incubated with different concentrations of glucose in 2 different media: a medium containing peptone and a defined medium without peptone. Worms were grown in the presence of live or inactivated bacteria. Lifespan assays were performed. The relative amount of CML in the worms was quantified by dot-blot. Immunohistochemical analyzes were used to detect CML epitopes in the worms. Different oxidation markers were measured: expression of antioxidant genes by RT-qPCR and oxidation of proteins by oxyblot. The expression of genes encoding glyoxalases and scavenger receptors were measured by RT-qPCR.

Results: The longevity of the worms was reduced by the glucose diets in a dose-dependent manner. The pathophysiological effect of glucose was greater with live bacteria and in the medium without peptone. CML epitopes were detected in the apical part of the intestine of worms grown with glucose. The amount of CML epitopes depended on glucose concentration and was highest in worms incubated with inactive bacteria in medium without peptone.

The expression of genes encoding antioxidant proteins, including superoxide dismutase SOD-3 and glutathione-S-transferase GST-4, depended on on glucose concentration. These genes are more expressed when worms were grown with live bacteria in the medium without peptone.

Expression of the gene encoding the glutathione-independent glyoxylase, DJR-1.2, was induced by glucose in a dose-dependent manner. This gene was more expressed when worms were grown with inactivated bacteria. Some genes encoding scavenger receptors wee more expressed by worms grown in the medium with glucose and peptone.

Conclusion: An association between glucose-rich diet, oxidative markers, endogenous glycation and worms' longevity has been demonstrated in *C. elegans* under certain culture conditions, especially in media with inactive bacteria. These results show that different factors modulate the pathophysiology of glucose. To study the physiopathological mechanisms of glucose linked to endogenous glycation, it is essential to grow *C. elegans* in a medium with inactive bacteria and preferably without peptone.

Poster 60

Title: Advanced glycation end products (AGEs) in relation to COPD and related lung function

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Background and aims: COPD is a progressive lung disease causing a large burden of morbidity and mortality worldwide. Previous studies found higher advanced glycation end product (AGE) levels in lung tissue, plasma and skin in COPD. However, it is still unclear if and how AGEs are involved in the development or progression of COPD. More studies in large populations are needed. We investigated the association between skin AGEs measured by skin autofluorescence (SAF) and COPD prevalence and relevant lung function parameters in the Rotterdam Study, a prospective population-based cohort.

Materials and methods: In 2594 participants including 615 prevalent COPD patients, AGEs were measured by SAF using the AGE ReaderTM (DiagnOptics B.V., Groningen, The Netherlands). Spirometry (Forced Expiratory Volume in one second [FEV1], expressed as % predicted) and diffusing capacity of the lung for carbon monoxide (D_{LCO} and D_{LCO}/alveolar volume [V_A]) were measured according to ATS/ERS guidelines. D_{LCO} and D_{LCO}/V_A values were corrected for haemoglobin levels ($D_{LCO}c$ and $D_{LCO}c/V_A$). COPD prevalence and spirometry data were collected at the same time point of SAF measurement. The associations of SAF with COPD prevalence, COPD severity (GOLD stage), and lung function were analysed cross-sectionally in logistic and linear regression models respectively. Stratified analyses by COPD status and smoking status were performed.

Results: SAF (in arbitrary units [AU]) was associated with COPD prevalence (OR=1.256, p=0.029), FEV_1 % predicted (β=-3.517, p<0.001), and $D_{LCO}c$, (β= -0.245, p=0.005). SAF was associated with COPD GOLD stage (OR=4.037, p=0.001，stage 3&4 compared to stage1). Subgroup analyses showed that association between SAF and FEV_1 % predicted was stronger in COPD patients (β=-6.427, p<0.001) than that in non-COPD group (β=-1.873, p=0.021); SAF was associated with $D_{LCO}c$ and $D_{LCO}c/V_A$ only in COPD patients (β=-.608, p=0.001; and β=-0.065, p=0.015 respectively) but not in non-COPD group; SAF was associated with FEV_1 % predicted and $D_{LCO}c$ only in former smokers (β=-4.802, p<0.001; and β=-0.277, p=0.059 respectively) and current smokers (β=-4.516, p=0.022; and β=-0.416, p=0.005 respectively) but not in never smokers (β=-0.194, p=0.886; and β=-0.004, p=0.970 respectively).

Conclusion: Skin AGEs are associated with COPD prevalence and COPD severity and also with impaired lung function, especially in COPD patients, and in current and former smokers. Further analyses are needed to investigate whether smoking explains the observed associations between skin AGEs and COPD and impaired lung function.

Key words: advanced glycation end products (AGEs), skin autofluorescence (SAF), chronic obstructive pulmonary disease (COPD), spirometry, lung function.

Poster 61

Title: The glycolytic by-product methylglyoxal is present in very high concentrations in human leukocytes and increases after an oral glucose tolerance test.

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Background and aims: Immune cell activation has been identified as a key driver of diabetic complications. Activation of immune cells is linked with increased glycolysis. The dicarbonyl compound methylglyoxal (MGO) is formed as a byproduct of glycolysis. Plasma MGO levels peak after a glucose load in individuals with diabetes. It is currently unknown what the levels of MGO are in circulating immune cell subsets and whether MGO accumulates in these cells after a glucose load. We therefore investigated levels and accumulation of MGO in circulating leukocytes.

Materials and methods: MGO was quantified in human blood fractions using ultra-performance liquid chromatography-tandem mass spectrometry (whole blood, plasma, erythrocytes, and leukocytes) and in lymphocytes, granulocytes, and monocytes isolated from healthy donors using fluorescence-activated cell sorting (FACS). Potential peroxidase interference and consequent MGO formation during the pre-analytical procedure was avoided by performing the derivatization directly in perchloric acid at a low pH and by using NaN_3. Next, we estimated MGO content in the specific circulating immune cell subsets with flow cytometry using a fluorescent probe that detects MGO (mean fluorescence intensity/MFI). MGO concentrations are presented as mean ± standard deviation.

Results: MGO levels were much higher in whole blood than in plasma (3.31±0.54 μM vs 0.076±0.023 μM). Leukocyte and erythrocyte fractions contained comparable amounts of MGO and contributed to the majority of whole blood MGO. Further measurements in purified leukocyte subsets showed that intracellular MGO concentrations were extremely high in lymphocytes (2698±1888μM), granulocytes 1706±925μM), and monocytes (2873±427μM). Intracellular MGO concentrations were 1000-fold lower in erythrocytes (2.53± 1.46μM). During an oral glucose tolerance test, the glucose load increased intracellular MGO levels in granulocytes with 6.5%, in monocytes with 18% and in lymphocytes with 26%.

Conclusion: MGO is present in a very high concentration in leukocytes and increases in leukocytes after a glucose load in abdominally obese individuals.

Poster 62

Title: Glyoxal-induced formation of carboxymethyl arginine in type 1 collagen decreases both its strength and flexibility *in vitro*

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Background and aims: Glycation of proteins induces the formation of advanced glycation end products (AGEs), such as carboxymethyl arginine (CMA), which is preferentially generated in glycated collagen type I, an essential component of bone tissue. However, the effect of AGE formation on the characteristics of type I collagen remains unclear due to the lack of suitable *in vitro* experimental systems analyzing type I collagen. The aim of this study is elucidation of the effect of glyoxal, the inducer of collagen-specific CMA, on the characteristics of type I collagen.

Materials and methods: Similar to mammalian bones, fish scales are composed of osteoblasts, osteoclasts, and calcified bone matrixes, including type I collagen, and they use a parathyroid hormone and calcitonin to regulate calcium metabolism in osteoblasts and osteoclasts. The present study isolated type I collagen from fish scales to analyze the formation of AGEs in type I collagen and the effect of glyoxal on the characteristics of type I collagen *in vitro*.

Results: Type 1 collagen molecules comprise $\alpha 1$ and $\alpha 2$ chains. Biochemical studies found that glyoxal induced the crosslinking in two or three of $\alpha 1/\alpha 2$ chains, and that it also induced the formation of CMA in type 1 collagen. In addition, a three-point bending test of type 1 collagen provided evidence that glyoxal significantly decreased the strength and flexibility of type I collagen.

Conclusion: Our study using the goldfish scale model provides evidence that the AGE formation in type I collagen induced by glyoxal, the CMA inducer, facilitates the crosslinking of type I collagen, decreasing both its strength and flexibility.

Poster 63

Title: Change in AGEs and 2SC in mice tissues with aging

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Background and aims: A nonenzymatic post-translational modification, including the Maillard reaction and succination, leads to decreased enzymatic function and hyposecretion of enzymes by modifying the physiological proteins, thereby causing onset and development of diseases such as diabetes and arteriosclerosis. Previous studies have reported that advanced glycation end-products (AGEs) were gradually elevated with increasing age and pathology. Moreover, marked accumulation of N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(carboxyethyl)lysine (CEL), and pentosidine was observed in human articular cartilage collagen and skin collagen with increasing age; additionally, CML and CEL were increased in human lens proteins. Aging is a risk factor for cognitive decline and neurodegeneration; however, only a few studies have reported AGEs variation in brain with increasing age. Furthermore, S-(2-succinyl)cysteine (2SC) is a product of succination formed by the modification of cysteine in protein with fumarate, an intermediate of TCA cycle in mitochondria. Although the involvement of 2SC with diabetes and mitochondrial disease has been reported, the measurement of 2SC in physiological samples by instrumental analysis is still difficult. Few studies have reported the relationship between aging and the accumulation of 2SC in tissues. In the present study, the change in 2SC and AGE accumulation in aging mice tissues was evaluated by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to clarify the variance of post-translational modification products with aging.

Materials and methods: Male C57BL/6J mice of 4, 12, 96 weeks of age were dissected, serum and each tissue sample were collected. Pretreatment procedures such as homogenization, deproteinization, delipidation, and hydrolysis were performed; thereafter, 2SC, CML, and N^{δ}-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1) were measured by LC-MS/MS.

Results: The levels of 2SC and CML were remarkably increased in mice brains aged 12-96 weeks, compared with those at 4 weeks.

Conclusion: The present study reveals that the amount of 2SC was the highest in mice brains than in other tissues, indicating that the brain requires high energy and may easily reflect mitochondrial dysfunction. Furthermore, the mitochondrial function is likely to be impaired by increased oxidative stress along with aging, thereby resulting in the formation of abundant 2SC and CML in mice brains.

Poster 64

Title: Dietary advanced glycation endproducts (AGEs) increase their concentration in plasma and tissues, result in inflammation and modulate gut microbial composition in mice; evidence for reversibility

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Background and aims: Dietary advanced glycation endproducts (AGEs) are associated with negative biological effects, possibly due to accumulation in plasma and tissues and through modulation of inflammation and gut microbiota. Whether these biological consequences are reversible by limiting dietary AGE intake is unknown.

Materials and methods: Young healthy C57BL/6 mice were fed a standard chow (n=10) or a baked chow high AGE-diet (n=10) (~1.8-6.9 fold increased protein-bound N_ε-carboxymethyl)lysine (CML), N_ε-(1-carboxyethyl)lysine (CEL), and N_δ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1)) for 10 weeks or a switch diet with baked chow for 5 weeks followed by 5 weeks of standard chow (n=10). We assessed accumulation of AGEs in plasma, kidney, and liver and measured inflammatory markers and gut microbial composition.

Results: After 10 weeks of baked chow, a substantial panel of AGEs were increased in plasma, liver, and kidney. These increases were normalized after the switch diet. The inflammatory z-score increased after the baked chow diet. Gut microbial composition differed significantly between groups, with enriched *Dubosiella spp*. dominating these alterations.

Conclusion: A high AGE-diet led to an increase of AGEs in plasma, kidney, and liver and to more inflammation and modification of the gut microbiota. These effects were reversed or discontinued by a diet lower in AGEs.

Keywords: dietary advanced glycation endproducts, gut microbiota, 16S rRNA sequencing, ultraperformance liquid chromatography tandem mass spectrometry.

LB Posters 1

Title: Age-dependent accumulation of dicarbonyls and advanced glycation endproducts (AGEs) associates with mitochondrial stress

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Background and aims: Aging is a strong risk factor for brain dementia and cognitive decline. Mitochondria are a hub for metabolic activities and a key player for both brain aging and neurodegeneration. Age-related accumulation of metabolites such as advanced glycation end products (AGEs) could serve as danger signals to initiate and accelerate disease process and neurodegeneration. The causes and consequences of cerebral AGEs accumulation remain largely unknown. Here, we comprehensively investigate age-related cerebral accumulation of AGEs and reactive AGEs precursors dicarbonyls, including methylglyoxal (MG), glyoxal (GO), and 3-deoxyglucosone (3-DG), and their effects of mitochondrial respiratory function and reactive oxygen species (ROS) in the aging human and mouse brains. Our study addresses whether increased levels of AGEs and dicarbonyls correlate with bioenergy failure in aging human and mouse brain and whether scavenging mitochondrial reactive oxygen species (ROS) facilitates clearance of AGEs and restores mitochondrial function and ATP levels.

Materials and methods: Normal aging human brains and C57B/6 mouse brains at 3-30 months of age were used in this study. Levels of AGE-adducts (total AGEs, MG-H1, CML, and CEL) and dicarbonyls (MG, GO, and 3-DG) were determined by quantification of immunodot blot, ELISA, and RP-HPLC, respectively. To determine the effect of mitochondrial ROS on AGEs accumulation, 21-month-old mice were given by Mito-TEMPO (0.25 mg/kg/day) via intraperitoneal injection for 3 months, or brain slices were pre-treated with Mito-TEMPO (2 µM) followed by treatment with AGEs (100 µg/ml) or MG (1 µM) for 1 h during perfusion. Mitochondrial function and oxidative stress were measured by enzyme activities associated with mitochondrial respiratory chain complex I & IV, ATP levels, and the amount of hydrogen peroxide.

Results: We demonstrated that AGEs, including arginine and lysine derived N(6)-carboxymethyl-lysine (CML), Nε-(1-Carboxyethyl)-L-lysine (CEL), and methylglyoxal-derived hydroimidazolone-1 (MG-H1), were significantly elevated in the brains of both human and mice with advanced age compared to young cohorts. In parallel, levels of dicarbonyls, including MG, GO and 3-DG, were also elevated in the brains of aged mice and elderly humans. Accordingly, aging mouse and human brains revealed decrease in mitochondrial respiratory chain complex I & IV activities and ATP levels, and increased ROS. Notably, scavenging mitochondrial ROS by administrating Mito-TEMPO robustly reduced aged-induced accumulation of AGEs and dicarbonyls, improved mitochondrial respiratory function, and restored ATP levels.

Conclusion: Our findings provide evidence that supports the link between age-related accumulation of toxic metabolites (AGEs) and mitochondrial perturbation and oxidative stress. This highlights a novel mechanism by which AGEs-dependent signalling promotes carbonyl stress and sustains mitochondrial dysfunction, which initiates, promotes, and exacerbates age-related dementia, neurodegeneration, and cognitive decline. Thus, clearance and detoxification of AGEs and related metabolites may represent a new therapeutic avenue for combating cognitive decline and mitochondrial degeneration relevant to aging and neurodegenerative diseases including Alzheimer's disease.

LB Posters 2

Title: Evaluation of antiviral activity of Manuka honey against SARS-CoV-2.

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Background and aims: In 2020 a global pandemic was declared caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2). The pandemic is still ongoing and continues to cause considerable mortality and morbidity world-wide and new variants of the virus are emerging. Rapid development and rollout of vaccines for SARS-CoV-2 is in progress to counter the pandemic but has been tempered by the emergence of new SARS-CoV-2 variants, many of which (e.g. beta, gamma and delta variants) exhibit reduced vaccine effectiveness. Therefore, an effective prevention and treatment is in an urgent need. Up to date there is no approved antiviral treatment for coronavirus disease 2019 (COVID-19). Several studies have shown that honey has virucidal/antiviral effect on multiple enveloped viruses such as HIV, influenza virus, herpes simplex, and varicella-zoster virus. Methylglyoxal (MG), a bioactive component in Manuka honey, has antiviral activity *in vitro*. Our previous *in silico* analysis suggested vulnerability of SARS-CoV-2 to inactivation by MG. MG may modify arginine residues in the functional domains of viral spike and nucleocapsid proteins, resulting in loss of charge, protein misfolding and inactivation. The aim of this study was to characterize the antiviral activity of Manuka honey against SARS-CoV-2 *in vitro*

Materials and methods: Wild-type SARS-CoV-2 with titers of multiplicities of infection (MOI) 0.1 and 0.05 were incubated with 2-fold serial dilutions of 250+ Manuka honey (equivalent to 250 to 31 μM) in infection medium (Dulbecco's Modified Eagle Medium + 2% fetal bovine serum + 100 units/ml penicillin + 100 μg/ml streptomycin) for 6 h. Manuka honey treated and untreated control SARS-CoV-2 was incubated with confluent cultures of Vero cells *in vitro* for 1 h, cultures washed with phosphate-buffered saline and incubated in fresh infection medium at 37°C for 4 - 5 days until 70% of virus control cells displayed cytopathic effect. We also studied the effect of scavenging MG in Manuka Honey with aminoguanidine (AG; 500 μM) on virucidal activity. The antiviral activity of MG was judged by median tissue culture infectious dose ($TCID_{50}$) assays.

Results: Manuka honey inhibited viral replication at the 250 μM MG equivalent, corresponding to *ca.* 20-fold dilution of commercial 250+ grade honey. Two-fold greater dilution, 125 μM MG equivalent, corresponding to *ca.* 40-fold dilution of Manuka honey, was ineffective. Prior scavenging of MG by addition of AG resulted in virus replication levels equivalent to those seen in the virus control without AG.

Conclusion: Manuka honey has virucidal activity against SARS-CoV-2 when incubated with the virus in cell-free media at dilutions of no greater than *ca.* 20-fold 250+ grade. Anti-viral activity was inhibited by AG, consistent with the anti-viral effect being mediated by MG. Manuka honey was less effective than expected, given the stated MG content and compared to antiviral activity of authentic MG against SARS-CoV-2 *in vitro*. This may indicate that either commercial calibration of MG content requires reappraisal or MG in Manuka honey is complexed with other substances and decreased bioavailability.

Keywords: SARS-CoV-2; COVID-19; methylglyoxal; antiviral; Manuka honey.

LB Posters 3

Title: Ameliorative Potential of cereal grasses in streptozotocin-nicotinamide induced diabetic rats

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Background and aims: Cereal grasses are used as source of food since times immemorial. Besides impacting high nutritive value they contain an array of bioactive compounds. The bioactive compounds scavenge free radicals thereby attributing to high antioxidant activity. Keeping this in view, the study was planned to evaluate the antioxidative potential of cereal grasses in diet induced obese rats and NA-STZ induced diabetic rats.

Materials and methods: The study was carried out in three phases.

Phase I comprised of in vitro trials encompassing physicochemical analysis involving proximate analysis, phytochemical screening, antioxidant and antimicrobial assay of cereal grasses.

The second phase involved in vivo trials using wistar strain as experimental models. The animals were divided into two groups viz. diet induced obese rats and diet and NA-STZ induced diabetic rats. The former comprised of obesic trials induced by building up lipids and oxidative stress levels by feeding cafeteria diet to animals. The latter dealt with diabetobesic trials induced by combitorial approach of cafeteria diet and diabetes induced by pharmacological means (nicotinamide-streptozotocin injection, intraperitoneally). The ameliorative effect of cereal grasses (singly and in blends) in modulating dyslipidemia and oxidative stress biomarkers was studied in experimental animals for a period of 9 weeks.

The third phase comprised of food product development incorporating cereal grasses followed by organoleptic evaluation.

Results: The cereal grasses showed high nutritional index with a wide array of bioactive compounds attributing to their therapeutic potential. Administration of cafeteria diets in obese and diabetic rats resulted in metabolic aberrations in lipidemic, glycaemic and oxidative stress biomarkers. The effect was counteracted by supplementation of cereal grasses (singly and in blends) and was more or less similar to the reference drugs treated animals. The histopathological evaluation conferred the antioxidative potential of cereal grasses ascribing to its restorative and hepatocellular protection in animals. One variant of wheat grass and blend of all three cereal grasses incorporated products viz., jeera biscuits, parantha and flavoured milk were more or less acceptable as control while all variants of food sprinkler were equally accepted as control

Conclusion: The study demonstrated anti-obesity and antidiabetic activity of cereal grass, hence they can be used as effective nutraceuticals and functional foods with therapeutic efficacy for amelioration and management of these diseases and their associated complications.

LB Posters 4

Title: Health and safety consideration from methylglyoxal exposure of Manuka honey,
Authors: Mohamed Ahmed Kamal Elahtam, Mohamed Saeed Ibrahim Mohamed and Naila Rabbani
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Background and aims: Methylglyoxal (MG) is a major component of Manuka honey and contributes to antiviral and antibacterial properties of the honey. MG is a reactive alpha-dicarbonyl compound that is produced endogenously during glycolysis inside cells and is metabolised by the glyoxalase system. Increased level of MG is damaging to the proteome and genome in physiological system and is said to be involved in the development of diabetic complications and disease. MG is also found in food and beverages during food and drink processing and naturally occurs in Manuka honey. This raised question if the ingested MG in food product is as damaging to the proteome and genome as produced endogenously. The aim of this study was to assess the health and safety consideration from MG exposure of Manuka honey.
Materials and methods: This is a literature review. We search the literature by using key words such as "methylglyoxal and Manuka honey" "Methylglyoxal toxicity in food" "Antibacterial properties of Manuka honey" and other. We included research articles published during 2009 -2021. We also used the report of The Committee on Toxicity of Chemicals in Food published by UK government in November 2009.
Results: MG is found in various foodstuffs either formed during food processing as shown in the table below. The level of MG found in food products is very low compare to normally produced endogenously. Typical concentration of MG in a healthy human plasma is between 100 – 130 nM and it increase 2 fold in obesity, 3-4 fold in diabetes and 5 fold in renal failure.

Table 1   Methylglyoxal contents of some food

Food or beverage	MG content
Sweetened cola (sucrose-sweetened)	0.1 μmol per 330 ml serving
Sweetened cola (high fructose corn syrup-sweetened)	1 μmol per 330 ml serving
Fruit juice	0.7 μmol per 330 ml serving
Bread and cookies	4 μmol per 100 g serving
Jams, jellies and sweeteners	5 μmol per 100 g serving
Commercial honey	0.3 μmol per 7 g serving (teaspoon)
Manuka honey	4–74 μmol per 7 g serving

Henle *et al.* found that the ingested MG has low bioavailability due is degradation in intestine
Conclusion: The dietary exposure to MG in food and beverages is usually <0.03 mmol of MG per day or <1 % of total MG exposure. An adult healthy human subject is estimated to produce ca. 3 mmol MG per day. To date, studies have indicated that there is limited metabolic transit of ingested MG into the body – likely due to metabolism of MG by intestinal bacteria. MG exposure to a 7 g serving of Manuka honey represents exposure of 0.004 – 0.074 mmol MG and so represents a minor increased total exposure to MG (endogenous and exogenous sources) of only 1 – 2% and is unlikely to be deleterious to health.
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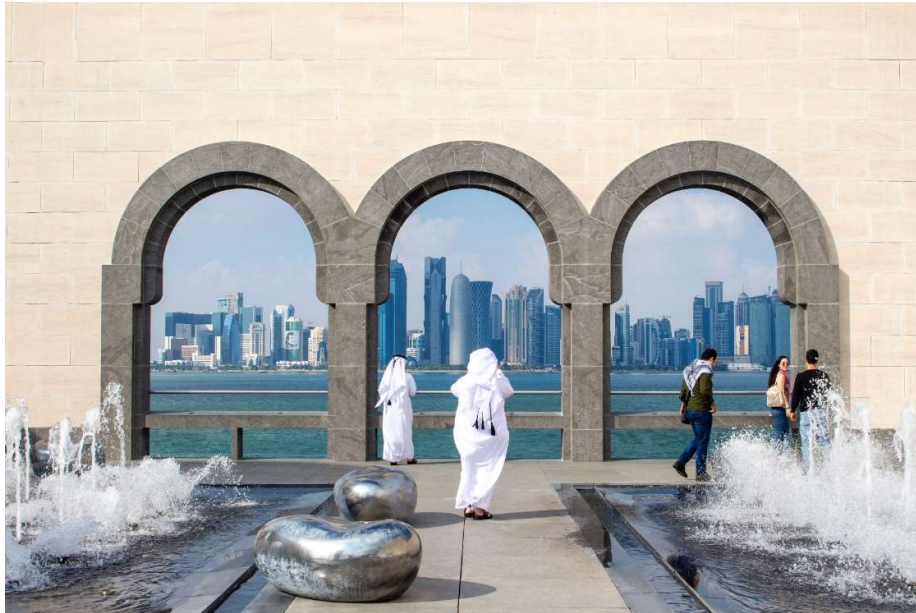## Protein Glycation in Food, Nutrition, Health and Disease

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