12th International Symposium on the Maillard Reaction 2015
Tokyo, JAPAN

Program & Abstract

Dates
September 1 [Tue] – 4 [Fri], 2015

Venue
ITO International Research Center,
The University of Tokyo,
Tokyo, Japan

Conference Chairperson
Teruo Miyazawa
New Industry Creation Hatchery Center,
Tohoku University,
Japan
# CONTENTS

Message from the Conference Chairperson .............................................. 1

Symposium History and Symposium Committee ..................................... 2

General Information

- Access .................................................................................................. 4
- Congress Information ........................................................................... 7
- Instruction for Chairpersons & Speakers ............................................ 10
- At a glance program .......................................................................... 14

Program ................................................................................................. 15

Abstract ............................................................................................... 33
Welcome to Tokyo

First of all, I would like to thank all of you for continuing contribution and support on International Maillard Reaction Society (IMARS). I am honored to serve as the President of IMARS and hold the 12th International Symposium on the Maillard Reaction (12th ISMR).

The 1st ISMR was held in 1979, in Uddevalla Sweden. Since then, we have had fulfilling symposiums in many different countries. This time, I would like to organize the next symposium in 2015, in Tokyo Japan. I believe that the next one will provide a great opportunity for an active discussion and contribute further study. In addition, you could explore chic city Tokyo and experience “the washoku”, traditional Japanese food, which is now UNESCO world intangible cultural heritage. I surely believe that it will benefit your career outputs and will also leave you with some wonderful memories. I look forward to meeting you in Tokyo.

September 1, 2015

Teruo Miyazawa
Conference Chairperson
## Symposium History

<table>
<thead>
<tr>
<th>No.</th>
<th>Place</th>
<th>Country</th>
<th>Year</th>
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<tbody>
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<td>1</td>
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<td>Sweden</td>
<td>1979</td>
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<td>1982</td>
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<td>3</td>
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<td>4</td>
<td>Lausanne</td>
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<td>1989</td>
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<td>5</td>
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<td>6</td>
<td>London</td>
<td>UK</td>
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<td>7</td>
<td>Kumamoto</td>
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<td>8</td>
<td>Charleston</td>
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<td></td>
<td>COST-IMARS Joint Workshop</td>
<td>Napoli</td>
<td>Italy</td>
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<tr>
<td>9</td>
<td>Münche</td>
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<td>11</td>
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<td>12</td>
<td>Tokyo</td>
<td>Japan</td>
<td>2015</td>
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## Symposium Committee

**Honorary Conference Chairperson:** Naoyuki Taniguchi, Riken  
**Conference Chairperson:** Teruo Miyazawa, Tohoku University

**Local Organizing Committee**  
Atsuhiko Ichimura (*Kyoto University*)  
Fumitaka Hayase (*Meiji University*)  
Masatsune Murata (*Ochanomizu University*)  
Kiyotaka Nakagawa (*Tohoku University*)  
Yasuhiro Yamamoto (*Kanazawa University*)  
Hiroshi Nishida (Niigata University of Pharmacy and Applied Life Science)

**International Organizing Committee**  
John Baynes (*University of South Carolina*)  
Inés Birlouez-Aragon (*Spectralys Innovation*)  
Mark Cooper (*Baker IDI Heart &. Diabetes Institute*)  
Thomas Henle (*Technical University of Dresden*)  
Thomas Hofmann (*Technical University of Munich*)  
Vincent Monnier (*Case Western Reserve University*)  
Monika Pischetsrieder (*University of Erlangen*)  
Helen Vlassara (*Icahn School of Medicine at Mount Sinai*)
<table>
<thead>
<tr>
<th>Local Financial Committee</th>
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<tr>
<td>Fumitaka Hayase <em>(Meiji University)</em>            Atsuhiko Ichimura <em>(Kyoto University)</em></td>
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<td>Reiko Inagi <em>(The University of Tokyo)</em>          Satoshi Miyata <em>(JCHO Osaka Hospital)</em></td>
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<td>Toshio Miyata <em>(Tohoku University)</em>               Tatefumi Mori <em>(Tohoku University)</em></td>
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<td>Masatsune Murata <em>(Ochanomizu University)</em>            Ryoji Nagai <em>(Tokai University)</em></td>
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<td>Tomoko Oya-Ito <em>(Nagoya University)</em>                 Hirohiro Watanabe <em>(Meiji University)</em></td>
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<td>Keiichiro Suzuki <em>(Hyogo College of Medicine)</em>         Hiroshi Yamamoto <em>(Kanazawa University)</em></td>
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<td>Teruyuki Usui <em>(Kagawa Nutrition University)</em>           Yasuhiko Yamamoto <em>(Kanazawa University)</em></td>
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<td>Tadao Kurata <em>(Niigata University of Pharmacy and Applied Life Sciences)</em></td>
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<td>Hiroshi Nishida <em>(Niigata University of Pharmacy and Applied Life Science)</em></td>
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<td>Vincent Monnier <em>(Case Western Reserve University)</em></td>
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<td>Paul Thornalley <em>(University of Warwick)</em></td>
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<th>Scientific Advisory Committee</th>
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<td>Norma Frizzell <em>(University of South Carolina)</em>                        Yukio Fujiwara <em>(Kumamoto University)</em></td>
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<td>Reiko Inagi <em>(The University of Tokyo)</em>                                      Tomoko Oya-Ito <em>(Nagoya University)</em></td>
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<td>Satoshi Miyata <em>(JCHO Osaka Hospital)</em>                                       Ryoji Nagai <em>(Tokai University)</em></td>
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<td>Ram Nagaraj <em>(University of Colorado)</em>                                        Yasuhiko Yamamoto <em>(Kanazawa University)</em></td>
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<td>Vincent Monnier <em>(Case Western Reserve University)</em></td>
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<tr>
<td>Hiroshi Nishida <em>(Niigata University of Pharmacy and Applied Life Science)</em></td>
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General Information

Access

- Root map (ITO International Research Center, The University of Tokyo)

Clear Tokyo Metro Map is available attached QR code
Access by Subway

- Hongo-sanchome (Marunouchi Line) / 8 minutes’ walk
- Hongo-sanchome (Oedo Line) / 6 minutes’ walk
- Kasuga (Mita Line) / 10 minutes’ walk

Access by Train and Subway

- Ochanomizu Station (JR Chuo-Line, JR Sobu-Line)
  - Transfer to Subway Marunouchi-Line (for Ikebukuro) -> get off at Hongo-sanchome Station.

Access by Train and Bus

Ueno (JR Yamanote-Line, other lines)
  - Transfer to Toei bus [学01]-Line (for the University of Tokyo)
    -> get off at Tatsuokamon, Todai-Byoin-Mae or Todai-Konai stops.

Okachimachi (JR Yamanote-Line, other lines)
  - Transfer to Toei bus [都02]-Line (for Otsuka Station)
    -> get off at Hongo-sanchome Eki-mae stop or Yushima-yonchome stop.
  - Transfer to Toei bus [上69]-Line (for Otakibashi-shako-mae)
    -> get off at Hongo-sanchome Eki-mae stop or Yushima-yonchome stop.

Ochanomizu Station (JR Chuo-Line, JR Sobu-Line)
  - Transfer to Toei bus [茶51]-Line (for Komagome Station South Exit)
    -> get off at Todai-Akamon-Mae, Todai-Seimon-Mae or Todai-Nogakubu-Mae stops.
  - Transfer to Toei bus [東43]-Line (for Arakawa-dote-soshajo)
    -> get off at Todai-Akamon-Mae, Todai-Seimon-Mae or Todai-Nogakubu-Mae stops.
  - Transfer to Toei bus [学07]-Line (the University of Tokyo)
    -> get off at Tatsuokamon, Todai-Byoin-Mae or Todai-Konai stops.
**Access by Train and Subway**

- Takebashi Station (Tozai Line) / Directly connected from Exit 3B
- Tokyo Station (JR Line) / 20 minutes' walk
- Otemachi Station (Chitoda Line) / 5 minutes' walk
- Jinbocho Station (Hanzomon Line and Shinjuku Line) / 5 minutes' walk
Congress Information

Welcome Reception
Date and Time: September 1 (Tue)  19:00-20:30
Place: Event Space, ITO International Research Center (B2F)

Banquet
Date and Time: September 3 (Thu)  19:00-21:00
Place: KKR Hotel (See Access Map on page 6)

JMARS Meeting
Date and Time: September 1 (Tue)  15:00-16:30
Place: Gallery 1, ITO International Research Center (B1F)

IMARS Meeting
Date and Time: September 2 (Wed)  19:00-20:30
Place: Session hall, ITO International Research Center (B2F)

Registration
Place: In front of Main Hall, ITO International Research Center (B2F)
Opening Hour: September 1 (Tue):  14:00 - 19:00
September 2 (Wed):  8:30 - 18:00
September 3 (Thu):  8:30 - 13:00
September 4 (Fri):  8:30 - 15:00

Registration fee

<table>
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<tr>
<th></th>
<th>IMARS Member</th>
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Acknowledgements

This symposium is supported in part by Tokai University Educational System General Research Organization. The organizers sincerely appreciate the generous support and participation for 12th International Symposium on the Maillard Reaction 2015 by the following foundations and groups.

Foundation
The Naito Foundation
Daiichi Sankyo Foundation of Life Science
The Tokyo Biochemical Research Foundation

Mizutani Foundation
Yakult Bio-Science Foundation

Donation
Mishima Kaiun Memorial Foundation
TOHMEI SCIENCE Co., Ltd.
NISSHIN SEIFUN GROUP INC.
Astellas Pharma Inc.
MARUDAI FOOD CO., LTD.
NIPPON SHINYAKU CO., LTD.
ASAI Germanium Research Institute Co., Ltd.
Amway Japan G.K.

Kewpie Corporation
Nissei Marine Industrial Co., Ltd
ASAHI KASEI PHARMA CORPORATION
Eisai Food & Chemical Co., Ltd.
T.HASEGAWA CO., LTD.
ARK Resource Co., Ltd.
Kao Corporation
Fuji Chemical Industries Co., Ltd.
KOBUAYASHI Pharmaceutical Co., Ltd.

Package Sponsor
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Nippi, Incorporated
Kikkoman Corporation

Mishima Kaiun Memorial Foundation
HORUS Co., Ltd.

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Morinda, Inc.

Sharp Corporation

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COSMO BIO CO., LTD.
KAC Co., Ltd.
Nippi, Incorporated
SHISEIDO CO., LTD.
Japan Institute for the Control of Aging (JaICA), Nikken SEIL Co., Ltd.

Greiner bio-one Co., Ltd.
Morinda, Inc.
Selista Inc.
Sharp Corporation

-8-
Supporting Societies

Japan Society of Nutrition and Food Science
The Japanese Biochemical Society
The Japanese Society for Food Science and Technology
The Japanese Society of Carbohydrate Research
The Japan Society for Bioscience, Biotechnology, and Agrochemistry
The Vitamin Society of Japan
The Japanese Conference on the Biochemistry of Lipids
Japanese Society of Nephrology
The Japanese Society for Dialysis Therapy
Japanese Society of Anti-Aging Medicine
Society for Free Radical Research Japan
Japan Atherosclerosis Society
Japan Consortium for Glycobiology and Glycotechnology
The Japan Diabetes Society
Instruction for Chairpersons & Speakers

Instruction for Chairperson

1) All chairpersons are asked to be in the lecture room no later 15 minutes prior to the beginning of the session.
2) Chairpersons should make efforts to maintain the time schedule in cooperation with time keeper, and give warning speakers if needed.
3) Careful time keeping is vital to ensure the smooth operation of the entire program.

Instruction for Speakers

Presentation Time

The time allocated for each presentation is as follows:

- **Keynote**: 45 minutes
- **Plenary**: 30 minutes (25 minutes presentation, 5 minutes for Q & A)
- **YIA session**: 15 minutes (12 minutes presentation, 3 minutes for Q & A)
- **Oral session**: 15 minutes (12 minutes presentation, 3 minutes for Q & A)

In order to ensure the smooth operation of the sessions, we ask you to keep to the time allocation as stated above.

Visual Aids

PowerPoint will be the only method of presentation available for oral presentation in this symposium. Please provide slides with sufficient font size and contrast to ensure your text can be seen clearly from the back of the large session room.

<For making presentation slides>

1. The following projection medium is available for presentation.
   - OS: Windows 7
   - Application: PowerPoint 2010, 2013, and PDF Adobe Reader XI (version 11.0.2)
If you would like to use Mac PC, please bring your own PC and the adapter for VGA cable (Mini D-sub 15 pin).

We will prepare a computer, a monitor, a microphone and a laser pointer on the podium in session rooms. If you need to use movies, please bring use your own PC.

2. PowerPoint presentation files should be prepared in English.
3. Please ensure that every page is clear and does not contain too much information.
4. Pictures should be saved as reasonable sizes. You should not use too large data to avoid troubles.
5. Please be sure to bring the presentation data in USB flash drive, and also prepare back-up data to avoid unexpected troubles. We recommend you to save the back-up data as a PDF file.
6. You cannot use any sound nor internet connection in presentation.

Submission of Presentation Slide Data

We ask you to meet our audio-visual staffs at the PC desk by in the foyer to check, rehearse and upload slide data prior to your presentation. Please come to the desk at least 1 hour before your presentation. You can’t use any sound nor internet connection in presentation. If you have movies embedded in your presentation, please bring your own PC and AC adapter to the PC desk.

On Presentation

You can control the slides with a mouse and keyboard provided on the podium.

Presentation Slides After The Symposium

Your presentation slides will be deleted after the symposium, and will not be provided to any attendees nor opened to public.
**Poster Presentation**

**Place:** Event Space, ITO International Research Center (B2F)

Abstracts scheduled for presentation in poster session will appear in the Symposium Program. Please refer to the table below for setup, tear down and presentation times for posters:

<table>
<thead>
<tr>
<th>Set-Up</th>
<th>Sep. 2nd, 9:00-13:00</th>
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</thead>
<tbody>
<tr>
<td><strong>Poster Session</strong></td>
<td>Sep. 4th, 12:00-14:00</td>
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<td></td>
<td>Presenters given an odd number: 12:00-13:00</td>
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<td>Presenters given an even number: 13:00-14:00</td>
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<tr>
<td><strong>Tear Down</strong></td>
<td>Sep. 4th, 16:00-17:30</td>
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</tbody>
</table>

**Poster Boards**

1. The poster board surface area is 210 cm high by 90 cm wide.
2. Each poster should indicate the title of presentation, name of authors and affiliations.
3. Text should be printed in a font large enough to be read comfortably from a distance of 4-5 feet (160 cm).
- Posters should be displayed on the boards using pushpins that will be available at the Poster Session room. No other adhesive method is permitted on the boards.
- Contain the title and name(s) of author(s) and affiliation(s) to identify your presentation easily.
- Prepare and bring all illustrations needed for your presentation to the meeting, such as figures, tables, schemas, equations, etc.
- Do not write or draw on the poster boards.
# At a Glance Program

<table>
<thead>
<tr>
<th>Date</th>
<th>Room</th>
<th>AM</th>
<th>Lunch</th>
<th>PM</th>
<th>Evening</th>
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<tr>
<td>Sep. 1 (Tue)</td>
<td>Hall</td>
<td>9:00-10:00 Plenary Lectures</td>
<td>12:00-13:00 Lunch/Seminar</td>
<td>14:00-16:45 Registration Start</td>
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<td>10:00-12:00 YIA Presentations</td>
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<td>17:00-18:30 Keynote Lectures</td>
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<td>18:30-19:00 President's Lecture</td>
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<td>19:00-20:30 Welcome Reception</td>
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<td>Sep. 2 (Wed)</td>
<td>Hall</td>
<td>9:00-10:00 Plenary Lectures</td>
<td>12:00-13:00 Lunch/Seminar</td>
<td>13:00-15:00 YIA Presentations</td>
<td>IMARS Meeting</td>
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<td></td>
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<td>10:00-11:45 Oral Presentations</td>
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<td>15:00-17:00 Plenary Lectures</td>
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<td>Foyer</td>
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<td>9:00-10:00 Plenary Lectures</td>
<td>12:00-13:00 Lunch/Seminar</td>
<td>13:00- Excursion</td>
<td>19:00- Banquet</td>
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<td>10:00-11:45 Oral Presentations</td>
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<td>14:00-15:00 Plenary Lectures</td>
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<td>17:00-                  Closing Remarks</td>
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-14-
Program

September 1 (Tuesday)

14:00- Registration

16:45- Opening Remark
    Teruo Miyazawa (Conference Chairperson)

Keynote Lecture
    Chairperson: Reiko Inagi (The University of Tokyo, Japan)

17:00-17:45  Protein Quailty Control by the Unfolded Protein Response
    Kazutoshi Mori (Kyoto University, Japan)

    Chairperson: Vincenzo Fogliano (Wageningen UR, The Netherlands)

17:45-18:30  Multi-omics Approach for the Elucidation of Food Functionality
    Hisanori Kato (The University of Tokyo, Japan)

President’s Lecture
    Chairperson: Naoyuki Taniguchi (Riken, Japan)

18:30-19:00  Why is the discrepancy between healthy “WASHOKU” and mystic “AGE/RAGE axis” ?
    Teruo Miyazawa (Tohoku University, Japan)

19:00-20:30  Welcome Reception
September 2 (Wednesday)

Plenary Lectures

Chairperson: Masanobu Kawakami (Nerima Hikarigaoka Hospital, Japan)

PL-1
9:00-9:30  Glyco-redox: new Sake in an old bottle
Naoyuki Taniguchi (Riken, Japan)

Chairperson: Varoujan Yaylayan (McGill University, Canada)

PL-2
9:30-10:00  Physiological relevance of dietary melanoidins
Vincenzo Fogliano (Wageningen UR, The Netherlands)

YIA Presentations-1 (Medical)

Chairpersons: Josephine Forbes (University of Queensland, Australia)
Motoko Takahashi (Sapporo Medical University, Japan)

YIA-M1
10:00-10:15  Immunochemical detection of MG-H1 in human serum by specific monoclonal antibody and its correlation with LC-MS/MS analysis
Kota Hatano (Tokai University, Japan)

YIA-M2
10:15-10:30  Post-glucose load plasma α-dicarbonyl concentrations are increased in individuals with impaired glucose metabolism and type 2 diabetes: the codam study
Dionne Maessen (Maastricht University, The Netherlands)

YIA-M3
10:30-10:45  Plasma levels of Ne-(carboxymethyl)lysine are inversely associated with central obesity and inflammation, and significantly explain a part of the central obesity-related increase in inflammation: the hoorn and codam studies
Katrien Gaens (Maastricht University, The Netherlands)

YIA-M4
10:45-11:00  RAGE mediates the vascular aging induced by dietary carboxymethyllysine
Nicolas Grossin (Lille University, France)

YIA Presentations-1 (Food)

Chairpersons: Kiyotaka Nakagawa (Tohoku University, Japan)
Varoujan Yaylayan (McGill University, Canada)

YIA-F1
11:00-11:15  Effect of prenatal dietery load of maillard reaction products on metabolic status of female wistar offspring
Radana Gurecká (Comenius University, Slovakia)

YIA-F2
11:15-11:30  New insights into bread melanoidins
Cynthia Helou (LaSalle Beauvais, France)
Creatine is a scavenger for methylglyoxal in vivo and in food

Jürgen Löbner (Technische Universität Dresden, Germany)

The role of amino acid metal complexes in the maillard and akabori reactions

Ossanna Nashalian (McGill University, Canada)

Luncheon Seminar (Morinda, Inc.)

Antiglycation activity of iridoids and their food sources

Brett West (Morinda, USA)

Protein damage markers in diagnosis, progression and treatment of arthritis

Usman Ahmed (Queen Elizabeth Hospital, UK)

Alagebrium chloride (ALT-711) short-term therapy for the treatment of experimental type I diabetes

Danielle J Borg (University of Queensland, Australia)

RAGE-DNA aptamer improves aldosteron-induced renal injury possibly via inhibition of Rac1-MR axis in mice with hypertensive nephropathy

Kensei Taguchi (Kurume University, Japan)

AGER1 overexpression in glomerular podocytes results in renal disease which is exacerbated by diabetes

Aowen Zhuang (University of Queensland, Australia)

Multiresponse kinetic modelling of maillard reaction and caramelisation in hazelnuts during roasting

Neslihan Göncüoğlu Taş (Hacettepe University, Turkey)

Amadori products formation in emulsified systems

Antonio Dario Troise (Wageningen University, The Netherlands)
A kinetic approach to evaluate inhibition of protein glycation during heating

H. Gül Akıllıoglu (Hacettepe University, Turkey)

The maillard reaction: A source for the formation of desired aroma-active compounds and undesired foodborne toxicants

Michael Granvogl (Technical University of Munich, Germany)

Plenary Lectures

Chairperson: Toshio Miyata (Tohoku University, Japan)

PL-3
15:00-15:30 Physiological glycation by methylglyoxal, glyoxalase 1 and developments in disease mechanisms and clinical therapeutics
Paul Thornalley (University of Warwick, UK)

Chairperson: Vincenzo Fogliano (Wageningen UR, The Netherlands)

PL-4
15:30-16:00 Proteomic methods to study the maillard reaction in food
Monika Pischetsrieder (University of Erlangen-Nürnberg, Germany)

Chairperson: Satoshi Miyata (JCHO Osaka Hospital, Japan)

PL-5
16:00-16:30 Skin AGEs, fluorescence and the progression of macrovascular complications in type 1 diabetes: The plot thickens
Vincent Monnier (Case Western Reserve University, USA)

Chairperson: Ryoji Nagai (Tokai University, Japan)

PL-6
16:30-17:00 The role of sugar conjugated binary complexes of amino acids with Cu (II) in controlling the maillard reaction
Varoujan Yaylayan (McGill University, Canada)

Oral Presentations ~ Glycation and inflammation ~
Chairpersons: Eric Boulanger (Lille University, France)
Takefumi Mori (Tohoku University, Japan)

OP-1
17:00-17:15 Role of RAGE during intestinal inflammation and its therapeutic potential in IBD
Nicolas Grossin (Lille University, France)

OP-2
17:15-17:30 Acrylamide, a dietary glycated product implicated in intestinal inflammation
Eric Boulanger (Lille University, France)

OP-3
17:30-17:45 Possible role of glycation in skin aging, inflammaging and photoaging as evidenced by a reconstructed skin model
Pageon Hervé (L’Oreal, France)
High dietary intake of advanced glycation end products during pregnancy and lactation produces insulin secretory defects in offspring of nonobese diabetic 8.3 TCR transgenic mice

Danielle Borg (University of Queensland, Australia)

**Oral Presentations ~ Chemistry ~**

**Chairpersons: Siyun Wang** (University of British, Canada)
**Tatiana Bilova** (University of Leipzig, Germany)

**OP-5**
18:00-18:15 Amide advanced glycation endproducts – Mechanisms and Relevance
**Marcus Glomb** (Martin-Luther-University, Germany)

**OP-6**
18:15-18:30 A proposal of a browning mechanism via 4-hydroxy-5-methyl-3(2H)-furanone (HMFO) in xylose-lysine maillard reaction system
**Masatsune Murata** (Ochanomizu University, Japan)

**OP-7**
18:30-18:45 Model optimization and antioxidant activity of blue maillard reaction products
**Hao Jing** (China Agricultural University, China)

**OP-8**
18:45-19:00 The potential of carbohydrates and corresponding early glycated peptides for the formation of advanced glycation end-products
**Andrej Frolov** (Universität Leipzig, Germany)
September 3 (Thursday)

Plenary Lectures

Chairperson: Kei Fukami (Kurume University, Japan)

**PL-7**
9:00-9:30  The critical role of reactive dicarbonyls in diabetic complications  
Mark Cooper (Baker IDI Heart & Diabetes Institute, Australia)

Chairperson: Frederik Tessier (LaSalle Beauvais, France)

**PL-8**
9:30-10:00  Acrylamide: Formation in Foods and Reactions during Digestion  
Vural Gökmen (Hacettepe University, Turkey)

Oral Presentations ~ AGES and fibrosis ~

Chairpersons: Mark Cooper (Baker IDI Heart & Diabetes Institute, Australia)  
Reiko Inagi (The University of Tokyo, Japan)

**OP-9**
10:00-10:15  RAGE: A new frontier in intestinal fibrosis  
Eric Boulanger (Lille University, France)

**OP-10**
10:15-10:30  Overexpression of the AGE detoxification receptor AGER1 results in hepatic fibrosis and hepatic insulin resistance  
Aowen Zhuang (University of Queensland, Australia)

**OP-11**
10:30-10:45  Hypohalous acids contribute to extracellular matrix damage  
Paul Voziyan (Vanderbilt University Medical Center, USA)

**OP-12**
10:45-11:00  A novel in vivo approach to study endoplasmic reticulum stress induction by advanced glycation end products  
Christina Piperi (University of Athens, Greece)

Oral Presentations ~ Dietary AGEs and analysis ~

Chairpersons: Masatsune Murata (Ochanomizu University, Japan)  
Hao Jing (China Agricultural University, China)

**OP-13**
11:00-11:15  Dietary and lifestyle predictors of skin autofluorescence  
Mark Cooper (Baker IDI Heart & Diabetes Institute, Australia)

**OP-14**
11:15-11:30  Bread mrps and gut microbiota: focus on enterobacteriaceae  
Pascale Gadonna-Widehem (LaSalle Beauvais, France)

**OP-15**
11:30-11:45  Computer vision prediction of furan content in fried starchy model systems  
Franco Pedreschi (Pontificia Universidad Católica de Chile, Chile)

-20-
12:00-13:00  Luncheon Seminar  (Sharp Corporation)

Non-invasive measurement of skin AGEs to evaluate diabetic complications
Ryoji Nagai (Tokai University, Japan)

13:00-   Excursion

19:00-   Banquet at KKR Hotel
September 4 (Friday)

Oral Presentations ~ Disease biomarker ~

**Chairpersons:** Naila Rabbani (University of Warwick, UK)
Yukio Fujiwara (Kumamoto University, Japan)

**OP-16**
9:00-9:15  Excess consumption of dietary advanced glycation end products induce changes in gut microbiota which is associated with inflammation
Melinda Coughlan (Baker IDI Heart & Diabetes Institute, Australia)

**OP-17**
9:15-9:30  MS based quantification of individual glycation sites in plasma proteins as potential type 2 diabetes biomarkers
Sandro Spiller (Universität Leipzig, Germany)

Oral Presentations ~ Kinetetics ~

**Chairpersons:** Marcus Glomb (Martin-Luther-University, Germany)
Andrej Frolov (Universität Leipzig, Germany)

**OP-18**
9:30-9:45  Kinetic of furan formation in fried starchy food model systems: influence of oil adsorption and non-enzymatic browning
María Mariotti (Pontificia Universidad Católica d Chile, Chile)

**OP-19**
9:45-10:00  Multiresponse kinetic modeling of 1,2-dicarbonyl compound formation in wheat flour-glucose model dough system under dry heating conditions
Tolgahan Kocadağ (Hacettepe University, Turkey)

Oral Presentations ~ Clinical evidence and therapeutic approach ~

**Chairpersons:** Paul Thornalley (University of Warwick, USA)
Ram Nagaraj (University of Colorado, USA)

**OP-20**
10:00-10:15  Alpha dicarbonyl derived advanced glycation end products correlate with progression of diabetic nephropathy in type 2 diabetes
Kevin Wheelock (NIDDK, USA)

**OP-21**
10:15-10:30  Accumulation of advanced glycation endproducts is associated with macrovascular complications and mortality, and glycaemic control with microvascular complications in type 2 diabetes mellitus: the AURORA follow-up study
Kadir Yozgatlı (University of Groningen, The Netherlands)

**OP-22**
10:30-10:45  Methylglyoxal metabolism and lipoprotein modification and link to risk of cardiovascular disease
Naila Rabbani (University of Warwick, UK)

**OP-23**
10:45-11:00  Development of screening technology for discovery of glyoxalase 1 inducers
Mingzhan Xue (University of Warwick, UK)
Oral Presentations  ~ Glycation ~

**Chairpersons:** Fumitaka Hayase (Meiji University, Japan)
David Kitts (University of British, Canada)

**OP-24**
11:00-11:15  Maillard glycation of proteins
**Hugo Cardoso** (Wageningen University, The Netherlands)

**OP-25**
11:15-11:30  Monitoring Maillard Reaction during storage of skim milk powders by measuring available lysine
**Kataneh Aalaei** (Lund University, Sweden)

**OP-26**
11:30-11:45  Quantitative correlation between maillard induced glycation and protein enzymatic hydrolysis
**Yuxi Deng** (Wageningen University, The Netherlands)

**OP-27**
11:45-12:00  Plant protein glycation: possible effects on physiology and food quality
**Tatiana Bilova** (University of Leipzig, Germany)

12:00-14:00  Poster presentation

Plenary Lectures

**Chairperson:** Yuichi Kaji (Tsukuba University, Japan)

**PL-9**
14:00-14:30  AGEs in basement membrane proteins promote tgb-mediated epithelial to mesenchymal transition of cells: implications for age and diabetes associated fibrosis
**Ram Nagaraj** (University of Colorado, USA)

**Chairperson:** Vural Gökmen (Hacettepe University, Turkey)

**PL-10**
14:30-15:00  Absorption, distribution in the organs and elimination of dietary carboxymethyllysine
**Frederik Tessier** (LaSalle Beauvais, France)

Oral Presentations  ~ Glycation and obesity ~

**Chairpersons:** Vincent Monnier (Case Western Reserve University, USA)
Yasuhioko Yamamoto (Kanazawa University, Japan)

**OP-28**
15:00-15:15  Dicarbonyl stress in adipose tissue of mice fed an obesogenic diet
**Jinit Masania** (University of Warwick, UK)

**OP-29**
15:15-15:30  Dicarbonyl stress in clinical obesity and effect of a low calorie diet
**Jinit Masania** (University of Warwick, UK)
Delayed intervention with pyridoxamine improves metabolic function and prevents adipose tissue inflammation and insulin resistance in high-fat diet-induced obese mice

**Mathias Van den Eynde** (Maastricht University, The Netherlands)

Anti-sRAGE autoimmunity in obesity: downturn after bariatric surgery is independent of previous diabetic status

**Eric Boulanger** (Lille University, France)

**Oral Presentations ~ Antioxidants / Health effects ~**

**Chairpersons:** Pascale Gadonna-Widehem (LaSalle Beauvais, France)  
Hiroshi Nishida (Niigata University of Pharmacy and Applied Life Science, Japan)

Characterization of anti-inflammatory activity of an advanced glucose-lysine maillard reaction product

**David Kitts** (University of British, Canada)

Effects of maillard reaction products on salmonella enterica under stress conditions

**Siyun Wang** (University of British, Canada)

The association between dietary intake of advanced glycation endproducts and plasma levels of advanced glycation endproducts: The CODAM study

**Jean Scheijen** (Maastricht University, The Netherlands)

Long term modification of microbiota and antioxidant defenses in colon by high formula derived ages in iugr individuals

**Abdennebi-Najar Latifa** (LaSalle Beauvais, Beauvais, France)

**17:00-21:30 YIA Ceremony**

**Motoko Takahashi** (Sapporo Medical University, Japan)

**Closing Remark**

**Naoyuki Taniguchi** (Honorary Conference Chairperson)
**Poster Session**

Presentation time:  Presenters given an **odd** number: **12:00-13:00**  
Presenters given an **even** number: **13:00-14:00**

**P1**  
Possibility of cell transplantation therapy for peritoneum injured by glucose degradation products  
**Yusuke Ohsaki** (Tohoku University, Japan)

**P2**  
Carbonyl stress and inappropriate renin angiotensin system activity associate with blood pressure elevation  
**Emiko Sato** (Tohoku University, Japan)

**P3**  
Anti-inflammatory effect of glycation products derived from high hydrostatic pressure enzymatic hydrolysate of flatfish byproduct and its mechanism  
**YoonSook Kim** (Korea Food Research Institute, Korea)

**P4**  
Formation of membrane-bound hemoglobin under influence of nitric oxide metabolites in presence of methylglyoxal  
**Alexey Topunov** (Russian Academy of Sciences, Russia)

**P5**  
Involvement of autophagy-regulated apoptosis in the advanced glycation end products-induced renal mesangial cell injury  
**Chih-Kang Chiang** (National Taiwan University, Taiwan)

**P6**  
Advanced glycation end products downregulated heat shock protein 60 in islet β-cell hypertrophy and dysfunction  
**Shing-Hwa Liu** (National Taiwan University, Taiwan)

**P7**  
Stability of advanced glycation and oxidation end products (OPs) in stored blood and urine samples from diabetes outcome studies  
**Paul Beisswenger** (PreventAGE Healthcare, USA)

**P8**  
Amadori-glycated phosphatidylethanolamine induces cellular telomerase activity  
**Takahiro Eitsuka** (Niigata University of Pharmacy and Applied Life Sciences, Japan)

**P9**  
Immune cell-specific RAGE deficiency exacerbates the inflammatory response in murine NASH  
**Mitchell Bijnen** (Maastricht University, The Netherlands)
Quantification of CMA in acid hydrolysate in tissues of STZ mice
Michi Sasaki (Nippi Research Institute of Biomatrix, Japan)

Immunohistochemical and instrumental detection of collagen-specific AGE
Masatoshi Shinagawa (Tokai University, Japan)

Instrumental detection of GA-pyridine, one of glycolaldehyde derived AGEs, in human atherosclerosis lesions
Ikuya Obata (Tokai University, Japan)

MG-H1, which is derived from methylglyoxal, is related to renal dysfunction in patients with chronic kidney disease independently of diabetes mellitus
Kenji Ito (Fukuoka University, Japan)

Plasma sRAGE, esRAGE but SVAP-1 decrease with number of cardiometabolic risk factors in apparently healthy adolescents
Katarína Šebeková (Comenius University, Slovakia)

The effect of 1,25-dihydroxyvitamin D on gene and protein expression of enzymes protecting from glucolipotoxicity in vitro
Katarina Kuricova (Masaryk University, Czech Republic)

The effect of glucose variability on the expression of genes involved in hyperglycaemia-induced tissue damage
Lukas Pacal (Masaryk University, Czech Republic)

Dicarbonyl stress impairs function of periodontal ligament fibroblasts in model hyperglycaemia
Amal Ashour (University of Warwick, UK)

GLO1 copy number increase in the lexicon glyoxalase 1 mutant mouse preserved the wild type phenotype
Alaa Shafie (University of Warwick, UK)

Enhanced uptake of a glycated food allergen by IL-4-treated human cultured macrophages
Abdul Zalikha (Paul-Ehrlich-Institut, Germany)

RAGE promoter methylation in vitro and in vivo
Anna Pleskacova (Masaryk University, Czech Republic)
Serum corboxymethly lysine, a dominant advanced glycation end product, is increased in women with gestational diabetes mellitus

**Vendula Bartáková** (Masaryk University, Czech Republic)

Structural studies on the sugar moiety of collagen fluorophore LW-1 implicates the presence of glucuronic acid

**David Sell** (Case Western Reserve University, USA)

The comparison of skin autofluorescent markers for future risk in the development and progression of subclinical cardiovascular disease in type 1 diabetes

**David Sell** (Case Western Reserve University, USA)

Specific detections of the fructated proteins in diabetic rat lens

**Emi Ito** (RIKEN, Japan)

Kynoxazine: a novel fluorescent compound in human lens derived from the maillard reaction of 3-hydroxykynurenine and erythrulose

**Stefan Rakete** (University of Colorado, USA)

Involvement of the Maillard reaction product of liver fibrosis by oxidative stress

**Haruhiko Sakiyama** (Hyogo College of Medicine, Japan)

Co-localization of AGEs and receptor for AGE in pterygium

**Yuichi Kaji** (University of Tsukuba, Japan)

Localization of advanced glycation end products in Exfoliation Syndrome

**Rie Sato** (University of Tsukuba, Japan)

Molecular basis for lens crystallin denaturation and precipitation by vitamin C oxidation products in AGE-related cataracts

**Vincent M Monnier** (Case Western Reserve University, USA)

Effect of pentosidine in homeostasis imbalance during the global skin aging process

**Pageon Hervé** (L’Oreal, France)
Effect of a topical dicarbonyl scavenging gel on skin autofluorescence and elasticity, markers of advanced glycation end product load in human skin  
Brett West (Morinda, USA)

Measurements of fluorophore in skin for non-invasive evaluation of AGEs accumulation  
Rei-ichi Ohno (Tokai University, Japan)

Mangosteen pericarp extract inhibits the formation of AGEs and improves skin conditions  
Kazuhiro Maejima (NIPPON SHINYAKU CO., LTD Japan)

Skin autofluorescence as a surrogate biomarker to predict retinal re-detachment after vitrectomy  
Bernadette Fokkens (University Medical Center Groningen, The Netherlands)

Skin AGE are associated with arterial calcification but not to arterial inflammation in recently diagnosed type 2 diabetes patients  
Andries Smit (University Medical Center Groningen, The Netherlands)

Insufficient leptin action induces RAGE expression and triggers pancreatic beta-cell failure in type 2 diabetes  
Dong Han (Kanazawa University, Japan)

Neuroprotective effects of endogenous secretory RAGE in ischemic cerebrovascular diseases  
Yu Shimizu (Kanazawa University, Japan)

RAGE of donor islets is a novel target to improve the efficiency of islet transplantation  
Yohichi Yasunami (Fukuoka University, Japan)

Renal effects of dietary carboxymethyllysine and implication of RAGE, Receptor for Advanced Glycation End-products  
Éric Boulanger (Lille2 University, France)

RAGE-KO murine model is protected against endothelial dysfunction induced by antiphospholipid antibodies  
Éric Boulanger (Lille2 University, France)
Effects of *osteomeles schwerinae* extracts on TGF-β1 expression and on binding to RAGE of mesangial cells under diabetic conditions

Young Sook Kim (KIOM, Korea)

Lysine-galactose maillard reaction products (MRPs)-induced anti-inflammatory effect in a co-culture system

Kwang-Won Lee (Korea University, Korea)

Formula milk derived CML induces rage activation, long term inflammation and oxidative stress in IUGR piglets

Elmhiri Ghada (LaSalle Beauvais, France)

RAGE mediates myocardial dysfunction and defects in mitochondrial energetics in high fat fed mice

Éric Boulanger (Lille2 University, France)

Glyceraldehyde-derived pyridinium-type glycation product cause the RAGE-related cytotoxicity in PC12 cells

Teruyuki Usui (Kagawa Nutrition University, Japan)

Immuonochemical and instrumental evidence of the increase of Nω-carboxyethyl arginine (CEA) in accordance with kidney failures

Mime Nagai (Tokai University, Japan)

Multiple AGES monitoring by LC-MS/MS is an effective means to evaluate a metabolic environment *in vivo*

Jun-ichi Shirakawa (Tokai University, Japan)

Establishment of method for the determination of Advanced glycation end products (AGEs) in human bone and cartilage using liquid chromatography tandem mass spectrometry (LC-MS/MS)

Shoutaro Arakawa (Tokai University, Japan)

Dietary AGES intake and metabolic syndrome in young adults

Claudia Luevano-Contreras (University of Guanajuato, México)

Isoferulic acid action against glycation-induced changes in structural and functional attributes of human high density lipoprotein

Deeba Jairajpuri (Arabian Gulf University, Kingdom of Bahrain)
Maillard reaction products in follow-up formula for infants and toddlers

Eden Tareke (Lund University, Sweden)

Is fat or process-induced chemicals the culprit?

Eden Tareke (Lund University, Sweden)

Formation of amino sugars in the maillard reaction

Ossanna Nashalian (McGill University, Canada)

Inhibitory effect of rhodiola rosea extracts on AGEs formation

Hikari Sugawa (Tokai University, Japan)

Anti-glycation activity of kaempferia parviflora extract (sirtmax®) and its evaluation in clinical trials

Jin Tatsuzaki (Tokiwa Phytochemical Co, Japan)

Factors affecting development of reductants during sugar transformation

Karel Cejpek (University of Chemical Technology, Czech Republic)

Receptor for advanced glycation end products on endothelium affect vascular smooth muscle cell dysfunction

Mi-Hyun Nam (Korea University, Korea)

Protective effects of plantamajoside on AGEs and UV-B-induced damaged in HaCaT keratinocyte

Ah Ram Han (Korea University, Korea)

The protective effect of Maillard reaction products of fish protein hydrolysate with ribose against oxidative liver damage

Sung-Yong Yang (Korea University, Korea)

Hepatoprotective effect of maillard reaction products of whey protein concentrate against oxidative stress through the Nrf2-dependent antioxidant pathway in HepG2 cells

Min Cheol Pyo (Korea University, Korea)

Plantamajoside attenuated advanced glycation end-products-induced adhesion molecules and RAGE via NF-κB translocation modulating

Won-rak Son (Korea University, Korea)
Identification of Immunomodulatory active compound from glycated whey protein concentrate by MALDI-TOF/TOF MS/MS
Su-Hyun Chun (Korea University, Korea)

Acrylamide and HMF formations in chitosan containing model systems during heating
Burse Ataç Mogol (Hacettepe University, Turkey)

Effect of in vitro gastrointestinal digestion on α-dicarbonyl compounds in biscuits
Aytül Hamzahoğu (Hacettepe University, Turkey)

Heat-induced toxicants and odorants in cooked meat
Maïa Meurillon (INRA, France)

Influence of variety and agronomic factors on the lysine content in chicory roots, and on Nε-carboxymethyl-lysine formation during roasting
Grégory Loaëc (LaSalle Beauvais, France)

Effects of odor from the glycine/glucose maillard reaction on human mood and brainwaves
Lanxi Zhou (Kitasato University, Japan)

The formation and browning mechanisms of blue pigments, melanodin intermediates
Fumitaka Hayase (Meiji University, Japan)

Quantitative analysis of D-amino acids in sweet rice wine (Mirin) and studies on the mechanism of their formation during maturation
Yutaka Inoue (Meiji University, Japan)

Vitamin C degradation products induce non-enzymatic protein glycation
Haruna Miyakawa (Ochanomizu University, Japan)

Browning of cheese during storage happens by the maillard reaction
Yui Sato (Ochanomizu University, Japan)

Novel yellowish maillard pigment, pyrrolothiazolate, formed from cysteine and glucose
Kyoko Noda (Ochanomizu University, Japan)
P73
Hydrothermal aggregation behavior of bovine serum albumin regulated by gloxal-derived glycation
Ling Chen (South China University of Technology, China)

P74
Changes in N\textsuperscript{ε}-(carboxymethyl) lysine content of soy sauce during storage
Guoqin Liu (South China University of Technology, China)

The following is Late Breaking Presentations.

P75
Discovery of anti-glycation agents: studies towards the molecular treatment of diabetes
Saima Rasheed (University of Karachi, Pakistan)

P76
Capsaicin, an active ingredient from chili peppers, attenuates glycaitive stress and restores srage levels in diabetic rats
Chi-Hao Wu (Taipei Medical University, Taiwan)

P77
Determination of glyceraldehyde in plasma and organ
Mona Sato (Kyoto University, Japan)

P78
Microbiological screening to degrade model melanoidin and detection of the enzymes involved
Seiichi Homma (Ochanomizu University, Japan)

P79
Analysis of a glycated lipids present in milk powder
Ai Kodake (Tohoku University, Japan)

P80
Isolation, identification, and formation condition of a novel maillard reaction product derived from pyridoxamine and sugars
Yuri Nomi (Niigata University of Pharmacy and Applied Life Sciences, Japan)

P81
Effect of rutin supplementation in rye-buckwheat ginger cakes on maillard reaction development and antioxidative properties
Małgorzata Przygodzka (Institute of Animal Reproduction and Food Research, Poland)

P82
Preparation of liposomes using glycated lipids for the rapeutic purposes
Reina Kamiyoshihara (Tohoku University, Japan)

P83
Detection of methylglyoxal derived hydroimidazolone of creatine (MG-HCr) in meat
Jürgen Löbner (Technische Universität Dresden, Germany)

P84
Anti-hypertensive effect of soy sauce in salt-sensitive hypersensitive rats
Megumi Kato (Tohoku University, Japan)
President’s Lecture
WHY IS THE DISCREPANCY BETWEEN HEALTHY “WASHOKU” AND MYSTIC “AGE/RAGE AXIS”?  

Teruo Miyazawa  
Tohoku University

Japan is now in a long life society in the world. “WASHOKU” diet is recognized as one of the reason why we can keep the health and longevity. “WASHOKU” diet contains a large amount of Maillard products like browning, flavor and aroma compounds which stimulates our appetite and increases nutritional significance. For example, soy sauce, containing melanoids and we take it every day as liquid seasoning, moderates the Na-dependent increase in blood pressure. Maillard meal seems contributing to keep our health. On the other hand in our body, there are many Amadori products such as glycated phosphatidylethanolamine (gPE), HbA1c, and glycated albumin. These products are used for biomarkers and show generally increase depend on aging, but not disease-causing. In our body, gPE concentration is around 0.08 mol% of PE molecules, and carboxymethylated-PE is only 0.02 mol% and carboxyethylated-PE is 0.01 mol%. We have to consider these AGE concentrations as to physiologically significant or not. How does membrane lipid peroxidation proceeds depend on Maillard reaction in our body? 1-3) What kind of reactive oxygen species are evoked from glycation reaction in our body? We have to confirm what is the most important AGE/RAGE axis including a single receptor fitting multiple ligands. More quantitative investigation and chemistry-based discussion should be required for the Maillard reaction for our body.

1) J. Ito, S. Mizuochi, K. Nakagawa, S. Kato, T. Miyazawa  
2) S. Kato, K. Nakagawa, Y. Suzuki, A. Asai, M. Nagao, K. Nagashima, S. Oikawa, T. Miyazawa  
3) J. Ito, K. Nakagawa, S. Kato, T. Hirokawa, S. Kawahara, T. Nagai, T. Miyazawa  
Keynote Lectures
PROTEIN QUALITY CONTROL BY THE UNFOLDED PROTEIN RESPONSE

Kazutoshi Mori
Department of Biophysics, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan

Proteins must gain correct tertiary and quaternary structures to fulfill their functions as assigned by the genetic code. Folding and assembly of newly synthesized secretory and transmembrane proteins occur in the endoplasmic reticulum (ER), the first organelle they encounter after synthesis on the membrane-bound ribosome, and are assisted or promoted by a number of molecular chaperones and folding enzymes (collectively termed ER chaperones hereafter) constitutively expressed quite abundantly in the ER. This process of productive folding in the ER is indispensable to life of all eukaryotes. Importantly, proteins still unfolded or misfolded even after assistance of ER chaperones are targeted to the cytosol across the ER membrane for ubiquitin-dependent degradation by the proteasome, a series of processes collectively termed ER-associated degradation (ERAD). Thus, these two mechanisms, productive folding and ERAD, ensure the quality of proteins that pass through the ER.

Furthermore, all eukaryotic cells have developed a way to adjust the expression levels of ER chaperones and ERAD components according to demands in the ER. Thus, when unfolded proteins accumulate in the ER, this ER stress signal is sensed by a transmembrane protein(s) in the ER and transmitted to the nucleus to induce the transcription of genes coding for ER chaperones and ERAD components, leading to maintenance of the homeostasis of the ER. This unfolded protein response (UPR) consists of transcriptional control only in yeast but of both transcriptional and translational controls in metazoans, as the number of ER stress sensors/transducers has increased with evolution, from one (IRE1) in *Saccharomyces cerevisiae*, three (IRE1, PERK, and ATF6) in *Caenorhabditis elegans* and *Drosophila melanogaster*, and five (IRE1α, IRE1β, PERK, ATF6α and ATF6β) in mammals. Thus, yeast ER expresses IRE1, a type I transmembrane protein, for transcriptional control only, whereas PERK, a type I transmembrane protein which has emerged in metazoan ER, is able to attenuate translation generally in response to ER stress to decrease the burden on the ER when ER quality control system is compromised.

Interestingly, transcriptional induction of ER chaperones and ERAD components is achieved by the IRE1 pathway in non-vertebrates, but by the ATF6 pathway in mammalian cells, thus revealing a switch in the principal regulator from IRE1 to ATF6 during evolution. We have shown that the ATF6 pathway has gained a function in the UPR probably with the advent of vertebra using medaka fish (*Oryzias latipes*). Our results clearly showed that ER chaperone levels must be adjusted according to increased demands in the ER, which is as critical as an ER chaperon function.

I will summarize the mechanism and evolution of the UPR, and then discuss what is physiological ER stress and why we need so many ER stress sensors; vertebrates have five more ATF6-like ER membrane bound transcription factors, which are regulated by Regulated intramembrane proteolysis.

References
MULTI-OMICS APPROACH FOR THE ELUCIDATION OF FOOD FUNCTIONALITY

Hisanori Kato
Sponsor Endowed Chair “Food for Life”, Organization for Interdisciplinary Research Projects, The University of Tokyo, Tokyo, Japan

Transcriptomics, proteomics, and metabolomics are three major platforms of comprehensive omics analysis in the science of food and nutrition. In recent years, many researchers have put the combination of multiple omics analysis (integrated omics) into practice to exhaustively understand the functionality of food components. Nutrigenomics research will be further facilitated by promoting the integrated omics research of food functionality.

As one of the attempts of our group for the application of integrated-omics, the evaluation of functionality of coffee will be presented. Lines of evidence from epidemiological studies and other studies have suggested that coffee intake could reduce the risk of obesity and diabetes. However, the detailed mechanisms associated with coffee consumption are still poorly understood. C57BL/6J mice were fed a normal diet (ND), a high-fat diet (HF), and HF containing 2% powders of coffee (CC), 2% decaffeinated coffee (DC) or 2% unroasted green coffee (GC) for 9 weeks. Liver fat accumulation, which is one of the important upstream events of type 2 diabetes, was suppressed by the consumption of each coffee. To reveal hepatic metabolic alterations underlying the anti-obesity and anti-diabetic effects of coffee consumption, we obtained multiple-omics profiles (transcriptomics, proteomics, and metabolomics) of the liver. Transcriptomics revealed down-regulation of PPARg and its target molecules which are related to lipid metabolism, in coffee intake groups. Metabolomics showed up-regulation of metabolites involved in urea cycle, with which the transcriptome data was highly consistent, suggesting accelerated energy expenditure. On the other hand, proteomics showed up-regulation of isocitrate dehydrogenase, a key enzyme in TCA cycle, and its related proteins, suggesting promoted energy generation. The TCA cycle and the urea cycle are directly connected as they mutually provide the other’s intermediates. Therefore, the up-regulation of both is thought to be a metabolic shift causing increased ATP turnover, which should be related to the alterations of lipid metabolism. The mechanism may play an important part in the suppressive effects of coffee consumption on obesity, inflammation, and hepatosteatosis. This study revealed global metabolic alterations induced by coffee intake providing significant insights into the association of the coffee intake and the prevention of type 2 diabetes. It also demonstrated the benefits of combined omics approaches in food and nutrition science.
Plenary Lectures
Both glycosylation and glycation are common posttranslational modification reactions of proteins. We have been interested in redox-related molecules such as glutathione, and superoxide and anti-oxidant enzymes, superoxide dismutases (SODs) under conditions of oxidative stress. We first determined that Cu, Zn-SOD purified from human erythrocytes is glycated, and its site-specific and random fragmentation was then observed following the glycation reaction. The fragmentation proceeded in two steps.

In the first step, the peptide bond of Cu,Zn-SOD between Pro62 and His63 was cleaved, which yielded a large (15 kDa) and a small (5 kDa) fragments. In the second step, random fragmentation occurred. The ESR spectrum of the glycated Cu,Zn-SOD suggested that reactive oxygen species are implicated in the both steps. The same fragmentations were observed upon exposure of the enzyme to an H$_2$O$_2$ bolus. Catalase completely blocked both steps of the fragmentation process, whereas EDTA blocked only the second step. Incubation with glucose resulted in a time-dependent release of Cu$^{2+}$ from the Cu,Zn-SOD molecule. The released Cu$^{2+}$ then likely participated in a Fenton type of reaction to produce hydroxyl radicals, which may contribute to nonspecific fragmentation. This represents the first report of the site-specific fragmentation of a protein caused by reactive oxygen species formed by the Maillard reaction. The glycation of Cu,Zn-SOD was also observed in diabetic rats and human type 2 diabetic patients.

Glutathione, a major redox regulator is another interest of our research. The glutathione degrading enzyme, g-glutamyl transpeptidase was found to undergo aberrant glycosylation in cancer tissues. We purified an enzyme called GnT-III which is thought to be responsible for the aberrant glycosylation, cloned its cDNA, and observed its pathophysiological significances. Our group has been also interested in various other glycosyltransferases that are involved in the formation of branching in N-glycans and that these enzymes have been implicated in various pathophysiologial events. Aberrant glycosylation leads to oxidative stress and these events are involved in the development of Alzheimer's disease and the pathogenesis of chronic obstructive pulmonary disease (COPD).

In conclusion, oxidative stress is caused by the glycation of anti-oxidative enzymes whereas oxidative stress regulates glycan expression via glycosyltransferase genes, subsequently resulting in structural and functional changes of glycoproteins. These changes may lead to the development of various diseases. We propose glyco-redox research, a new paradigm in relation to glycosylation and glycation research.
PHYSIOLOGICAL RELEVANCE OF DIETARY MELANOIDINS

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Considering our evolutionary heritage it is not surprising that humans love cooked foods and they are attracted by their texture, colour and aroma. These attributes are mainly related to the presence of the so called Maillard Reaction Products (MRPs). MRPs are formed during cooking of foods and are responsible for the pleasant colour and aroma we associate to baked, fried and roasted foods. In the last 30 years studies on the correlation between MRPs and health mainly focused on their possible negative effects due to so-called dietary Advanced Glycation Endproducts (AGEs), which were considered a risk factor for many metabolic diseases. Moreover, some MRPs such as acrylamide and heterocyclic amines are known as potentially hazardous compounds although epidemiological evidence does not support this concern, thus far.

The history of humans evolution tells us that our ancestors made a good decision when they started to consume cooked foods. Scientific literature supports this view: many epidemiological studies, and also some intervention studies designed at investigating the effects of food processing, indicated several beneficial effects of cooked foods. In some cases these beneficial effects were related to the presence of melanoidins which are the brown polymers formed by the Maillard reaction: they constituted from 1% to 30% of the weight of some cooked foods resulting in a dietary intake of 10-20 grams per day.

The main sources of dietary melanoidins are coffee and bakery products; however also other processed foods such as cocoa, black beer, roasted potatoes, roasted pulses and seeds, soy sauces, possess a significant melanoidins moiety.

Surprisingly, the formation of melanoidins which is the main quantitative chemical modification induced by food cooking, has been relatively poorly investigated and its physiological relevance is still largely unknown.

The studies of the past 10 years demonstrated that melanoidins behave like an antioxidant dietary fibre: they are used by microbiota modifying the equilibria among species in the gut environment and, thanks to the catechol or reductones groups, they are able to keep a favourable reducing environment along the gut.

In this lecture the main evidence about melanoidins biological effects will be presented and the hypothesis about their overall physiological relevance will be discussed.

7. Vitaglione P, Napolitano A., Fogliano V Cereal dietary fibre as natural functional ingredient to deliver phenolic compounds into the gut. Trends in Food Science and Technology, 2008, 19, 451-463
In 2013 we celebrated the centenary of the discovery of the glyoxalase system. The glyoxalase system is comprised of two enzymes, glyoxalase 1 (Glo1) and glyoxalase 2, and glutathione co-factor, catalyzing the conversion of methylglyoxal to sequentially S-D-lactoylglutathione and D-lactate. It is the primary enzymatic defense in the cytosol of cells providing protection against damaging glycation of proteins and nucleotides by endogenous dicarbonyls – particularly methylglyoxal. Major methylglyoxal-derived glycation adducts of protein and DNA are hydroimidazolone MG-H1 and imidazopurinone MGdG, respectively. Proteins modified by methylglyoxal, the dicarbonyl proteome, may be detected directly by high resolution Orbitrap mass spectrometry proteomics analysis.

Accumulation of methylglyoxal - “dicarbonyl stress” - occurs in ageing and metabolic and vascular disease. It is linked to both increased formation of methylglyoxal and down-regulation of Glo1, with consequent increased protein damage and dysfunction by dicarbonyl glycation. Dicarbonyl stress is mild in obesity, moderate in diabetes and severe in renal failure. Experimental studies suggest a role for dicarbonyl stress in insulin resistance and inflammation of obesity. Increased formation of methylglyoxal occurs in hyperglycaemia associated with diabetes and Glo1 is down-regulated in the kidney, retina and nerve. Dicarbonyl stress thereby contributes to the development of diabetic nephropathy, retinopathy and neuropathy. Experimental and clinical studies also indicate Glo1 deficiency is a driver of cardiovascular disease. Increased methylglyoxal modification of LDL and HDL may exacerbate dyslipidaemia and vascular inflammation in atherosclerosis.

Genomic studies revealed GLO1 has rare (2%) duplication in the human population and higher prevalence amplification (10 – 20%) in breast and lung cancer where it produces multidrug resistance. Recent adaptive genomic studies suggest GLO1 has low level copy number alteration in experimental and clinical dicarbonyl stress. This, with clonal selection, may explain dominant GLO1 amplification in advanced stages of difficult-to-treat triple negative breast cancer and malignant melanoma.

Transcription of Glo1 is under stress responsive control via transcription factor Nrf2. This provided a strategy to develop Glo1 inducers through screening of dietary bioactives. We recently completed a clinical trial of a Glo1 inducer formulation in overweight/obese subjects. The Glo1 inducer is now available for Phase 2 clinical trial in established disease.

In studies of the glyoxalase system there is now the opportunity of health benefits for the general population from Glo1 inducers for improved metabolic and vascular health. Similar benefits may be available in established diabetes and renal failure. Further effort on Glo1 inhibitors may provide improved chemotherapy of refractory tumours.
The dimension of the chemical space of the Maillard reaction in food is still not sufficiently solved. Although only a few Maillard reaction products are commonly monitored, we have to be aware that a considerably greater variety of products is actually formed due to the plethora of possible reaction partners and processing conditions. In order to evaluate the physiological, toxicological and technological relevance of the Maillard reaction in food, a comprehensive overview of all possible products would be necessary. For this purpose, untargeted analytical methods, the so called “omics” techniques can be applied. However, up to now, the technical possibilities do not allow to cover all reaction products, but usually only a subset with similar properties, such as volatile compounds or protein bound AGEs.

In the present talk, recent studies will be summarized which aim for the comprehensive analysis of protein bound MRPs in heated milk products using proteomic tools. Whereas MALDI TOF MS gives an excellent and simple overview over protein adducts in heated milk, its relatively low sensitivity allows only the detection of some high abundant products, such as the Amadori product, CML of methionine sulfoxide. However, it has to be assumed that also minor product can be of physiological or technological relevance. In contrast to MALDI, LCMSMS can reveal also minor modification. For this approach, milk proteins are heated in the presence and absence of lactose, and are analyzed by LC-MSMS after partial enzymatic hydrolysis. Finally, differences between the two sample groups are determined by bioinformatic tools.

In a first semi-untargeted approached based on possible protein modifications reported in literature, 14 different modifications were identified, which are formed during the processing of milk. Thus, it was shown that the Maillard reaction results not only in glycation and glycoxidation products, such as lactulosyllysine, CML or imidazolinones, but promotes also considerably the oxidation of cysteine, methionine or tryptophan. Furthermore, oxidative deamination of the N-Terminus was observed or loosely bound methylglyoxal hemiaminals.

In the next step, untargeted methods were applied, to identify also novel modifications. Here all differences between the two sample groups are detected by bioinformatic tools after LCMSMS analysis. Since the main problem of shot gun proteomics is often a mass of data which are difficult to interpret, a proper data management is essential here. Untargeted analysis indicate on the one hand, that the majority of the protein modification which are formed by Maillard reaction seemed to be elucidated. However, also few novel modifications were detected, which have not been described in literature before.

Furthermore, methods will discussed which allow a systematic evaluation of the properties of products formed during the Maillard reaction.
SKIN AGES, FLUORESCENCE AND THE PROGRESSION OF
MACROVASCULAR COMPLICATIONS IN TYPE 1 DIABETES : THE PLOT
THICKENS

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Collaborative research with the DDCT/EDIC group carried out in the past 25 years has
shown that skin collagen-linked AGEs are potent predictors of the future risk of developing
microvascular disease in Type 1 diabetes. Using multivariate regression analysis adjusting
for age and diabetes duration we found that retinopathy is very strongly predicted by
fructose-lysine (furosine) and glucosepane, nephropathy by furosine, and neuropathy by
furosine and methylglyoxal hydroimidazolone MG-H1, all of which in spite of adjustment
for past and future A1c. These findings implicate a very strong role of hyperglycemia in
microvascular disease progression and perhaps glycation itself as a pathogenetic mechanism.
In support of the latter argument, the relationship between past glycemia and complication
progression, while very robust, became non-significant upon adjustment for AGEs, implying
that glucotoxicity might be indeed mediated by the latter. In contrast, skin AGEs were less
specifically associated with the progression of macrovascular disease. While several AGEs
were associated with very long-term thickening of the intima-media (Carotid ultrasound),
fluorescent "AGEs" stood out. Total collagen-linked fluorescence was robustly associated
with Coronary Artery Calcium (CAC) deposition and cardiac MRI data, while both the novel
fluorophore LW-1 and MG-H1 were associated with IMT, all of which in spite for A1c. In
this population, noninvasive skin fluorescence behaved similarly. Thus clearly, whatever is
responsible for fluorescence appears to be more strongly related to subclinical CVD
progression than traditional AGEs themselves, expect for MG-H1 that was associated with
IMT. Moreover, it is unclear to what extent the 370/440 fluorescence that is measured by
either type of assay (CLF, total fluorescence, LW-1) is related to nonenzymatic glycation
itself. Ongoing studies on the structure of LW-1 will be presented that suggest presence of
complex chemistry that involves enzymatic glucuronidation of a yet elusive precursor. These
findings call for intensified research into the molecular origin of skin fluorescence in the
progression of CVD in diabetes.
THE ROLE OF SUGAR CONJUGATED BINARY COMPLEXES OF AMINO ACIDS WITH Cu (II) IN CONTROLLING THE MAILLARD REACTION

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Main Objectives: Transition metal ions are known to play an important role in the Maillard reaction in catalyzing redox reactions. However, they can also form strong binary complexes with amino acids with increased reactivity towards aldehydes. It is expected that such complexes can serve as molecular scaffolds upon which different sugar moieties can accumulate and eventually be released over time generating colors and aromas. This fact can create the opportunity for controlled release of aroma and flavor precursors. The main objectives of this study were (1) to explore the role of various amino acid metal complexes in the Maillard reaction with sugars such as glucose, fructose, galactose, mannose, sorbose and ribose, (2) to study their impact on the generation of total volatiles and on (3) the extent of browning under the Maillard reaction conditions.

Strategy and Methods: Aqueous model systems containing synthetic amino acid metal complexes - (AA)\textsubscript{2}Cu - and various monosaccharides or their isotopically enriched (\textsuperscript{13}C and \textsuperscript{15}N-) counterparts were heated in open vials at 110°C for 2 hours and the residues were analyzed by ESI/qTOF/MS, orbitrap MS, MALDI/TOF, APCI MS and by Py/GC/MS. Absorbance values at 420 and 550nm were used to compare the extent of browning.

Main Results: Isotope labeling studies have indicated the ability of (AA)\textsubscript{2}Cu complexes to undergo multiple reactions with reducing sugars to generate various mono- and di-conjugated sugar complexes as Amadori/Heyns or Schiff intermediates. Furthermore, under the reaction conditions these sugar conjugates underwent oxidative decarboxylation and dissociation reactions to generate free and copper-complexed Amadori compounds in addition to fructosamine, deoxy-fructosamine and their derivatives. Furthermore, at the end of the reaction, the (AA)\textsubscript{2}Cu/sugar model systems retained almost 94% of their total ninhydrin active amino acid content, whereas, sugar/AA models showed only 55.2% retention. These changes in amino acid active moieties indicated that after 2 hours of heating at 100°C the mixture containing (AA)\textsubscript{2}Cu was still rich in aroma precursors such as Amadori, Amadori metal complexes and fructosamine derivatives relative to AA/sugar samples where nitrogen atoms from ninhydrin active moieties were reacted and were incorporated as N-heterocycles.

Conclusions: Due to their stability relative to free Amadori compounds, formation of sugar conjugated and copper-complexed intermediates, allowed for the slower release of aroma and browning precursors such as Amadori products and their derivatives during heating as assessed by the extent of browning and total volatile release.
Diabetes is the world's leading cause of cardiovascular disease, blindness, amputation and end stage renal disease. Although glucose itself is relatively inert, excessive metabolic flux in diabetes leads to the generation of highly reactive toxic intermediates. Chief among these are reactive oxygen species (ROS) and equally reactive dicarbonyls, leading to the accumulation in diabetes of oxidative modifications and Advanced Glycation End-products (AGEs) respectively. The importance of these synergistic pathogenic pathways in diabetic complications is demonstrated by the vasculo-protection achieved by blocking them, even when plasma glucose levels remain persistently elevated. Moreover, increased levels of dicarbonyls are able to generate diabetes-like changes, in the absence of hyperglycemia. For example we have shown that mice treated with a glyoxalase (Glo)-1 inhibitor develop atherosclerosis and nerve damage, comparable to that seen in diabetes. Similarly Glo-1 KO mice develop diabetes-like renal damage. These effects are largely mediated through the Receptor for AGEs (RAGE). However, dicarbonyls also lead to the generation of mitochondrial ROS and contribute to diabetic complications independent to RAGE. Recent trials intensifying glucose control in diabetes have achieved only modest benefits, chiefly on microvascular outcomes. Moreover, the majority of patients who develop complications do not have poor glucose control. This implies other factors must be involved that determine the vulnerability to glucose toxicity, rather than hyperglycemia itself. Indeed in the ADVANCE trial, AGEs and RAGE were predictors of adverse outcomes, independent to achieved glycemic control. Taken together these data provide a strong rationale for targeting the generation of dicarbonyls to prevent, treat and potentially reverse diabetic complications.
Ingestion of food is considered as the major route of exposure to many contaminants in human health risk assessment. Besides, total amount of a contaminant found in the ingested food does not always reflect the amount that is available to the body. Therefore, determination of the bioaccessibility of a contaminant from the matrix, and the fate of ingested contaminant during digestion is an important issue for human health. Acrylamide is one of the most widely encountered thermal process contaminants in foods such as bakery products, fried potatoes and coffee. However, information about its fate during the digestion of processed foods is lacking.

This presentation describes the fate of acrylamide in bakery and fried potato products during in vitro multistep enzymatic digestion process simulating gastric, duodenal and colon phases. Acrylamide levels gradually decreased through gastric, duodenal and colon phases during in vitro digestion of biscuits. At the end of digestion, acrylamide reduction ranged between 49.2% and 73.4% in biscuits. Binary model systems composed of acrylamide and amino acids were used to understand the mechanism of acrylamide reduction. High-resolution mass spectrometry (HRMS) analyses confirmed Michael type addition of amino acids to acrylamide during digestion. Contrary to bakery products, acrylamide levels increased significantly during gastric digestion of fried potatoes. The Schiff base disappeared rapidly meanwhile acrylamide level increased during the gastric phase. This suggests that intermediates like the Schiff base accumulated in potatoes during frying are potential precursors of acrylamide under gastric conditions. Due to its elimination and formation potential during in vitro digestion process, levels of acrylamide ingested with foods may not directly indicate its absorption rate through gastric, duodenal and colon.
AGEs IN BASEMENT MEMBRANE PROTEINS PROMOTE TGFβ-MEDIATED EPITHELIAL TO MESENCHYMAL TRANSITION OF CELLS: IMPLICATIONS FOR AGE AND DIABETES ASSOCIATED FIBROSIS

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Main Objectives: Tissue fibrosis is a major factor in age and diabetes associated complications. However, mechanisms for such fibrosis are poorly understood. Proteins in the basement membrane (BM) of tissues are long-lived and they accumulate AGEs during aging and at a higher rate in diabetes. The human lens capsule is a BM secreted by lens epithelial cells. After cataract surgery, lens epithelial cells adhering to anterior capsule migrate to posterior capsule of the lens and attain a mesenchymal phenotype, which leads to fibrosis and eventually to posterior capsule opacification (PCO) that blocks vision. We investigated the effect of aging and cataracts on the AGE levels in the human lens capsule and determined the role of capsule AGEs in lens epithelial cell transformation to mesenchymal phenotype.

Strategy and Methods: AGEs in lens capsules were quantified by an LC-MS/MS method. Role of AGEs in lens epithelial cell mesenchymal transformation was assessed by qPCR in cells grown on AGE-modified extracellular matrix and in AGE-modified lens capsule.

Main Results: We found age-dependent increases in several AGEs and significantly higher levels in cataractous lens capsules than in normal lens capsules. The TGFβ2-mediated upregulation of the mRNA levels of mesenchymal markers was significantly enhanced in lens epithelial cells cultured on AGE-modified lens capsule and basement membrane extract (BME) compared with those on unmodified lens capsule and BME. Such responses were also observed for TGFβ1. In the human capsular bag model for posterior capsule fibrosis, the AGE content of the capsule proteins was correlated to TGFβ2-mediated α-smooth muscle actin (αSMA) synthesis, which is a major mesenchymal cell marker.

Conclusions: Taken together, our data imply that AGEs in the lens capsule promote TGFβ-mediated fibrosis of lens epithelial cells after cataract surgery and could play a role in PCO. Our results also suggest that AGEs in basement membranes could have a broader role in aging and diabetes-associated fibrosis.
The formation of carboxymethyllysine (CML) in food, its intestinal absorption, biodistribution and elimination and its pathophysiological implication has been widely researched and debated but grey areas still remain. The long term aim of this research is to establish correlations and causal relationships between the level of CML in the diet (dCML) or in the circulation, and human pathologies.

The main topic here, though, is the measurement of dCML at the different stages of its presence in the blood and the organs.

Our knowledge of the pharmacokinetics of dCML is still limited but our data clearly indicate that dCML is characterized by a partial, but rapid, absorption and elimination. Our findings showed that only the free CML level increased in animal blood while the protein-bound CML concentration remained unchanged. It is most likely that the apparent low level, or lack of association between dCML and serum CML could be accounted for by the fact that most scientists, including ourselves for a time, were performing the analysis on fasting blood samples instead of postprandial blood samples and have, additionally, quantified protein-bound CML rather than free CML. We recommend therefore that a duplicate analysis of blood (1h postprandial & fasting) be carried out using only validated LC-MS/MS methods which result in precise reading of free CML alone.

Other studies have tried to measure how much dCML remains in tissues by using intravenous injections of a $[^1]F$fluorobenzoyl derivative of CML. However the results are questionable because of the high chemical modification of the CML. In order to overcome this bias and others a protocol which differentiates dCML from endogenous CML (eCML) was developed using 3 CML isotopes with different mass-to-charge ratios (m/z) (e-CML $N^\varepsilon$-carboxymethyl-L-lysine: 205; dCML $N^\varepsilon$-[1$^{13}$C]carboxy[1$^{13}$C]methyl-L-lysine: 207; internal standard $N^\varepsilon$-carboxymethyl-L-[4,4,5,5-2H$_4$]lysine: 209). Wild-type and RAGE$^{-/-}$ mice were fed either a control diet including BSA or a dCML diet (200$\mu$gd-CML/g) for 30 days. After sacrifice the organs were analysed for eCML and dCML using an LC-MS/MS method. Mice exposed to dCML showed an accumulation in all organs tested, compared to the control mice. The rate of deposition was high in the kidneys, ileum, colon and lungs, medium in the brain and testicles, and low in the heart, muscle, liver and fat. This accumulation was not RAGE-dependent.

The conclusion is that the kidney is not the only organ affected by the accumulation of non-metabolized dCML. The high accumulation of dCML found by our new method of measurement in the gut and the lungs might also have important physiological consequences.

A fuller study of the subject is still needed. We believe it is risky to establish any nutritional recommendation unless validated LC-MS/MS methods and CML isotopes have been employed for the purposes of the measuring dCML in vivo.
YIA Presentations
YIA-M1 IMMUNOCHEMICAL DETECTION OF MG-H1 IN HUMAN SERUM BY SPECIFIC MONOCLONAL ANTIBODY AND ITS CORRELATION WITH LC-MS/MS ANALYSIS

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Main Objectives: Nd-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1) is reported as a major methylglyoxal (MG)-derived AGE structure. Several group developed the antibodies against MG-H1 by immunizing MG-modified protein. In the present study, MG-H1-conjugated protein was immunized to mice in order to prepare the specific monoclonal antibody.

Strategy and Methods: Monoclonal antibody named 16GF7 was purified from ascitic fluid by protein G column. MG-modified bovine serum albumin (MG-BSA) was prepared by incubating MG (0.01-1 mM) with BSA for up to 1 week. MG-H1 content of MG-BSA was also measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) after enzymatic digestion, and compared with ELISA. Furthermore, MG-H1 content in serum was measured by competitive ELISA.

Main Results: Competitive ELISA demonstrated that 16GF7 reacted with MG-H1 in dose dependent manner, whereas the antibody did not cross-react with other AGEs such as CML and CEL. The reactivity of 16GF7 increased in time dependent manner when BSA was incubated with 0.01 mM MG. MG-H1 content in MG-BSA measured by ELISA was highly correlated with MG-H1 content measured by LC-MS/MS. Furthermore, serum MG-H1 content measured by competitive ELISA increased by the pathogenesis of kidney failure compared with control subjects.

Conclusions: 16GF7 increased minimally modified MG-BSA, and comparison between ELISA and LC-MS/MS analysis demonstrated that 16GF7 significantly detected MG-H1 in dose dependent manner without pretreatment with heating. Taken together, 16GF7 can detect physiologically low concentration of MG-H1 and would be a simple tool for the detection of metabolic disorders.
POST-GLUCOSE LOAD PLASMA $\alpha$-DICARBONYL CONCENTRATIONS ARE INCREASED IN INDIVIDUALS WITH IMPAIRED GLUCOSE METABOLISM AND TYPE 2 DIABETES: THE CODAM STUDY

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Main Objectives: Type 2 diabetes and impaired glucose metabolism (IGM) are associated with an increased risk of microvascular complications and cardiovascular disease (CVD). Currently, there is increasing evidence that postprandial glucose excursions, rather than fasting glucose concentrations or mean glucose levels, play an important role in the development of these vascular complications. A possible mechanism through which they have a more damaging effect than high fasting or mean glucose levels, might be via the formation of reactive $\alpha$-dicarbonyls. In the current study, we investigated whether $\alpha$-dicarbonyls are increased after a glucose load in individuals without and with IGM and type 2 diabetes.

Strategy and Methods: Cross-sectional, linear analyses were performed in the Cohort study on Diabetes and Atherosclerosis Maastricht (CODAM, n=574, 61% men, 60 years old). Individuals with normal glucose metabolism (n=279), IGM (n=120) and type 2 diabetes (n=92) who had complete data on an oral glucose tolerance test (OGTT) and were not on insulin treatment were included in the study population. Plasma $\alpha$-dicarbonyl (methylglyoxal (MGO), glyoxal (GO) and 3-deoxyglucosone (3-DG)) levels were measured in the fasting state and in samples of the OGTT by the state-of-the-art technique UPLC-MS/MS.

Main Results: The presence of both IGM and type 2 diabetes was significantly associated with higher $\alpha$-dicarbonyl incremental area under the curves (iAUC), as calculated from the OGTT (for IGM: MGO $\beta=0.190$, 95% CI=0.106-0.274; GO $\beta=0.287$, 95% CI=0.172-0.401; 3-DG $\beta=0.285$, 95% CI=0.221-0.349; for type 2 diabetes: MGO $\beta=0.293$, 95% CI=0.180-0.405; GO $\beta=0.536$, 95% CI=0.382-0.689; 3-DG $\beta=0.542$, 95% CI=0.456-0.628). Adjustment for glucose iAUC attenuated these associations. Moreover, iAUCs of the $\alpha$-dicarbonyls correlated highly with glucose iAUC, but not with fasting glucose levels and HbA1C.

Conclusions: The increased levels of $\alpha$-dicarbonyls during an OGTT in individuals with IGM and type 2 diabetes underline the potential importance of $\alpha$-dicarbonyl stress as a candidate to explain the increased risk of diabetic complications in individuals with postprandial hyperglycemia.
PLASMA LEVELS OF N°-(CARBOXYMETHYL)LYSINE ARE INVERSELY ASSOCIATED WITH CENTRAL OBESITY AND INFLAMMATION, AND SIGNIFICANTLY EXPLAIN A PART OF THE CENTRAL OBESITY-RELATED INCREASE IN INFLAMMATION: THE HOORN AND CODAM STUDIES

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Main Objectives: Adipose tissue (AT) inflammation contributes to the development of complications such as insulin resistance and type 2 diabetes. Factors responsible for the AT inflammation in obesity are not fully clear. In this context, we recently reported that obesity is characterized by an accumulation of the advanced glycation/lipoxidation endproduct (AGE/ALE), N°-(Carboxymethyl)lysine (CML) in AT. In addition, we demonstrated that plasma CML levels were strongly reduced in obese subjects due to trapping of CML in AT, where it activates inflammatory signaling pathways leading to insulin resistance.

The objective of this study is to investigate the inter-relationships between measures of (central) obesity (i.e. BMI and waist circumference), plasma CML (as an inversely correlated marker of CML trapping in adipose tissue) and low-grade inflammation (LGI) in a large sample of individuals whose weight states ranges from normal to morbid obesity.

Strategy and Methods: We studied 1270 individuals (532 participants of the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) Study and 738 participants of the Hoorn Study), in whom protein-bound CML levels were measured by UPLC-Tandem MS and levels of circulating inflammatory markers (i.e. CRP, SAA, sICAM-1, sVCAM-1, IL-6 and TNF-a) were measured with multi-arrays. These inflammatory markers were compiled into a LGI score by averaging the respective z-scores (standard deviations of differences from the population mean). Multiple linear regression analyses, adjusted for age, sex, cohort and other covariates, were used to investigate 1) the association between obesity and LGI; 2) the association between obesity and plasma CML; and 3) plasma CML levels and LGI (see figure 1).

Main Results: We demonstrated that: 1) waist circumference, more strongly than BMI, was associated with the LGI score [standardized regression coefficient β=0.197 (95% CI: 0.139; 0.254) and β=0.262 (0.203; 0.321), respectively, p<0.001 for both], 2) Waist circumference and BMI were inversely associated with plasma CML [β=-0.357 (-0.414; -0.301) and β=-0.279 (-0.332; -0.226) for BMI, p<0.001 for both], 3) Plasma CML was inversely associated with the LGI score [β=-0.073 (-0.130; -0.015), p=0.002]. Finally, to investigate to what extent plasma CML contributes to the association between obesity and LGI, we further adjusted the association between BMI/waist circumference and LGI for CML plasma concentration. The association between waist circumference and LGI [β=0.262] was attenuated after adjustment for plasma CML [to β=0.202] with CML explaining the greatest portion of the association (about 12%). Other well-known risk factors contributing to obesity-related inflammation only explained 0-10% of the association between obesity and inflammation, indicating that CML is a significant factor leading to inflammation in obesity.

Conclusions: Obesity, in particular central obesity, is characterized by greater levels of low-grade inflammation but by lower levels of CML in plasma, the latter significantly explaining a portion of the positive association between central obesity and inflammation.
RAGE MEDIATES THE VASCULAR AGING INDUCED BY DIETARY CARBOXYMETHYLLYSINE

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Main Objectives: Arterial aging is characterized by a series of changes that take place in the vessel wall including metabolic, functional and structural modifications, e.g. the development of endothelial dysfunction and vascular stiffness with accompanying elastin disruption. Diet can affect arterial aging. Dietary AGEs are more and more described as potentially deleterious neo-formed compounds in food. This work investigated the effects of a carboxymethyllysine (CML)-enriched diet and RAGE involvement in aortic aging in mice.

Strategy and Methods: After 9 months of a control diet or CML-enriched diets (50, 100 or 200µgCML/g of food), endothelium-dependent relaxation (EDR), RAGE, vascular cell adhesion molecule-1 (VCAM-1) and sirtuin-1 (SIRT1) expression, pulse wave velocity (PWV) and elastin disruption were measured in aortas of wild-type or RAGE−/− male C57BL/6 mice.

Main Results: Compared to the control diet, EDR was reduced in the wild-type mice fed the CML-enriched diet (200µgCML/g) (66.8±12.26 vs 94.3±2.6%, p<0.01), but RAGE−/− mice were protected against endothelial dysfunction induced by dietary CML. RAGE and VCAM-1 (p<0.05) expression were increased in the aortic wall. RAGE−/− mice were protected against CML-enriched diet-induced endothelial dysfunction. Compared to control diet, the CML-enriched diet (200µgCML/g) increased the aortic PWV (86.6±41.1 cm/s vs 251.4±41.1 cm/s, p<0.05) in wild-type animals. Elastin disruption was found to a greater extent in the CML-fed mice (p<0.05). RAGE−/− mice fed the CML-enriched diet were remarkably protected from aortic stiffening and remodeling. The aging marker SIRT1 was found reduced in a CML-intake-dependent manner in wild-type mice (p<0.05), but remained unchanged in RAGE−/− mice.

Conclusions: Chronic CML ingestion induced endothelial dysfunction, arterial stiffness and aging in a RAGE dependent manner.
**Main Objectives:** To explore the use of protein glycation, oxidation and nitration biomarkers for early-stage diagnosis, progression and treatment monitoring of arthritis.

**Strategy and Methods:** Osteoarthritis (OA) and rheumatoid arthritis (RA) is the most common cause of chronic disability worldwide and is increasingly important in current ageing populations. We recently introduced a simple biochemical test to detect and discriminate early-stage OA (eOA), early-stage RA (eRA) and other non-RA inflammatory joint disease (non-RA) based on plasma hydroxyproline, citrullinated protein (CP) and anti-CP-antibodies (Ahmed et al., Scientific Reports 5: 9259, 2015). Protein glycation, oxidation and nitration may also provide useful biomarkers in arthritis.

Patients and healthy controls were recruited by collaborators from orthopaedic and rheumatology clinics in hospitals of Coventry, Birmingham, Exeter and Ipswich in the UK and synovial fluid and plasma samples collected. Protein damage markers (PDM) were quantified by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry. Data were used to train and test a diagnostic algorithm combining plasma PDMs and hydroxyproline to detect and discriminate 4-classes of skeletal health: good skeletal health, eOA, eRA and non-RA inflammatory joint disease. Data are mean ± SD or median [lower-upper quartile].

**Main Results:** There was little change of plasma protein glycation in eOA except CEL of plasma protein was increased 2-fold in patients with eOA, compared to healthy controls (0.046 [0.033 - 0.018] versus 0.019 [0.003 - 0.007] mmol/mol lys; p<0.05). In advanced OA, hydroimidazolones derived from glyoxal and methylglyoxal, G-H1 and MG-H1, were increased in synovial fluid protein 6-fold and 3-fold, respectively, with respect to eOA (G-H1, 0.293 ± 0.114 versus 0.052 ± 0.020; MG-H1, 0.90 [0.45 - 1.30] versus 0.35 [0.28 - 0.42] mmol/mol arg; P<0.001). Similarly changes were observed in advanced RA and eRA patient groups.

Protein oxidation in eRA and advanced OA and RA. Methionine sulfoxide free adduct of plasma was increased 3-fold in eRA, 4-fold in non-RA, 7-fold in advanced OA and 10-fold in advanced RA, compared to healthy controls (67 [55 – 82], 95 [62 – 119], 140 [100 – 300], 225 [134 – 283]) versus 22 (15 – 38) nM, respectively; P<0.001).

Combination of protein glycation, oxidation and nitration analytes in a diagnostic algorithm detect and discriminate between good health, eOA, eRA and non-RA.

**Conclusions:** This study provides first quantitation of protein damage markers in early and advanced joint disease with a first-in-class plasma-based biochemical assay for diagnosis and type discrimination of early-stage arthritis to facilitate improved clinical treatment and outcomes.
Main Objectives: Advanced glycation end products (AGE) are considered independent predictors of type 1 diabetes (T1D) progression in islet autoantibody positive children. Alagebrium(4, 5-dimethyl-3-(2-oxo2-phenylethyl)-thiazolium chloride (ALT-711) is a therapeutic agent which decreases the formation of AGEs. Using a murine model of T1D, we aimed to study the immune effects of ALT-711 therapy prediabetes.

Strategy and Methods: Female NODShiLt (n=10/group) were randomised to receive 1) no treatment or 2) ALT-711 (1mg/kg/day s.c.) prediabetes from day 50 - 100 of life and were followed until diabetes diagnosis or day 200.

Main Results: ALT administration for 50 days prediabetes, conferred protection from autoimmune diabetes until day 200 of life compared to controls (20% vs 80% incidence, p=0.005). Toward the end of the treatment period, ALT treated mice had lower glycated haemoglobin (7.4% vs 11.1%, p=0.0005), improved insulin secretion during OGTT (11.8 vs 10.2 nmol/L/min, p=0.02) and less pancreatic islet infiltration compared to controls (p=0.0005). After 30 days of ALT-treatment, whole pancreas digests showed a modest reduction in CD45.1+ cell number (1.17x10^5 vs 3.87 x 10^5 cells, p=0.07) and decreased F4/80+ macrophages (4.7 x10^3 vs 20.5 x10^3 cells vs, p=0.03), compared to controls. No differences in CD4+, CD8+, CD4+CD25+Foxp3+ T cells, CD19+B220+ B cells or CD11b+CD11c+ dendritic cell numbers or proportions were observed in the pancreatic lymph node or spleen. Splenocyte function at this time was not different between groups after immune stimulation by ovalbumin (163.8 vs 213.63 interferon producing cells). Adoptive transfer of diabetes to NODScid recipients by splenocytes from ALT treated or control NODShiLt mice did not differ between groups (69% vs 62%). In vitro, AGE modified albumin enhanced antigenic peptide activity by the enzyme ERAP-1, part of the MHC Class I pathway.

Conclusions: Taken together, short-term ALT-711 therapy prediabetes can improve islet function and reduce immune infiltrate, which could be via effects on antigen presentation by pancreatic beta cells.
YIA-M7  RAGE-DNA APTAMER IMPROVES ALDOSTERON-INDUCED RENAL INJURY POSSIBLY VIA INHIBITION OF RAC1-MR AXIS IN MICE WITH HYPERTENSIVE NEPHROPATHY

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Introduction: Receptor for advanced glycation endproducts (RAGE) is a multi-ligand receptor that belongs to the immunoglobulin superfamily, which can stimulate the generation of reactive oxygen species (ROS) and subsequently enhance various intracellular pathways by the binding of ligands such as advanced glycation endproducts (AGEs). Although role of RAGE is mainly investigated in the pathogenesis of diabetic nephropathy, its regulation and function in the hypertensive nephropathy (HN) is unknown. In addition, recent evidence has suggested that aldosterone-mineralocorticoid receptor (MR) axis plays an important role for the pathogenesis of HN. Therefore, in this study, we examined whether RAGE system could be involved in the aldosterone-induced renal damage via MR axis in deoxycorticosterone acetate-treated HN in mice. Further, we explored the effect of DNA-aptamer directed against RAGE (RAGE-aptamer) on the progression of HN.

Methods: Protocol 1: Uninephrectomized 8-week-old C57Bl/6J male mice were divided into three groups as follows; 4% salt diet group (control), 4% salt diet with DOCA (DOCA/salt) (50mg, 21days release, Innovation Research®) group, and DOCA/salt with hydralazine (Hyd, 1mg/kg/day) group. DOCA was administrated subcutaneously in the right flank region. Protocol 2: We constructed RAGE-aptamer by Systematic Evolution of Ligands by EXponential enrichment (SELEX) method. RAGE-aptamer was administrated in DOCA/salt mice continuously by osmotic mini pump for 21days.

Results: Systolic blood pressure was significantly increased in DOCA/salt mice compared with control, which was decreased by Hyd treatment. Urinary albumin excretion (UAE) was exacerbated in DOCA/salt mice compared with control group, which was affected by Hyd treatment. RAGE and connective tissue growth factor (CTGF) protein expression, ROS generation (8-hydroxy-2'-deoxyguanosine; 8OHdG), and extracellular matrix (ECM) accumulation were increased in the renal cortex, and plasma carboxy-methyl lysine (CML) levels were elevated in DOCA/salt mice. Further, GTP-bound Rac1 activity and MR overexpression were observed in the glomeruli of DOCA/salt mice. The treatment with RAGE-aptamer significantly suppressed aldosterone-induced increase in UAE, ROS generation, RAGE and CTGF overexpression, plasma CML levels, and ECM accumulation independent of blood pressure in DOCA/salt mice. In addition, RAGE-aptamer could suppress GTP-bound Rac1 activity and MR overexpression in glomeruli of DOCA/salt mice as well.

Conclusion: RAGE may play a pivotal role in the progression of aldosterone-induced HN through Rac1-mediated MR activation. RAGE-aptamer might be a novel therapeutic strategy for the progression of aldosterone-related HN.
Main Objectives: The accumulation of advanced glycation end products (AGEs) is implicated in the pathogenesis of diabetic nephropathy. The role of AGE receptors in preventing AGE accumulation at sites of diabetic complications has received much interest. We investigated the effects of overexpressing the advanced glycation end product receptor 1 (AGER1) in glomerular podocytes on the development of kidney disease in diabetes.

Strategy and Methods: Male heterozygous global AGER1 knock-in mice (AGER1), podocyte-specific (AGER1Pod) and littermate controls (WT) (N=8/group) were randomised to either control or diabetes induced by low dose streptozotocin (50mg/kg/day for 5 days) and followed for 24 (AGER1) or 12 weeks (AGER1Pod). Kidney function was determined by 24 hour urinary albumin excretion rate (UAER) and creatinine clearance (CrCl) and renal structural abnormalities assessed (glomerulosclerosis and tubulointerstitial fibrosis). Additionally we examined the localisation of AGER1 in subcellular fractions of the podocyte and concentrations of renal AGEs.

Main Results: Non-diabetic mice with podocyte-specific overexpression of AGER1 developed advanced kidney disease, matching the pathology seen in diabetic WT mice, while global AGER1 KI mice were not protected against renal injury in the context of diabetes. This was evidenced by increased glomerulosclerosis, tubulointerstitial expansion, increased UAER and a decline in CrCl. Interestingly there was greater renal clearance of AGEs seen in non-diabetic mice with podocyte-specific overexpression of AGER1 when compared with both global AGER1 KI and WT mice.

Conclusions: Increased expression of AGER1 in podocytes results in kidney disease which was exacerbated with diabetes. Efforts to increase the expression of AGER1 in an attempt to facilitate renal clearance of AGEs in diabetes may have undesirable side-effects leading to kidney dysfunction.
EFFECT OF PRENATAL DIEATARY LOAD OF MAILLARD REACTION PRODUCTS ON METABOLIC STATUS OF FEMALE WISTAR OFFSPRING

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Main Objectives: Maternal dietary habits affect the fetus, the outcome of pregnancy, and long term health of the child. Maternal obesity and improper prenatal nutrition provide maladaptive intrauterine cues to developing offspring, predisposing organs for chronic disease later in life. A direct relationship between newborn’s and maternal serum levels of several Maillard reaction products (MRPs) at the time of delivery has been reported, suggesting their maternal transmission. In pregnancy-associated pathologies impacting fetus development, such as preeclampsia and preterm birth, axis of advanced glycation end products (AGEs, in vivo analogues of MRPs) and their receptors is activated. These findings raise the question on the role of MRPs-rich diet in prenatal programming. To the best of our knowledge potential effects of MRPs-rich diet during pregnancy on prenatal programming have yet not been investigated. In this study we investigated the metabolic status and an early neurobehavioral development of young adult rats – offspring of dams consuming MRPs-rich diet during pregnancy.

Strategy and Methods: At the first day of pregnancy, rats were randomized into 2 groups, pair-fed with either standard rat chow or chow enriched with bread crusts as a source of MRPs until delivery. Female offspring from both groups, fed after weaning by a standard rat chow ad libitum, were investigated. At the age of 12 weeks, oral glucose tolerance test was performed, blood pressure was measured by tail plethysmography, and circulating markers of inflammation, oxidative and carbonyl stress, glucose and lipid metabolism, and semicarbazid-sensitive aminooxidase activity were assessed.

Main Results: The offspring of dams fed with MRPs-rich diet gained more weight during the last 2 weeks before sacrifice compared with the control group. These offspring presented higher circulating advanced oxidation protein products (markers of protein oxidation), while their levels of thiobarbituric acid reactive substances (markers of lipid peroxidation) and total antioxidant capacity were decreased. Prenatally affected rats presented higher levels of fasting glucose and lower quantitative insulin sensitivity check index (QUICKI). Other investigated markers were not affected significantly in prenatally challenged offspring. Data on the effects of prenatal load with MRPs-rich diet on early neuromotoric behavior of the offspring are under current evaluation.

Conclusions: This is the first study demonstrating that in rats maternal consumption of MRPs-rich diet during pregnancy imposes adverse metabolic effects in young adult female offspring. The potential prenatal programming effect of the consumption of MRPs-rich diet in humans remains to be elucidated.

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NEW INSIGHTS INTO BREAD MELANOIDINS

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Main Objectives: Melanoidins are complex brown polymers generated in foods during the final stage of the Maillard reaction. Requiring severe heating conditions, bread melanoidins (BMs) are only found in the crust of breads and not in the crumb where the temperature does not exceed 100°C. The aim of this work was to develop a new technique for measuring BMs and gaining more insight into their structure and role in breads.

Strategy and Methods: A new technique was developed for measuring BMs combining size exclusion chromatography for molecular weight separation and fluorescence measurement at wavelengths characteristic of certain Maillard reaction products (MRPs) (ex-em: 350-430nm). This technique was tested on bread crust and crumb throughout baking at 220°C (every 4min for 16min). A fiber-free crust-like model system was prepared using gluten, glucose and starch. It was analyzed using this newly developed technique throughout baking in the same conditions. This model was also used to assess the potential correlation between BMs and dietary fibers by measuring fiber amounts throughout baking. Bread crust, crumb and the crust-like model samples were enzymatically digested in simulation of gastro-intestinal digestion and BMs evolution was monitored throughout the procedure.

Main Results: Fluorescent MRPs with molecular weights between 1.7 and 5.6 kDa were found in the soluble fraction of bread crusts and in the crust-like model system but not in the crumb of the breads. Their amounts were proportional to the baking duration of the crust and the model system but did not evolve in the crumb. These unknown MRP polymers are therefore considered as soluble BMs. The quantification of dietary fibers in crust samples revealed a strong positive correlation between the amounts of BMs and the apparent amounts of soluble fibers. The gastro-intestinal digestion simulation showed a gradual release of BMs into the soluble fraction of the batches for bread crust and the crust-like model. This release was noticeable in the presence of proteases, but increased drastically in the presence of amylases. This finding is an indicator of the importance of carbohydrates in the BMs structure.

Conclusions: These fluorescent polymers can be used as indicators of the total melanoidins present in the cereal-based products. Bread melanoidins seem to contain important amounts of intact carbohydrates in their structure and seem to interfere with the measurement of dietary fibers.
Main Objectives: Diseases like diabetes and uremia are linked to elevated dicarbonyl concentrations in vivo. In particular, methylglyoxal shall play a pivotal role for irreversible damage of proteins or DNA. To inhibit consequences of this “dicarbonyl stress”, pharmacological strategies have been examined. Synthetic dicarbonyl-trapping compounds such as aminoguanidine received particular attention, but due to massive side effects this compound is no longer under development as a drug. Creatine is present in most tissues and body fluids and known to be linked to energy metabolism, especially in muscle and brain. Here, we followed the hypothesis that creatine may act as “natural” scavenger for dicarbonyl compounds under physiological conditions.

Strategy and Methods: The reaction between methylglyoxal and creatine under physiological conditions was studied using LC-MS/MS. The main reaction product N-(4-methyl-5-oxo-1-imidazolin-2-yl)sarcosine (MG-HCr) was isolated and characterized via NMR and mass spectrometry. The content of MG-HCr in incubation mixtures and in meat was determined using hydrophilic interaction chromatography coupled to mass spectrometry. MG-HCr was then quantified in urine of vegetarians and non-vegetarians.

Main Results: From the reaction of methylglyoxal and creatine under physiological conditions MG-HCr was isolated and identified by NMR and mass spectrometry. Due to its rapid formation, MG-HCr represents a specific product following “scavenging” of methylglyoxal by the guanidino moiety of creatine. MG-HCr was analyzed in urine samples of healthy volunteers. Daily MG-HCr excretion of non-vegetarians ranged from 0.35 to 3.84 µmol/24 h urine (median: 0.90 µmol/24 h urine) and of vegetarians from 0.11 to 0.31 µmol/24 h urine (median: 0.19 µmol/24 h urine), indicating that elevated excretion of MG-HCr is either influenced by the enhanced formation in vivo following dietary intake of creatine or by the ingestion of MG-HCr present in the diet. MG-HCr was quantified in raw meat (< 0.05 µmol/kg) and in cooked meat to 16-22 µmol/kg.

Conclusions: The increase of MG-HCr after cooking of meat emphasizes the role of creatine as a scavenger for dicarbonyl compounds in food. Under physiological conditions creatine can act as a protection molecule by the detoxification of methylglyoxal. Therefore it can be of special importance in situations when the body has to deal with pathophysiologically increased amounts of dicarbonyl compounds (“carbonyl stress”), for instance in diabetic or uremic patients.
THE ROLE OF AMINO ACID METAL COMPLEXES IN THE MAILLARD AND AKABORI REACTIONS

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Main Objectives: The transformation of α-amino acids into their hydroxymethyl derivatives is catalyzed by metal salts in the presence of Strecker aldehydes and the process is commonly known as the Akabori reaction. The main objective of this study was to understand the role of Akabori transformation within the Maillard reaction system. Moreover, considering the fact that the Akabori reaction requires amino acid metal salts as precursors, various amino acid metal salt mixtures were prepared and their chemistry under the Maillard reaction was explored in the absence of aldehydes.

Strategy and Methods: The metal-amino acid interactions in the absence of aldehydes or sugars were studied through heating of various alanine or glycine metal salts (Cu$^{2+}$, Fe$^{3+}$, Zn$^{2+}$, Ca$^{2+}$) and synthetic (Gly)$_2$Cu or (Ala)$_2$Cu complexes under pyrolytic conditions and analyzing their thermal degradation products by GC/MS. Subsequently, the reaction of the above complexes were also studied in the presence of paraformaldehyde as Akabori model systems with or without glucose. The aqueous mixtures of the model systems were heated at 110°C for 2 hours and analyzed by qTOF/ESI/MS and GC/MS. Isotope labeled precursors were used for confirmation purposes.

Main Results: Heating alanine metal salts indicated that Cu$^{2+}$ and Fe$^{3+}$ due to their high oxidation potentials were the only metals able to induce oxidative decarboxylation of amino acids and formation of Strecker aldehydes. Furthermore, studies performed with synthetic (Gly)$_2$Cu or (Ala)$_2$Cu complexes indicated that they constituted the critical intermediates undergoing free radical oxidative degradation followed by the loss of CO$_2$ and generation of Strecker aldehydes detected as stable Schiff base adducts or as pyrazine or pyridine derivatives. Furthermore, heating (Gly)$_2$Cu in the presence of formaldehyde led to its Akabori transformation into serine. Isotope labeling studies of the various products identified have provided for the first time mass spectrometric evidence for the detailed mechanism of Akabori transformation through initial Schiff base formation with formaldehyde followed by nucleophilic addition at the α-carbon. The first step is necessary for the activation of the complex and the conversion of glycine into serine and hydroxymethyl-serine; furthermore, the results indicated that sugars do not interfere with such transformations and on the contrary the presence of glycine copper complexes in the Maillard model systems can enhance the production of Maillard reaction products.

Conclusions: These results highlight a possible way to generate new amino acids during the Maillard reaction from precursors such as Strecker aldehydes that are either present or generated during processing.
Main Objectives: The objective of this study is to understand the kinetics of the chemical reactions, Maillard reaction and caramelisation, occurring in hazelnuts during roasting by multiresponse modeling.

Strategy and Methods: Hazelnuts were roasted at 150, 160, and 170°C for 15, 30, 60, 90, and 120 min. In order to monitor changes in concentrations of reactants and products, analysis of sucrose, fructose, glucose, 3-deoxyglucosone, 1-deoxyglucosone, methylglyoxal, glyoxal, 3,4-dideoxyglucosone, 5-hydroxymethylfurfural, dimethylglyoxal and amino acids were performed. To understand which of the reaction mechanism is mostly dominant during hazelnut roasting, a set of differential equations compiled and solved by numerical integration. Numerically solved equations were fitted to the experimental data obtained at different roasting temperatures. Numerical integration and determination of rate constants of the model was estimated by Athena Visual Studio software version 14.2.

Main Results: Maillard reaction, caramelisation and sucrose hydrolysis were considered as the main chemical reactions involving in hazelnut roasting. Sucrose hydrolyzed to glucose and fructose at all temperatures studied. However, there was no increase in glucose and fructose concentrations as Maillard reaction proceeds between these sugars and excess amino acids found in hazelnuts. Concentration of sucrose, glucose, fructose and amino acids were decreased during roasting at all temperatures while concentration of 3-deoxyglucosone, 1-deoxyglucosone, methylglyoxal, glyoxal, 3,4-dideoxyglucosone, 5-hydroxymethylfurfural, dimethylglyoxal increased. Fructose was found to play leading role in the proceeding of Maillard reaction and contributed most to the formation of 1-deoxyglucosone and 3-deoxyglucosone. The reason for that could be attributed to lower melting point of fructose compared to glucose. In the proposed roasting model, glucose also took part in Maillard reaction but after isomerization to fructose. Contribution of fructofuranosyl cation, that was known to form during sucrose degradation, was found to be more than the contribution of 3,4-dideoxyglucosone on the formation of 5-hydroxymethylfurfural.

Conclusions: Multiresponse modeling of reactions of foods is a challenging task as all the reactions are proceeding together and several other factors involves. In this study, statistically most possible pathways in sugar degradation and Maillard reaction, including alpha-dicarbonyl compounds formation, during hazelnut roasting at 150, 160, and 170°C at 15-120 min was proposed.
AMADORI PRODUCTS FORMATION IN EMULSIFIED SYSTEMS

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Main Objectives: The complex chemical network of Maillard reaction can lead to the formation of both potentially toxic molecules and desired aroma, flavor and color. Emulsified systems have been used by food scientists not only to deliver lipophilic bioactive components, but also to control their degradation and to improve their functional properties. The formation of Amadori products is the key step determining the fate of the reaction and it has been widely studied in water or in dry systems, however little information are available on the chemical behavior of amino acids and reducing sugars in emulsified systems during thermal treatments.

Strategy and Methods: In this work, a microemulsion consisting of water, glyceryl trioctanoate (10%) and Tween 20 (1%) was prepared by using a high pressure homogenizer. Two model systems were monitored: glucose was reacted at 140 °C for 2, 4, 6 and 8 min in presence of leucine and phenylalanine in emulsified systems or in aqueous system. Along with the thermodynamic parameters, the formation of \(N-(1\text{-Deoxy-D-fructos-1-yl})\text{-L-phenylalanine}\) and \(N-(1\text{-Deoxy-D-fructos-1-yl})\text{-L-leucine}\) and the degradation of the precursors was evaluated via liquid chromatography Orbitrap high resolution mass spectrometry (HRMS).

Main Results: Results revealed that there were only slight differences in the formation of \(N-(1\text{-Deoxy-D-fructos-1-yl})\text{-L-phenylalanine}\) in aqueous or dispersed system, while \(N-(1\text{-Deoxy-D-fructos-1-yl})\text{-L-leucine}\) was dramatically reduced up to 50% in presence of dispersed systems. The dimension of the droplets remained constant during the thermal treatment and the thermodynamic parameters were not significantly influenced.

Conclusions: The partition coefficient of amino acids, determining the reactants location is a prominent factor able to influence the final extent of the Maillard reaction. The investigation of Maillard reaction in emulsified system will further extend the possibility to elucidate its mechanism and to improve the quality of thermally treated emulsion containing foods and ingredients. The reaction mechanism proposed introduced new insights in the control of Maillard reaction end products formation by switching the reaction rates according to the reactants location in presence of emulsion.
Main Objectives: Although many researchers studied the anti-glycation activity of several compounds, there is a lack of appropriate approach to express the degree of inhibition taking into account the mechanism of glycation reaction. The objective of this study is to examine the compatibility of the enzyme inhibition kinetics in order to calculate the inhibitory activity of protein anti-glycation agents in the early stage of the Maillard reaction.

Strategy and Methods: We proposed that the kinetic data related to the glycation reaction and the effect of anti-glycation agents could be analyzed as in enzyme inhibition kinetics, because glycation is a site-specific reaction and inhibitors affect glycation rate through non-covalent interactions. In this respect, model systems composed of ovalbumin, glucose, and anti-glycation agent (tannic acid or calcium ion) at different molar ratios were heated at 90°C for different times in dry state or in solution. Control model systems were composed only of ovalbumin and glucose, and heated under the same conditions. Heated samples were analyzed for furosine, acid derivative of N-ε-fructoselysine (FL), to monitor the progression of early glycation stage.

Main Results: Comparing to control, presence of calcium ions and tannic acid decreased FL formation significantly (p<0.05) in the model systems during heating in dry state. Evaluation of the kinetic data by Lineweaver-Burk equation revealed that calcium inhibited glycation of ovalbumin by a mixed non-competitive mechanism in both dry and in solution conditions; while the mode of inhibition by tannic acid was found to be pure non-competitive in dry state.

Conclusions: This approach allowed us to evaluate the effects of calcium and tannic acid on the glycation potential of ovalbumin in early stage. It was possible to estimate the inhibitory activity of these anti-glycation agents comparatively having different modes of interaction with proteins. This kind of kinetic analysis is compatible for interpretation of any candidate agent for the inhibition of protein glycation reactions.
THE MAILLARD REACTION: A SOURCE FOR THE FORMATION OF DESIRED AROMA-ACTIVE COMPOUNDS AND UNDESIRED FOODBORNE TOXICANTS

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Main Objectives: During the Maillard reaction, both desired aroma-active compounds as well as undesired toxicologically relevant compounds might be formed. In the past, many research groups have focused either on the positive or on the negative substances, but there are only rare studies available combining these both aspects.

Strategy and Methods: Quantitation experiments of the aroma-active compounds as well as of the toxicants were based on stable isotopically labeled compounds used as internal standards (stable isotope dilution analysis) either for GC-MS or LC-MS/MS.

Main Results: The first part of the lecture will place emphasis on the simultaneous formation of aroma compounds, e.g., Strecker aldehydes and pyrazines, and of foodborne toxicants like acrylamide, furan, or “thermally formed” biogenic amines during heat-processing of food. The use of different labeled educts in combination with gas chromatography-mass spectrometry enabled the elucidation of formation pathways. With this information at hand, possible mitigation strategies for the toxicologically relevant compounds can be suggested. But, these recommendations have to come along with the maintenance of the desired aroma compounds to ensure the typical food flavor, which is expected by the consumers. Two studies dealing with potato chips and cocoa beans/chocolate will be explained in detail showing possibilities to reduce the formation of the undesired compounds by using specific processing conditions or the appropriate raw material (e.g., variety).

The second part of the lecture will highlight the proof of a novel class of Strecker aldehyde precursors, which are formed during Maillard reaction. These precursors (identified via syntheses of reference compounds and confirmed by MS and NMR experiments) are able to lead to a significant increase of Strecker aldehydes, e.g., 2- and 3-methylbutanal or phenylacetaldehyde, only by the addition of water to processed food. In addition, these precursors are also able to release the Strecker aldehydes during the preparation of food at home (e.g., by adding milk to cereals, water to cocoa or coffee powder, etc.) or during mastication.

Conclusions: In summary, knowledge about both desired and undesired compounds formed during the manufacturing process of food will offer new possibilities to produce foodstuff, which is healthier, but which still has the same sensory properties.
Oral Presentations
Background and main objectives: Inflammatory bowel diseases (IBD) are chronic and relapsing, life-long diseases of the gastrointestinal tract. Environmental factors which have a crucial role in the development of these diseases remain largely unknown. Few studies have described Advanced Glycation End-Products (AGE) as potential contributors of intestinal inflammation, and the role of AGE and their receptor RAGE in the pathophysiology of IBD are not yet fully elucidated. The global aim of our study was to address the link between AGE, RAGE, and IBD which might help in better understanding the role of nutrition, as a major source for AGE, in the pathophysiology of IBD.

Strategy and Methods: Three different models of intestinal and colonic inflammation (indomethacin, dextran sodium sulfate (DSS) and trinitro benzene sulfonic acid (TNBS)) were induced in C57BL/6 wild type (WT) and RAGE null mice. In a second set of experiments, mice were orally administered with the food derived ligand for RAGE, carboxymethyllysine-bovine serum albumin (CML-BSA) or control BSA for 30 days; then, intestinal and colonic inflammation were induced. Severity of inflammation was evaluated using macroscopic, histologic and molecular parameters in the small intestine and colon of mice.

Main Results: Following indomethacin administration, a significant decrease in ulcerations number and area was observed in the duodenum, jejunum and ileum of RAGE null mice compared to WT mice. Consistently, IL1β mRNA levels were significantly decreased in the three intestinal segments. RAGE null mice were protected from DSS- and TNBS-induced colitis, with a significant decrease of clinical and macroscopic parameters. Myeloperoxidase (MPO) activity, reflecting the neutrophil infiltration, was also significantly reduced in the colon of TNBS- treated RAGE null mice compared to TNBS- treated WT mice. IL1β and iNOS mRNA expression were significantly decreased in colitic RAGE null mice compared to colitic WT mice. Chronic BSA-CML administration to mice worsened indomethacin-induced enteritis, as evidenced by a significant increase in ulcerations number and area in the duodenum, jejunum and ileum compared to control BSA-treated mice. Consistently, MPO activity and oxidative stress assessed by anion superoxide dosage were significantly increased in the ileum of CML BSA-treated mice compared to control BSA-treated mice. Chronic CML-BSA administration did not induce any effect on colonic inflammation.

Conclusions: We demonstrated that RAGE signaling pathway is implicated in intestinal and colonic inflammation in mice. We showed that BSA-CML might be a dietary factor involved in intestinal inflammation. The role of RAGE and AGE in IBD now merits further investigations.
Main Objectives: A strong body of evidence supports the role of environmental influence in the development of Inflammatory Bowel Diseases (IBD). The increase in incidence of IBD during the 50’s in Europe and United States and since about 10 years in developing countries parallels the modification of alimentary habits and in particular, the consumption of refined food. Acrylamide is a chemical compound considered a potential carcinogen in humans. Acrylamide is produced naturally in food as a result of cooking starch rich food at high temperature (>120°C, baking, frying or grilling). High amounts of acrylamide are present in Western diet (in potato chips, pizza, French fries or pastries) with unknown effects on intestinal inflammation.

Strategy and Methods: Our aim was to determine the effects of acrylamide on intestinal homeostasis in mice. C57bl6 mice were given increasing doses of acrylamide in their drinking water (25; 50 and 100µg/kg of body weight/day) for 9 months, control mice receiving water only. Colon was then harvested and assessed for macroscopic, structural and histological modifications. Markers of intestinal barrier, inflammatory and immune responses were also quantified.

Main Results: No macroscopic lesions were observed in mice receiving acrylamide. However, the 3 doses of acrylamide induced structural abnormalities of the colon with a modification of the intestinal permeability compared to control mice. Crypts depth (148µm for control mice, 215µm for 25µg of acrylamide, and 211µm for 50µg, p=0.0317), number of goblet cells per crypt (respectively x3 for 25µg and 50µg, and x2 for 100µg, p=0.0195) and Muc 2 expression (+75% for 100µg, p=0.04) were higher in mice receiving acrylamide. Acrylamide also induced expression of inflammatory markers; a significant increase of myeloperoxidase activity (+48%, p=0.03 for 50µg, and +50%, p=0.04 for 100µg), and oxidative stress (+103% for 50µg, p=0.03 and +52% for 100µg, p=0.009 for iNOS expression, and +32% for 25µg, p=0.04 and +37% for 100µg, p=0.009 for NADPH oxydase expression) were observed in mice receiving acrylamide compared to control mice. The immune response was also disturbed; with an increased expression of Th1 cytokines (+57% for 50µg, p=0.02, for TNF expression, and +477%, p=0.009, +443%, p=0.01, and +408%, p=0.009, for 25µg, 50µg and 100µg of acrylamide respectively for IFNg expression), Th2 cytokines (+291%, p=0.02, +81%, p=0.03, and +82%, p=0.03 for 25µg, 50µg et 100µg respectively for IL-4 expression) and a decreased expression of Th17 cytokines (-24% for 100µg, p=0.02 for IL17f) in mice receiving acrylamide compared to control mice.

Conclusions: At low doses, acrylamide disturbed intestinal homeostasis with architectural modifications, altered permeability, increased inflammation and mucosal immune response. More studies are now needed to evaluate the role of acrylamide in IBD.
**OP-3**

**POSSIBLE ROLE OF GLYCATION IN SKIN AGING, INFLAMMAGING AND PHOTOAGING AS EVIDENCED BY A RECONSTRUCTED SKIN MODEL**

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**Main Objectives:** Glycation is a slow chemical reaction which takes place between amino groups in proteins and a reducing sugar. In skin, this reaction creates new residues or induces the formation of cross-links (Advanced Glycation End Products or AGEs) in the extracellular matrix of the dermis. Such cross-linking between macromolecules may be responsible for loss of elasticity or alterations of other properties of dermis observed along aging. In order to reproduce this phenomenon in vitro, a model of reconstructed skin was created using collagen modified by glycation to construct the dermal compartment.

**Strategy and Methods:** The effect of glycation upon skin homeostasis was investigated using the reconstructed skin model with glycated collagen. Collagen glycation was induced with sugar (ribose) or through specific chemical modification for reconstructed skin containing N epsilon-(carboxymethyl) lysine (CML) or pentosidine within the dermal part. Dermal equivalents were prepared using human dermal fibroblasts with or without CD45+ cells of the monocyte lineage (from peripheric blood) embedded into a glycated collagen gel. Epidermis was obtained by seeding human epidermal keratinocytes on the dermal equivalents. The cultures were kept submerged during one week and then raised at the air-liquid interface for one more week. At that time, some samples were exposed to UVA rays (10 J/cm²) to evaluate the UV effect. qRT-PCR for mRNA extracted from dermal cells and Elisa or immunostaining were performed to analyze the different conditions.

**Main Results:** This model allowed us to uncover biological alterations of dermal markers (type I pro-collagen, type IV collagen were increased) and an increase in metalloproteinases (MMP1, MMP2 and MMP9) responsible for degradation of the dermal matrix. Epidermal markers (α6 and β1 integrins sub-units) were also enhanced. Consequently the imbalance between synthesis and degradation that results from glycation, as shown in this model, may contribute to skin aging (1). A more detailed investigation also showed that: i) some specific AGEs may play a more important role than others in skin aging (example with CML and pentosidine which have opposite effects) (2), ii) when hematopoietic precursors were incorporated into the modified collagen, AGEs enhanced the differentiation of these cells into dendritic cells and macrophages (DC-SIGN, BDC1A, CD163 positive cells) to create a potentially inflammatory environment (“inflammaging”), iii) when the reconstructed skin was exposed to UV for more closely mimicking the actual situation of skin aging, the results suggest a possible involvement of glycation in the first steps of solar elastosis (mRNA coding for MMP1, MMP3, tropo-elastin, elastase and MMP12 was up-regulated).

**Conclusion:** Using the reconstructed skin model, our results taken altogether stress the importance and, possibly, a central role of glycation not only in global skin aging but also its implication in inflammaging and photo-aging that lead to several aspects of skin aging. Such reconstructed skin model appears useful as possibly integrating various factors linked to skin aging.

**Keywords:** Skin aging, glycation, CML, pentosidine, elastosis, chronicologic aging, photo-aging.


HIGH DIETARY INTAKE OF ADVANCED GLYcation END PRODUCTS DURING PREGNANCY AND LACTATION PRODUCES INSULIN SECRETORY DEFECTS IN OFFSPRING OF NONOBESE DIABETIC 8.3 TCR TRANSGENIC MICE.

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Main Objectives: Western diets contain high levels of modified proteins such as advanced glycation end products (AGEs). AGEs can be maternally transmitted in utero and to newborns and have been associated with type 1 diabetes progression in autoantibody positive children. We aimed to examine the carry over effect of lowering AGE content during pregnancy and lactation in NOD8.3 IGRP206-214 TCR transgenic offspring.

Strategy and Methods: NOD8.3 IGRP206-214 males were mated with NODShiLt females and fed either a diet low (AIN93G) or high (baked AIN93G) in AGE content during mating, pregnancy, and lactation. Weaned NOD8.3+ female pups were provided the same diet as their parents and followed to day 28 of life.

Main Results: NOD8.3 offspring fed a high AGE diet showed no difference in body weight (11.2 ± 1.6 vs 12.6 ± 3.1) or fasting blood glucose (7.9 ± 1.7 vs 8.1 ± 1.3). Immunohistochemistry revealed a high AGE diet reduced insulin content (10.8 ± 1.3 vs 38.3 ± 2.7; p<0.0001) and the AGE-receptor RAGE (7.9 ± 1.2 vs 12.5 ± 1.0; p=0.01), in comparison to a low AGE intake. Basal (2.8 ± 1.6 vs 10.3 ± 3.0; p=0.002) and glucose stimulated (2.1 ± 0.6 vs 8.6 ± 4.0; p=0.004) insulin secretion were reduced in isolated islets after high AGE feeding compared to a low AGE diet. Flow cytometry analysis indicated a reduction in total CD4+ T cells after high AGE feeding in the spleen (5.1 ± 1.5% vs 2.7 ± 0.9%; p=0.03) and pancreatic lymph node (7.6 ± 1.2% vs 6.2 ± 0.7%; p=0.03).

Conclusions: These results demonstrate improved insulin secretion in offspring exposed to low dietary AGE conditions. AGE levels may modulate adaptive immunity however further investigations are still required.
Main Objectives: Advanced Glycation Endproducts with an amide structure (Amide-AGEs) are formed within the non-enzymatic degradation of reducing sugars. This novel class of AGEs was verified in in vitro incubations, in biological samples and in foods, and compared to the formation of established AGEs.

Strategy and Methods: Insights to mechanistic aspects were drawn from incubations with 13C-isotopically labelled sugars in presence or absence of 18O2 atmosphere. An efficient enzymatic work-up was developed for the detection in protein isolates from human lens and from selected food samples. Quantitation was done by a highly sensitive multimethod based on coupled liquid chromatography mass spectrometry.

Main Results: The formation of amide-AGEs is related to the fragmentation of dicarbonyl compounds. These are reactive intermediates within the complex reaction cascades of the Maillard reaction. Specifically, oxidative α-dicarbonyl cleavage and hydrolytic β-dicarbonyl cleavage lead to carbonyl and carboxyl follow-up products. While low molecular weight carbonyl fragments are prone to further degradation, the resulting carboxylic acids are stable endproducts. In incubations of ascorbic acid at 37°C and pH 7.4 more than 75% of the Maillard induced degradation can be explained by above mechanisms. In presence of amines the very same mechanisms lead to stable amide modifications. In addition, some structures stem from isomerization as an alternative formation pathway. The formation of amides from formic, glycolic, acetic, oxalic and lactic acid and from acids with a C4 and C5 carbon backbone was verified based on authentic reference compounds. The structures were identified in free form in human plasma, in protein bound form in plasma and lens proteins with N6-lactoyl, N6-formyl and N6-acetyl lysine amide within the same range as established AGEs as N6-carboxymethyl lysine (CML). In chocolate, N6-formyl and N6-acetyl lysine were the most relevant structures beside CML.

Conclusions: Amides are quantitatively important AGE structures. Selected compounds are formed within the range of established AGEs. As their non-enzymatic mechanism is unique they can be used to dissect pathological issues relevant in vivo, but also technological questions relevant in food industry.
OP-6  A PROPOSAL OF A BROWNING MECHANISM VIA 4-HYDROXY-5-METHYL-3(2H)-FURANONE (HMFO) IN XYLOSE-LYSINE MAILLARD REACTION SYSTEM

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Main Objectives: During the research of the comparison of Maillard reaction systems between xylose and glucose, we found that a major peak showing an absorption maximum at 285 nm appeared on HPLC analysis of xylose and lysine system. The aim of this study was to identify this compound and clarify the role of this compound on the browning.

Strategy and Methods: A solution containing 0.3 M L-Cys, 0.3 M L-Lys, and 0.2 M phosphate buffer (pH 7) was heated at 100°C for 60 min. After the solution was extracted with EtOAc, the extract was concentrated. As a result, colorless crystal was obtained. A solution containing 1.8 mM HMFO, 3.4 mM L-Lys, and 0.2 M phosphate buffer (pH 6-8) was heated at 100°C for 0-6 h. The reaction solution was analysed with GPC equipped with a diode-array detector. Dicarbonyl compounds formed in the reaction solution were trapped with o-phenylenediamine, before the quinoxaline derivatives were analysed with HPLC.

Main Results: The major Maillard product between xylose and lysine showing the absorption maximum at 285 nm was obtained as a colorless crystal and identified as HMFO by such instrumental analyses as NMR, MS and X-ray crystallography. This compound occupied HMFO accounted for 60-80% of the total area of HPLC peaks (280 nm), and about 20 mg/100 ml of HMFO was produced from a solution containing 200 mg/100ml of xylose at pH 6.5. The decomposition and browning of HMFO were observed at the process of concentration during the purification procedure of HMFO from the reaction mixture. The concentration of HMFO reached the maximum after heating for 1 h at pH 7.0 and then decreased. This result suggested HMFO was decomposed or polymerized during heating. HMFO was produced not only from xylose, but also from arabinose and ribose, and ribose was the best precursor. When HMFO was heated in a buffer solution with or without Lys, colored and colorless polymers were appeared. Methylglyoxal and diacetyl were major decomposed dicarbonyl compounds from HMFO, these dicarbonyl compounds being considered to the major precursors for polymers.

Conclusions: We identified HMFO as a major intermediate compound in a reaction system containing xylose and lysine. HMFO was decomposed into dicarbonyl compounds, being polymerized to form brown pigments.
MODEL OPTIMIZATION AND ANTIOXIDANT ACTIVITY OF BLUE MAILLARD REACTION PRODUCTS

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Main Objectives: Blue colour can be formed in the xylose (Xyl) and glycine (Gly) Maillard reaction (MR) model system. However, there are fewer studies on the reaction conditions for the blue Maillard reaction products (MRPs). The objective of this study is to investigate characteristic colour formation and antioxidant activities in four different MR model systems and to determine the optimum reaction conditions for the blue colour formation in Xyl-Gly MR model system, using the Random Centroid Optimization program.

Strategy and Methods: Random Centroid Optimization (RCO) program, a sequential optimization technology by repeating a three-stage search cycle consisting of random search, centroid search and mapping, is designed for optimization of chosen parameters and give an optimum range without requirement for extreme accurate points. We applied RCO in optimizing the reaction conditions for blue colour formation in the Xyl-Gly MR model system, with consideration on variable factors of molar ratio of xylose to glycine, NaHCO3 concentration, ethanol concentration, initial pH, temperature and heating time.

Main Results: The blue colour with an absorbance peak at 630 nm appeared before browning in Xyl-Gly MR model system, while no blue colour formation but only browning was observed in xylose-alanine, xylose-aspartic acid and glucose-glycine MR model systems. Xyl-Gly MR model system showed also higher antioxidant activity than other three model systems. The optimum conditions for blue colour formation were as follows: xylose and glycine ratio 1: 0.16 (M:M), 0.20 M NaHCO3, 406.1 mL L⁻¹ ethanol, initial pH 8.63, 33.7°C for 22.06 h, which gave much brighter blue colour and higher peak at 630 nm.

Conclusions: Characteristic blue colour could be formed in Xyl-Gly MR model system and optimum condition for the blue colour formation was proposed and confirmed.
OP-8  THE POTENTIAL OF CARBOHYDRATES AND CORRESPONDING EARLY GLYCATED PEPTIDES FOR THE FORMATION OF ADVANCED GLYCATION END-PRODUCTS

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Main Objectives: Protein glycation is a non-enzymatic post-translational modification formed by reaction of reducing sugars with N-terminal and lysyl amino groups. Resulting Amadori compounds readily oxidize ('glycoxidation'), yielding advanced glycation end-products (AGEs), a heterogeneous group of compounds demonstrating a clear pro-inflammatory effect in mammals. Alternatively, carbohydrates can be involved in oxidative degradation and formation of α-dicarbonyls, highly-reactive towards lysyl and arginyl residues and also yielding AGEs ('autoxidative glycosylation'). Despite the pronounced physiological effects of AGEs, the potential of individual carbohydrates for their formation is still not estimated. Moreover, the mechanisms of advanced glycation under elevated temperatures are only partly characterized. Here, we characterize the glycation potentials of individual sugars of different origin and characterize the impact of free carbohydrate and its glycation product in AGE formation under the conditions stimulating cooking process.

Strategy and Methods: The in vitro sugar reactivities were estimated at 95 °C with model peptides using equimolar amounts of carbohydrates. The mechanistic studies were performed with the glycated peptide Ac-AKAmadoriASASFL-NH2 and its isotopically labeled (13C6,15N1-Leu) unglycated counterpart in presence of 13C6-D-glucose. The incubated mixtures were analyzed by UPLC-ESI-QqTOF-MS and differentially abundant products were characterized by MS/MS and quantified on the relative basis. The sugar-related products were identified and quantified by GC-EI-MS.

Main Results: Dihydroxyacetone-phosphate, glyceraldehyde-3-phosphate and fructose were (in terms of starting material consumption and product spectrum) the most reactive carbohydrates. Less reactive sugars (sugar mono phosphates, ribulose-1,5-bisphosphate, raffinose, maltose and lactose) formed specific products. Mechanistic studies revealed 25 peptide products, including six truncated sequences (two of which with modified residues), five oxidatively modified species (allysine, ortho-, meta- and para-tyrosine and an oxidative crosslink), 11 products with sugar-related mass increments (including carboxymethyl, formyl, pyrraline-related structures) and three compounds containing several modified residues. The analytes could be distributed to several groups: (i) those showing similar kinetics for “light” and “heavy” peptides in both pathways (α-amino semiadipic aldehyde-containing product and peptide fragments), (ii) analytes formed mostly via “glycoxidation”, i.e. Amadori degradation (carboxymethylated peptides), or (iii) “autoxidative glycosylation” (pyrraline and a truncated form of it), and (iv) those formed via both pathways but without clear preferences (peptides containing methylated and formylated lysine). Thus, we could assign formation pathways for Nɛ-carboxymethyllysine (CML) and pyrraline, to the best of our knowledge, for the first time.

Conclusions: The sugar reactivity patterns and glycation pathways were characterized. Individual AGEs were formed via specific (CML and pyrraline) and unspecific (formyl- and methyl lysine) mechanisms. CML and pyrraline were assigned to specific formation pathways at 95 °C (‘glycoxidation’ and ‘autoxidative glycosylation’, respectively) for the first time.

Keywords: AGEs, carbohydrates, glycation, mechanisms, pathways, tandem mass spectrometry
RAGE: A NEW FRONTIER IN INTESTINAL FIBROSIS

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Background: Intestinal fibrosis is a common and severe compliance of inflammatory bowel disease (IBD) characterized by excessive deposition of extracellular matrix components (ECM) and for which efficient and well-tolerated therapies are currently lacking. Inflamed colonic mucosa of patients with active IBD show a significant increase of the receptor for advanced glycation end products (RAGE), able to regulate chronic inflammation by activating the NF-κB pathway. In addition, a growing body of evidence in kidney, liver and lung fibrosis shows how the increase of myofibroblast activation and proliferation and the consequent ECM accumulation are regulated by RAGE. Our aim was to determine the role of RAGE in intestinal fibrogenesis.

Strategy and Methods: Fibrosis was induced in C57BL/6 wild type (WT) and RAGE null mice by administration of 2.5% (w/v) dextran sulfate sodium (DSS) in drinking water for 5 days followed by 7 days of water, for three cycles. Three days after the last cycle, the entire colon was excised and scored for the assessment of macroscopic lesions, including dilation, thickness and adhesion, on a 0-3 scale. Distal colon specimens were subject to Hematoxylin/Eosin staining, to assess the degree of inflammation, and Picrosirius red staining to assess collagen deposition. A total microscopic score was calculated according to the presence of ulceration, inflammatory degree, depth of lesions and fibrotic degree. mRNA expression of the main profibrotic mediator, Tgf-β1, and the expression of ECM components, mainly collagen types I-III (Col1A1 gene) and fibronectin (FN-1 gene), were evaluated by quantitative RT-PCR.

Main Results: Compared to WT mice, DSS-treated C57/Bl6 RAGE null mice showed a significant decrease of the colon weight/length ratio (29%, p<0.0001), an indicator of wall thickening. In RAGE null mice, the macroscopic score was significantly reduced compared to WT mice (6±0.92 vs 1.43±0.54, p<0.0001). DSS-treated RAGE null mice showed also a significant decrease of total microscopic score compared to WT mice (49%, p<0.001). Tgf-β1 mRNA expression was significantly increased by the DSS administration in WT mice colon, whereas it was unchanged in RAGE null mice. Col1A1 and FN-1 genes were upregulated in DSS-treated WT mice (5.52 fold, p= 0.0137 and 53 folds, p= 0.0016, respectively). Upregulation of these genes by DSS treatment was decreased in RAGE KO mice for Col1A1 (3.17 fold, p= 0.0341) and totally prevented for FN-1.

Conclusions: This study represents a first insight into the involvement of RAGE in the development of DSS-induced intestinal fibrosis in mice. The potential profibrotic role of RAGE in IBD could both shed light into the complex and dynamic fibrogenic processes in IBD and pave the way for new anti-fibrotic agents for this disease.
OP-10 OVEREXPRESSION OF THE AGE DETOXIFICATION RECEPTOR AGER1 RESULTS IN HEPATIC FIBROSIS AND HEPATIC INSULIN RESISTANCE

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Main Objectives: Advanced glycation end-products (AGEs) are known to contribute to diabetic-related complications and high serum levels of AGEs have been associated in patients whom have developed non-alcoholic steatohepatitis (NASH). Moreover a diet constituting only a higher level of AGEs can lead to the development of hepatic inflammation and fibrosis, thereby contributing to the disease progression of NASH. AGE receptor-1 (AGER1) is additional thought to be involved in the detoxification and clearance of AGES.

Strategy and Methods: C57/Bl6 mice were genetically modified via the Cre-loxP recombination system with a ubiquitous genetic knock-in of the human gene encoding AGER1 (DDOST) in the ROSA26 locus. Male heterozygous global AGER1 knock-in mice (AGER1) and littermate controls (WT) (N=7-8/group) were randomised to receive either AIN-93G (low AGE content diet) or baked AIN-93G (high AGE content diet; which contained a 5-fold higher AGE content of CML, CEL and MGO) and fed for 24 weeks. Metabolic function was determined by insulin and glucose tolerance testing, Echo-Magnetic Resonance Imaging (Echo-MRI) determined body composition and CLAMS (Columbus Instruments Comprehensive Lab Animal Monitoring Systems) measured calorimetry, activity, body core temperature and respirometry over a 24 hour day cycle. Serum and liver tissue was subsequently collected; proteomics was conducted by SWATH-MS analysis and metabolomics by HPLC. Biochemical and histological analysis identified changes in lipid accumulation and fibrosis in the liver.

Main Results: Here we show that mice with fed a macro- and micro-nutrient equivalent diet of only lowered AGE content slowed the progression of hepatic fibrosis, however over-expressing AGER1 in mice did not lead to the protective pathways involving detoxification and clearance of AGEs. Rather we observed hepatomegaly and increased omental fat deposition, coupled with increased utilization of fats and ketones rather than glucose. Moreover mice fed a diet of high AGE content exhibited an absence of fatty liver despite increased liver fibrosis.

Conclusions: A diet constituent of a high AGE content results in hepatic fibrosis which was exacerbated by an upregulation of AGER1. Hepatic fibrosis appeared to be a consequence of hepatic insulin resistance and cellular starvation.
Main Objectives: Diabetes is characterized by activation of toxic oxidative and glycoxidative pathways that are triggered by persistent hyperglycemia and contribute to diabetic complications. One of the major proposed pathogenic mechanisms is accumulation of protein modifications called advanced glycation end products (AGEs). However, other non-enzymatic post-translational protein modifications may also contribute to pathogenic protein damage in diabetes. One potential source of these modifications is hypohalous acids.

Strategy and Methods: Site-specific protein modifications derived from hypochlorous (HOCl) and hypobromous (HOBr) acids were analyzed in NC1 domains of collagen IV isolated from kidneys of control and diabetic animals using liquid chromatography-tandem mass spectrometry. The impact of these modifications on structure and function of collagen IV was determined using molecular dynamics simulations, limited proteolysis, denaturation/refolding, and solid phase ligand binding techniques.

Main Results: We report, for the first time, the increased levels of HOCl-derived hydroxylation and chlorination of tryptophan residues of renal collagen IV in experimental diabetes. These modifications specifically affected two residues, W28 and W192, within NC1 domain of collagen IV but not the other tryptophan or tyrosine residues. The levels of these modifications were comparable to the AGE levels reported in diabetic extracellular matrix (ECM). One particular modification, chlorotryptophan, has not been previously found in proteins. Molecular dynamics simulations including oxidized and chlorinated W28 and W192 residues predicted more relaxed NC1 hexamer tertiary structure and diminished assembly competence in diabetes. These predictions were confirmed experimentally using limited proteolysis and denaturation/refolding techniques. We further demonstrate that diabetic renal ECM exhibits diminished binding of α1β1 integrin consistent with modification of collagen IV by HOCl and HOBr. Finally, we show that structural and functional damage to collagen IV networks can be ameliorated by scavenging hypohalous acids in vitro and in vivo.

Conclusions: Our results suggest that hypohalous acid-derived modifications of renal ECM and specifically collagen IV networks contribute to functional damage in diabetes. Our findings are in agreement with the existence of the “hot spot” residues that accumulate the bulk of diabetic non-enzymatic protein modifications. Also, our results question a canonical view of a more rigid and proteolysis-resistant diabetic ECM.
A NOVEL IN VIVO APPROACH TO STUDY ENDOPLASMIC RETICULUM STRESS INDUCTION BY ADVANCED GLYCATION END PRODUCTS

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Main Objectives: Advanced glycation end products (AGEs) are modifications of proteins or lipids that cause molecular rearrangements, contributing to cell injury, inflammatory and cardiovascular diseases. AGEs have recently been associated with endoplasmic reticulum (ER) stress. Unfolded protein response (UPR) is a normal physiological reaction of a cell in order to prevent accumulation of unfolded and misfolded proteins in the ER and improve the normal ER function. However, ER function becomes impaired in pathological conditions, leading to the development of ER stress. We sought to explore the potential involvement of AGEs in the induction of ER stress-associated apoptosis in vivo.

Strategy and Methods: We used C57BL/6 mice and Chop knockout mice, 4-5 weeks old, which were provided for a month with a low- or high-AGE content diet and free access to water. At the end of one month several organs were removed and assessment of mRNA and protein levels of established UPR activators were performed. Fibroblasts were isolated from wild-type and Chop-deficient animals and exposed to AGE-bovine serum albumin (AGE-BSA) to confirm the induction of ER stress.

Main Results: Chop-deficient mice and wild-type controls fed either with high- or low-AGE diet were investigated for the expression of UPR chaperones, ER stress markers BiP, GRP94 and Chop. Wild-type mice were more sensitive to experimental diet, suggesting the stimulation of the UPR. In various organs such as the lungs, heart, liver, spleen and pancreas the levels of BiP and GRP94 chaperones were effectively induced. Chop knockout mice were more resistant to ER stress than their wild-type counterparts. Similar results were also obtained by in vitro experiments involving mouse embryonic fibroblasts (MEFs). Consistently with these findings MEFs were more sensitive to administration of AGEs than the Chop-deficient cells, but their sensitivity was restored by knocking down Chop expression by siRNA. Thus, siRNA-mediated inhibition of Chop levels inverted the sensitive to AGE-BSA wild-type cells.

Conclusions: Our findings indicate that AGEs induce ER stress and stimulate the modulators of the UPR. Accordingly, our study provides hints for understanding the role of AGEs in ER stress-mediated apoptosis in-as-much-as malfunction of this homeostatic mechanism has been implicated in a variety of common diseases such as diabetes and atherosclerosis.

*These authors contributed equally to this work
Background: Advanced Glycation Endproducts (AGEs) contribute to the development of diabetes complications and more recently it has been hypothesised that AGEs may play a major role in the pathogenesis of type 2 diabetes. AGEs are formed endogenously but also enter the body during cigarette smoking and the consumption of AGEs in heat-processed food. The fluorescent nature of many AGEs enables their long-term accumulation within body tissues to be detected due to their absorption of UV light at a specific wavelength. An AGE Reader non-invasively measures skin autofluorescence (SAF), which correlates to actual tissue AGE accumulation in skin biopsies from both healthy subjects and patients with diabetes. Identification of habitual dietary and lifestyle factors which influence tissue AGE levels may provide information about simple lifestyle modifications which could potentially prevent or slow the development of type 2 diabetes in susceptible individuals.

Objective: To identify dietary and lifestyle behaviours associated with SAF in a cross-sectional population sample.

Methods: 250 adult volunteers (mean age: 47±16 years) completed validated food frequency (EPIC-FFQ) and physical activity (International Physical Activity Questionnaire-Short Form) questionnaires, and a general health survey. Participant BMI, waist circumference, blood pressure and random blood glucose was also measured. SAF was measured by an AGE Reader (Diagnoptics, Netherlands). A Dietary AGE Score was calculated for each participant based on their consumption of high-AGE foods and use of cooking methods which promote AGE formation. Correlation analysis and backwards stepwise linear regression were used to identify significant predictors of SAF.

Results: Significant positive correlations were found between SAF and age, body weight, waist circumference, BMI and random blood glucose. Cigarette smokers also had a significantly higher SAF than non-smokers (2.4 U vs 2.0 U respectively, P<0.05). No correlations were found between SAF and any dietary or physical activity variables. Regression analysis identified age (beta coefficient: 0.019, P<0.001, 95%CI:0.014-0.025), cigarette smoking (beta coefficient: 0.4, P=0.001, 95%CI:0.11-0.61) and waist circumference (beta coefficient: 0.008, P<0.01, 95%CI:0.002-0.013) as the only significant predictors of SAF. Age, cigarette smoking and waist circumference explained 46% of the variation in SAF.

Conclusion: Age, cigarette smoking and waist circumference were independent predictors of SAF in this adult sample. Tissue accumulation of fluorescent AGEs was not associated with habitual physical activity or dietary intake of any macronutrients, micronutrients or AGEs in food. This may indicate that healthy adults have sufficient detoxification and/or excretion systems to prevent dietary AGEs and their precursors from accumulating in body tissues.
**Main Objectives:** Gut microbiota is a very complex ecological community that contributes to host health and particularly to digestion, protection and gut maturation. When disruption of the intestinal ecosystem equilibrium appears (dysbiosis), an expansion of some subdominant commensal bacteria like *Enterobacteriaceae* can occur. This increase is generally associated with an inflammation due to an excessive exposition to endotoxins. The aim of this work was to assess the impact of bread Maillard reaction products (MRPs) on microorganisms and particularly *Enterobacteriaceae* in various systems.

**Strategy and Methods:** Multiple experiments were carried out using *in vitro* and *in vivo* systems. Batch fermentations were made using culture media enriched with bread crust (rich in MRPs and melanoidins) and bread crumb (poor in MRPs and exempt of melanoidins). Pure enterobacteria strains as well as fecal material from healthy individuals were used to inoculate these fermentation media. *In vivo* models consisted of 4 weeks old male Sprague Dawley rats fed diets enriched with bread crust and bread crumb over a period of 30 days. Cecal contents were collected and bacterial counts were followed by plate counting and qPCR.

**Main Results:** Cultures of pure *E. coli* strains showed a strain-dependent effect in the presence of MRPs. Some strains had increased lag phases and decreased growth rates in the presence of these MRPs, while other strains were not affected. In the case of the fecal material fermentation, *Enterobacteriaceae* counts drastically decreased for all individuals after 2 days of fermentation in the presence of bread MRPs. However, this population was not affected for the control batches. In the case of the *in vivo* models, *Enterobacteriaceae* counts remained unaffected for the rats fed both types of diets rich or poor in bread MRPs.

**Conclusions:** Bread MRPs can affect gut microbiota and particularly *Enterobacteriaceae*. However, the decrease of this pro-inflammatory population in the presence of bread MRPs is of interest for the study of foodborne anti-inflammatory components. Further work is being undertaken for the study of the impact of these MRPs on *Enterobacteriaceae* in inflammatory models, as well as their impact on more advantageous floras such as *Bifidobacterium*. 
Neo-formed contaminants (NFCs) are toxic compounds formed during heating processes that exhibit potential harmful effects to humans. Among the several NFCs described in literature furan is mainly formed through Maillard Reaction (MR) in starchy based potato and cereal products during high thermal load unit operations such as frying and baking. Furan (C$_4$H$_4$O) is a small organic compound (Mw: 68 g mol$^{-1}$) with high volatility (boiling point: 31°C) and lipophilicity. The presence of furan in a broad range of heat processed foods (0–6000 µg kg$^{-1}$) has received considerable attention due to the fact that this heat induced contaminant is considered as a “possible carcinogenic compound to humans”. However, despite its high volatility, furan has also been found in low-moisture foods processed in open containers, such as potato chips, crackers, crisp breads, and toasted breads. MR could be not only the main responsible of furan formation in starchy foods, but also the main responsible of the development of attractive sensory attributes of this kind of heated foods. In this sense, the final color of heated potato derived products is the result of the MR that depends on the content of reducing sugars and amino acids or proteins at the surface and the temperature and time of the high temperature thermal operation.

Currently the costs of furan analysis are quite prohibitive and time consuming. Up to now, gas chromatography (GC) coupled to mass spectrometry (MS) is the most useful technique for furan determination in different food matrices. Although this method performs well for quality control purposes in a food analysis laboratory, it is laborious, costly, and cannot be adopted for process control purposes by the food industry. On the other hand, computer vision is playing an increasingly important role in automated visual food inspection for quality assurance issues. In the last years, computer vision applied to food quality evaluation have been concentrated on using or developing tailored made methods based on visual features that are able to solve a specific task. The general objective of this research was to assess the capability of predicting the furan content in starchy fried food models by using computer vision techniques.

For this mean, samples of dough circular pieces composed of wheat flour and water were deep-fat fried at different temperature-time combinations: 5 times (5, 7, 9, 11 and 13 min) and 5 constant temperatures (150, 160, 170, 180 and 190°C). Color images from the resulting samples were acquired in standard conditions in order to extract their textural, geometric and chromatic features (2915 totally). Simultaneously, corresponding samples were analyzed to determine their furan content by GC-MS. For improving furan predictive models, three strategies were tested: (i) Furan prediction using whole data set; (ii) Furan prediction selecting best features with genetic algorithms (GA); (iii) Furan prediction selecting the best features with interval partial least square regression (iPLS).

Predictive models were evaluated by comparison of correlation coefficient of prediction (Rp), root mean squared error of cross validation (RMSECV) and the ratio of standard error of prediction to sample standard deviation (RPD). Generally, the best Rp was 0.89 for furan after (ii). RMSECV for (ii), decreased averagely by 45%, compared to (i), while (iii) only decreased 23 % regarding to (i). In conclusion, RPD values for iPLS reached 2.2 meaning that is feasible to implement color image processing technique for sorting fried foods for furan using appropriate image features.

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**Main Objectives:** It is thought that over-consumption of food high in Advanced glycation end-products (AGEs) may activate pathways involved in chronic disease progression thereby exacerbating pre-existing pathology. The majority of diet-derived AGEs escape digestion and reach the colon, and previous studies suggest that dietary AGEs can modulate the gut microbiota, though a comprehensive profiling of gut microbiota using metagenomics has not been previously performed. The aim of this study was to characterise the effects of dietary AGEs on gut microbiota profile and inflammation.

**Strategy and Methods:** C57BL6/J mice (n=10/group) were randomised to receive a diet low in AGEs (unbaked rodent chow, AIN93G) or a diet high in AGEs (baked AIN93G rodent chow, 160 degrees C for 1h, resulting in a 5-fold higher AGE content) for 24 weeks. 16S rRNA sequencing was used to profile the gut microbiome. Inflammation was determined by plasma monocyte chemoattractant protein (MCP)-1 measured by ELISA. Bacterial translocation to the circulation was measured by plasma lipopolysaccharide (LPS) using the Limulus Amebocyte Lysate (LAL) assay. Tight junction proteins ZO-1 and occludin were determined in the ileum and jejunum by qPCR.

**Main Results:** Chronic consumption of excess dietary AGEs by healthy mice led to an increase in cecal bacterial diversity compared to the low AGE diet. Analysis of the operational taxonomic unit (OTU) at the family level showed an increase in Bacteroidaceae and Helicobacteraceae and a decrease in Lachnospiraceae and Saccharibacteria. There was also a decrease in Akkermansia muciniphila species and genus Ruminococcus. Plasma MCP-1 and LPS were increased after high AGE feeding. ZO-1 and occludin gene expression were downregulated in ileum and jejunum respectively.

**Conclusions:** These novel data indicate that excess dietary intake of AGEs alters the gut microbiome, induces intestinal permeability and bacterial translocation to the circulation, supporting the notion that diet-derived AGEs can promote inflammation.
MS BASED QUANTIFICATION OF INDIVIDUAL GLYcation SITES IN PLASMA PROTEINS AS POTENTIAL TYPE 2 DIABETES BIOMARKERS

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Main Objectives: Pathogenesis of type 2 diabetes mellitus (T2DM) is accompanied by intensive protein glycation, which refers to non-enzymatic reactions of reducing sugars with amino groups. The sugar moieties of resulting Amadori products can undergo oxidative degradation yielding a heterogeneous group of so-called advanced glycation end-products (AGEs). AGEs accumulate over time in tissues and thus became characteristic for advanced stages of the disease, whereas Amadori products are more suitable for early diagnostics and therapy control.

Nowadays, glycated haemoglobin and serum albumin are recognized as markers of hyperglycemia. However, quantification of individual glycation sites in multiple proteins might provide more reliable information about the glycemic status of patients. As glycated species represent only a minor protein fraction, sensitive analytical techniques for quantification of individual modification sites are required. So far reliable methods are still missing for this purpose. To fill this gap, we present here a precise and robust quantitative approach.

Strategy and Methods: Plasma samples obtained from diabetic patients and non-diseased individuals were separated from low-molecular weight compounds, tryptically digested, enriched for glycated peptides by boronic acid affinity chromatography (BAC), desalted by solid phase extraction (SPE), and separated by RP-HPLC coupled online to ESI-QqQ-MS. Absolute quantification relied on multiple reaction monitoring (MRM) of multiple glycation sites corresponding to plasma proteins and stable isotope dilution approach.

Main Results: Previously, we have identified 40 glycation sites as prospective biomarkers using a small number of plasma samples taken from T2DM patients and healthy individuals [1]. Here, we selected 30 candidates that were synthesized as standard compounds [2-3]. Additionally, 19 were synthesized with one or two ¹³C,¹⁵N-labeled amino acids as internal standards.

Furthermore, the whole sample preparation and analysis procedure, i.e. tryptic digestion, BAC, SPE, and RPC-MS, was optimized for recovery and precision and finally validated. The 30 glycation sites were quantified in plasma samples obtained from five male diabetic patients and matched (age: 35-65, BMI, gender) healthy individuals. Thereby, 27 tryptic glycated peptides corresponding to nine different plasma proteins were significantly increased (at least $P < 0.05$) in diseased subjects. Currently, two cohorts of 50 diabetic and non-diabetic samples are analyzed to judge their diagnostic value and to compare it to HbA₁c, which is well-established for long-term glucose control.

Conclusions: The presented approach enables sensitive quantification of prospective T2DM biomarkers and application in clinical diagnostics.

References:


Main Objectives: The presence of furan, a “possibly carcinogenic compound to humans”; in fried starchy foods has been related to non-enzymatic browning and lipid oxidation. Considering that furan is a volatile and low polar compound, we decided to explore the existence of some relationship between the furan formation, the oil absorption and non-enzymatic browning in this kind of food matrixes.

The present work studied the kinetics of furan formation, non-enzymatic browning development, lipid oxidation and oil absorption, as well as their associations in fried wheat flour model systems with the aim to clarify the mechanisms responsible of furan occurrence in fried starchy foods.

Strategy and Methods: Wheat flour dough with moisture content of 40% in wet basis was prepared and cut in circle slices (d: 40 mm; h: 2.3 mm) which were fried at 150, 160, 170 and 180 °C up to 13 min. Then, the furan content, lipid oxidation level and oil absorption were determined by GC-MS, polar compound determination and Bligh and Dyer extraction, respectively. Additionally, the colour development of fried samples was quantified in $L^*$, $a^*$ and $b^*$ units by using a computer vision system. After that, the kinetics of furan formation, lipid oxidation, oil absorption and non-enzymatic browning were determined and adjusted to mathematical models. Furthermore, the Arrhenius-type dependency of the four studied responses was evaluated.

Main Results: A logistic model was successfully used to explain the kinetic behavior of furan formation in fried starchy food model systems at the four different temperatures studied (R²: 0.934-0.999). Regarding to oil absorption and color development, both phenomena followed a first order kinetic model for all evaluated temperatures (R²≥0.995). On the other hand, no significant changes (p≥0.05) in the level of polar compound were observed under all evaluated conditions. An Arrhenius-type dependency was found, not only for furan formation but also for oil absorption and colour development.

Conclusions: As for Maillard reactions in general, for all samples, an increase in furan level was observed when the degree of non-enzymatic browning increased. Furthermore, at similar levels of non-enzymatic browning and lipid oxidation, higher furan content correlated with higher oil absorption level. Our results suggest that lipid oxidation is not responsible of furan formation in starchy fried foods. Moreover, these findings would confirm the retention role of oil over the furan formed by Maillard reaction.
Main Objectives: Formation of 1,2-dicarbonyl compounds in sugar rich foods during thermal processing is an important aspect providing flavor and color development. On the other hand their presence in heat-treated foods may cause health related concerns. Mechanism of 1,2-dicarbonyl compound formation in Maillard reaction and caramelization has a complex reaction network which multiresponse kinetic modeling may identify statistically more important and dominating pathways along with their kinetic and thermodynamic constants.

Strategy and Methods: A dough model was prepared comprising 10 g wheat flour, 1 g glucose and 6 mL water and then it was dried and finely ground. Reactions were performed with 100 mg of the mixture in screw capped glass tubes in an oil bath at 160, 180 and 200 °C. The following compounds were analyzed in the reaction mixtures: glucose, fructose, amino acids, glucosone, 3-deoxyglucosone, 3,4-dideoxyglucosone, 1-deoxyglucosone, glyoxal, methylglyoxal, diacetyl, 5-hydroxymethyl-2-furfural. The most appropriate mechanistic model was selected after testing several probable reaction networks. A set of differential equations compiled and solved by numerical integration to obtain kinetic parameters by using Athena Visual Studio software version 14.2.

Main Results: Maillard reaction and dehydration of sugars proceed simultaneously and they comprise same intermediates from sugar backbone. Glucose mainly isomerized to fructose and they both involved in Maillard reaction. Amino acids rapidly degraded leading to an increase in the concentrations of 3-deoxyglucosone and 1-deoxyglucosone until free amino acids completely used during heating. On the contrary, methylglyoxal and diacetyl concentrations were continued to increase afterwards. 5-hydroxymethyl-2-furfural accumulation was mainly started after consumption of amino acids. Several pathways of 1,2-dicarbonyl compound formation was investigated by building suggested mechanisms and the observed data was confronted with the predicted values. The results provided important aspects of the 1,2-dicarbonyl formation mechanism under dry heating conditions in dough model system.

Conclusions: Overall, multiresponse kinetic modeling of Maillard reaction and caramelization simultaneously provided key reaction routes under dry conditions at elevated temperatures.
OP-20  ALPHA DICARBONYL DERIVED ADVANCED GLYcation END PRODUCTS CORRELATE WITH PROGRESSION OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETES

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Main Objectives: Advanced glycation end products (AGEs) play an important role in diabetic nephropathy (DN) and can also serve as clinical markers of early kidney disease. We examined the association between serum AGE concentrations and structural lesions on kidney biopsies in Pima Indians with type 2 diabetes and early DN.

Strategy and Methods: Subjects participated in a 6-year randomized, placebo-controlled clinical trial to evaluate the renoprotective efficacy of losartan (ClinicalTrials.gov number NCT00340678). At baseline, free AGEs were measured in serum by LC-MS/MS and glomerular filtration rate (GFR) by iothalamate clearance. At the end of the trial, a kidney biopsy was performed. Associations between clinical characteristics, glomerular structural variables, and AGEs were explored by Spearman correlations. Associations with glomerular structural parameters, including glomerular basement membrane (GBM) width, mesangial fractional volume (VvMes), total filtration surface (TFS), and cortical interstitial fractional volume (VvInt) were also examined by linear regression after adjusting for age, sex, diabetes duration, HbA1c, treatment group, GFR, and urine albumin/creatinine ratio (ACR).

Main Results: Participants (n=109, mean age 40±10 years, diabetes duration 10.3±6.0 years, HbA1c 9.3±2.3%) had median GFR=165 ml/min (IQR=132-188) and median ACR=30 mg/g (IQR=12-66). Baseline levels of carboxyethyllysine (CEL), carboxymethyllysine (CML), 3DG hydroimidazolone (3DGH), and methylglyoxal hydroimidazolones (MGH1) correlated inversely with each other (r=-0.42 – 0.77, p<0.0001) and with baseline GFR (r=-0.21 – 0.32, p<0.05), but not ACR. CEL, 3DGH, and MGH1 correlated inversely with TFS, and CEL and MGH1 correlated positively with VvMes. After multivariable adjustment, MGH1 and 3DGH remained inversely associated with TFS, and MGH1 and CEL positively with VvMes. Parameter estimates (p-values) for the adjusted relationships, presented as the difference in the morphology measurement in standardized SD units per doubling of the biomarker, are shown in the table, and the adjusted correlations for MGH1 are illustrated in the figure.

<table>
<thead>
<tr>
<th>Biomarkers (µmol/L)</th>
<th>GBM width</th>
<th>VvMes</th>
<th>TFS</th>
<th>VvInt</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGH1</td>
<td>0.100 (0.316)</td>
<td>0.230 (0.018)</td>
<td>-0.269 (0.009)</td>
<td>0.190 (0.097)</td>
</tr>
<tr>
<td>CEL</td>
<td>0.263 (0.121)</td>
<td>0.355 (0.033)</td>
<td>-0.263 (0.140)</td>
<td>0.203 (0.298)</td>
</tr>
<tr>
<td>3DGH</td>
<td>0.221 (0.092)</td>
<td>0.140 (0.283)</td>
<td>-0.274 (0.047)</td>
<td>0.258 (0.089)</td>
</tr>
<tr>
<td>CML</td>
<td>-0.049 (0.743)</td>
<td>-0.145 (0.323)</td>
<td>0.137 (0.379)</td>
<td>-0.058 (0.733)</td>
</tr>
</tbody>
</table>

Conclusions: Higher baseline serum levels of dicarbonyl-derived AGEs were significantly associated with structural lesions of DN including increased mesangial fractional volume and loss of total filtration surface area measured 6 years later.
ACCUMULATION OF ADVANCED GLYCATION ENDPRODUCTS IS ASSOCIATED WITH MACROVASCULAR COMPLICATIONS AND MORTALITY, AND GLYCAEMIC CONTROL WITH MICROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS: THE AURORA FOLLOW-UP STUDY.

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Main Objectives: The UK Prospective Diabetes Study (UKPDS) showed that glycaemic control (HbA1c) can predict vascular complications in type 2 diabetes mellitus (T2DM). Later, the DCCT Trial showed that accumulation of Advanced Glycation Endproducts (AGE) from skin biopsies can predict vascular complications in type 1 diabetes mellitus. Nowadays, tissue AGEs can be measured non-invasively with the AGE-Reader using Skin Auto Fluorescence (SAF). Previously, we showed predictive values of SAF for macro- and microvascular complications in well-controlled T2DM patients in the primary care. The primary aim of the current study was to evaluate the associations between SAF and the development of both macrovascular complications and microvascular complications in secondary care patients with T2DM. Our secondary aim was to compare these results with the UKPDS risk score calculations.

Strategy and Methods: A prospective cohort study of 514 T2DM patients (mean age 69±11 years, T2DM duration 18 years) from five Dutch hospitals was performed. Clinical status was assessed from April 2007 till October 2015.

Main Results: After a median follow-up of 5.1 (IQR 4.3-5.9) years, 79 (15%) patients died and 49 (9%) were lost to follow-up. 189 (37%) patients developed a macrovascular complication, and 133 (26%) patients a microvascular complication. Tertiles of SAF were significantly associated with the development of macrovascular complications in Kaplan-Meier analysis (logrank \(p=0.003\)) but not for microvascular complications. However, tertiles of HbA1c were not associated with macrovascular complications, but only with development of microvascular complications (logrank \(p=0.022\)). Cox regression analysis for SAF gave a hazard ratio (HR) of 1.53 (95%CI 1.24-1.89), \(p=0.0007\) per unit (SAF) increase in the development of macrovascular complications. After correction for the UKPDS risk score the HR stayed significant: HR 1.28 (1.03-1.60), \(p=0.026\). For HbA1c and microvascular complications a crude HR 1.20 (1.06-1.36), \(p=0.004\) was found. The HR did not change after correction for the UKPDS score: HR 1.20 (1.06-1.36), \(p=0.004\).

Conclusions: This study shows that accumulation of AGEs is associated with development of macrovascular complications in patients with T2DM after 5-years of follow-up. Furthermore, glycaemic control (HbA1c) is associated with the development of microvascular complications.
Main Objectives: The aim was to assess the effect of MG metabolism, lipoprotein modification and dysfunction on the risk of cardiovascular disease (CVD).

Strategy and Methods: Increased atherogenic small dense low density lipoprotein (LDL) and decreased high density lipoprotein (HDL) are linked to increased risk of atherosclerosis and CVD. Major proteins of LDL and HDL, apolipoprotein B100 (apoB100) and apolipoprotein A-1 (ApoA-1) have functionally important arginine residues which may be glycated by MG leading to functional impairment. We isolated LDL and HDL from healthy subjects and patients with type 2 diabetes (T2DM), quantified MG-derived advanced glycation adduct (AGE) contents, located the major sites of modification and studied associated functional change. We also did a glyoxalase (Glo1) functional genomics study in diabetic, apolipoprotein E (ApoE) deficient mice on development of atherosclerosis.

Main Results: MG-derived hydroimidazolone MG-H1 was a major AGE of ApoB100 of LDL, accounting for ca. 3% LDL in healthy people and increasing 3-fold in T2DM patients. LDL minimally modified by MG was atherogenic – small, dense with increased binding to arterial wall proteoglycans and aggregation in vitro. Radiotracer studies in rats showed that it had normal plasma clearance but increased partitioning onto the aortal wall. Peptide mapping identified R18 as a hotspot site of apoB100 modification which modified by MG induced distortion, exposing a proteoglycan binding domain in the apoB100 N-terminus.

HDL modified by MG and related dicarbonyl metabolites accounted for 2.6% HDL in healthy people, increasing to 4.5% in T2DM patients. MG modification in vitro occurred at R27, R123, and R149 of apoA-1 linked to membrane fusion, intramolecular bonding and ligand binding. It induced re-structuring of HDL particles, decreasing stability and plasma half-life in vivo. Kinetic modelling predicted dicarbonyl modification produces 2 - 6% decrease in HDL clinically.

Diabetic ApoE deficient mice had increased MG-H1 in aortal protein and atherosclerosis at 20 weeks which was not prevented by overexpression of Glo1 in the endothelium. ApoE deficient mice Glo1 knockdown mice did not accumulate MG-H1 in aortal protein at 20 weeks nor have increased atherosclerosis.

Conclusions: Glycation of LDL and HDL by MG occurs at low levels in healthy people, increasing 2 – 3 fold in diabetes. Increased atherogenicity and decreased stability of MG-modified of LDL and HDL suggest a role in dyslipidaemia and CVD risk. Endothelial dicarbonyl stress does not drive atherosclerosis and MG potentiation of atherosclerosis may be a late event in diabetic ApoE deficient mice model of CVD.
Main Objectives: The aim of this study was to develop stable transfectant cell lines for screening of small molecules for glyoxalase 1 (Glo1) expression inducing activity or “Glo1 inducers”.

Strategy and Methods: Human glyoxalase 1 (Glo1) has a functional antioxidant response element (ARE) in its promoter. This may be exploited to develop small molecule Glo1 inducers as potent and safe anti-glycation pharmaceuticals to counter dicarbonyl stress. Glo1 expression is induced by activation of transcription factor Nrf2 which binds with accessory proteins to a functional, regulatory ARE in the GLO1 promoter and increases Glo1 transcription. To screen for Glo1 inducers we developed a GLO1-ARE luciferase reporter stable transfectant cell line and a similar quinone reductase NQO1-ARE luciferase reporter stable transfectant cell line as a control. NQO1 is a well-established ARE-regulated gene.

A pGL4.22[luc2CP/puro] reporter vector containing GLO1-ARE was transfected into the HepG2 cell line. Transfected HepG2 cells were selected with puromycin (1 µg/ml). After culture for 3 weeks, puromycin-resistant cells were screened for luciferase activity after treatment with 4 µM sulforaphane for 6 h. After validation of positive clones by measuring luciferase activity, the stable cell line was expanded in selection media and thereafter used in studies with chemical inducers. A similar procedure with a pGL4.22[luc2CP/puro] reporter vector containing NQO1-ARE produced the control stable transfectant cell line.

Main Results: After validation of the positive clones, the stable cell lines were expanded in selection media and used to screen chemical libraries to identify non-toxic Glo1 inducers. Dietary bioactive compounds known to induce expression of NQO1 were screened for ability to also induce Glo1. Not all activators of Nrf2, as judged by induction of NQO1 expression, are inducers of Glo1 expression. This relates to Nrf2 activation potency (level of functionally active Nrf2 achieved in the cell nucleus), recruitment of required accessory proteins and off-target effects of dietary bioactives. Several inducers of Glo1 expression were found. An example is trans-resveratrol (tRES). The median effective concentration EC50 of tRES for induction of Glo1 was 3.56 ± 0.25 µM; cf. the EC50 for induction of NQO1 was 5.18 ± 0.78 µM. Glo1 expression induction was validated in HepG2 cells in culture where incubation with 10 µM tRES increased Glo1 activity and protein by ca. 4-fold.

Conclusions: A GLO1-ARE luciferase reporter stable transfectant cell line was produced. It may be used in small molecule screening for discovery of Glo1 inducers for novel functional food and pharmaceutical development.
Main Objectives: The Maillard reaction is a non-enzymatic reaction that occurs naturally during food processing and storage, that results in the formation of compounds such as glycated proteins and melanoids. The effects of this reaction on food products range from changes in color and foaming properties, to a decrease in nutrient bioavailability and formation of toxic compounds. Even so, most research is focused on simple models with the use of amino acids or small peptides and one sugar. The aim of the project will be to study in depth the mechanisms behind the Maillard reaction, using 3 different proteins, namely α-Lactalbumin, β-Lactoglobulin and β-Casein, due to their wide distribution in milk based products. Regarding reactive saccharides, a selection of several different monomers (pentose/hexose) and oligomers (differing in size and structure) will be used.

Strategy and Methods: The first focus was to study the first steps of the Maillard reaction: the saccharide attachment, dependent on protein/saccharide combination used, it's kinetics as well as the glycation sites within the protein sequence. In addition, the levels of dehydration, possible crosslinking, and also, competition between different saccharides for attachment sites have been studied.

Main Results: Results demonstrate that α-Lactalbumin and β-Lactoglobulin possess similar reactivity when glycation level is compared to overall N-terminal groups, while β-Casein possess a slower reactivity. Mono-saccharide isomers exhibit differences when glycation rates are compared, as well as disaccharide isomers (α(1-4) linked maltose vs β(1-4) linked lactose). When the level of glycation surpasses the number of available Lysines, the loss of water molecules occurs, depending on buffering system used.

Conclusions: This suggests that the buffering system will affect the mechanism of glycation, but also that Arginine must possess a different mechanism then Lysine’s for glycation.
MONITORING MAILLARD REACTION DURING STORAGE OF SKIM MILK POWDERS BY MEASURING AVAILABLE LYSINE

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Main Objectives: Application of skim milk powders (SMP) has increased enormously in the food industry during recent years due to various reasons. With regard to composition of SMP the possibility of Maillard reaction after production and during storage is not ruled out. Therefore, investigating the factors which influence the formation of Maillard products is of crucial importance for both the manufacturers and consumers. Thus, in this study available lysine was used as a marker to monitor the extent of Maillard reaction in Freeze-dried, Spray-dried and Drum-Dried SMP during storage at highly controlled atmospheres.

Strategy and Methods: Three types of skim milk powders mentioned above were manufactured by using pilot-scale dryers and were stored over saturated salt solutions for 200 days. Two temperatures (20, 30°C) and two relative humidities (33, 52%) were selected as the storage variables. Available lysine was quantified in pre-determined intervals by a dye-binding method using Acid-orange 12 established by Hurrel, et al 1979. The method was validated for SMP and protein isolates by Aalaei, Rayner, Sjöholm, 2015. Water activity and water content of the samples were also useful tools to understand the water absorption during the storage.

Main Results: Based on the findings of this study, temperature and relative humidity of the storage have a profound influence on the rate of available lysine loss. Choice of the dryer as the other investigated variable also had a significant impact on the available lysine content of the product. Freeze-dried powders were the ones which their available lysine content least affected by the drying process. Regarding storage conditions, SMP stored at 52% relative humidity and 30°C indicated a 40% decrease on average in the available lysine content after 200 days, while the powders stored at 33% relative humidity and 20°C did not show a significant loss during the same period of time.

Conclusions: The importance of studying the storage stability of SMP and the affecting parameters has increased not only because of its application in infant formulas, but also as a multi-functional ingredient in various food industries including confectionary, meat, dairy and many other areas. Considering the long shelf-life of SMP and its possible exposure to non-favorable conditions during storage and transportation in various countries, the physical and nutritional changes due to Maillard reaction is of great concern.

Main objectives: Milk powders (e.g. infant formulas) with similar protein composition and protein content can have different protein digestibility. This variation in digestibility has been proven to be related to the Maillard reaction, which takes place during the production and/or storage of foods that contains proteins and carbohydrates. The reaction leads to changes in techno-functional properties of the proteins as well as their digestibility. While both Maillard reaction and protein digestion have received quite some attention, surprisingly few studies provide information about how much the Maillard reaction quantitatively affects the protein digestion. In addition, the Maillard reaction can also induce structural changes on the proteins, which can affect the digestibility. Therefore, in this research, proteins were glycated without inducing structural changes. The aim is to obtain a quantitative relation between the degree of glycation and their digestibility. Two enzymes were chosen for digesting the protein: 1) trypsin (specific for lysine and arginine, the same preference as the glycation) ; 2) Bacillus licheniformis protease (BLP) (specific for glutamic and aspartic acids). It is hypothesized that trypsin hydrolysis will be hindered by the blockage of cleavage sites while BLP hydrolysis will only be influenced when the glycation sites are close to the cleavage sites.

Strategy and methods: A pure protein (α-lactalbumin) and a monosaccharide (D-glucose) were heated (50 °C) for 0-8 hours to obtain samples without further structural changes (determined by size exclusive chromatography and circular dichroism). The average degree of glycation was measured by high definition mass spectrometry and confirmed by o-phtaldialdehyde method. The average degree of glycation was correlated with the degree of hydrolysis measured by a pH-stat.

Main results: The glycated α-lactalbumin only varies in the degree of glycation without other changes. For both enzymes, ~40-50 % loss of digestibility (expressed as degree of hydrolysis after 2 h incubation) was observed for the proteins with highest degree of glycation. For trypsin, a linear correlation between the degree of hydrolysis and average degree of glycation was observed. Even for BLP, a decrease in digestibility was observed but only when the average degree of glycation is > 40 %. This shows that the blocking of the lysines does not only influence the trypsic digestibility but also shows significant effects on digestibility by enzymes that are not specific for lysines.

Conclusions: Maillard glycation decreases not only trypsic digestibility and also digestibility by enzymes that are not specific for lysines.
PLANT PROTEIN GLYCATION: POSSIBLE EFFECTS ON PHYSIOLOGY AND FOOD QUALITY

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Main Objectives: Glycation is a non-enzymatic post-translational modification formed by reaction of reducing sugars (aldoses and ketoses) with amino groups of proteins, and resulting so-called Amadori and Heyns compounds, respectively. Their further oxidation and formation of $\alpha$-dicarbonyls (glycoxidation) yield advanced glycation end-products (AGEs) known for their pro-inflammatory effects in humans. Though AGEs readily form during thermal processing of foods, it may occur also during the life time of crop plants. In this context, it is important to know the patterns of AGEs in crop plants and effect of environmental stress on their dynamics.

Strategy and Methods: The models of high light, drought and metal stress were established with Arabidopsis thaliana and Brassica napus. The leaves were harvested before stress application and in multiple points throughout the stress period. The analytical strategy (applied to A. thaliana and B. napus) relied on the combination of LC-based bottom-up proteomics (LC x LC-ESI-Orbitrap-LIT-MS/MS data dependent acquisition experiments), untargeted and targeted metabolomics (GC-EI-Q-MS) and model glycation experiments with synthetic peptides (LC-QqTOF-MS and MS/MS).

Main Results: Even unstressed plants displayed rich patterns of glycated and glycoxidated proteins (up to 400 and 900 modified peptides, respectively) mostly involved in regulatory pathways, protein and nucleic acid metabolism. The product structures were comprehensively characterized with ESI-MS/MS. Light and metal stress resulted in increased Amadori/Heyns product (mostly triose- and pentose-derived) formation. The number of AGE-modified sites (dominating with arginine residues) was essentially increased under drought and high metal concentrations (44 and 65 unique sequences, respectively), while all stresses resulted in significantly higher abundances of corresponding products. However, the majority of AGE-modified sites did not resemble glycated ones. Remarkably, all stresses caused significant up-regulation of tissue carbohydrates. As it was not accompanied with the elevation of free $\alpha$-dicarbonyl levels, autoxidation of the carbohydrates followed by immediate binding of generated intermediates to cellular proteins and metabolites might be the most probable scenario. Reactivities, glycation and AGE-formation potentials of individual sugars were characterized by in vitro experiments with synthetic peptides. Thereby, D-glucoso-6-phosphate showed the highest reactivity, while dihydroxyacetone phosphate, D-glyceraldehyde and D-ribose demonstrated the highest potential for AGE formation.

Conclusions: The character and the scale of the changes in the protein glycation and glycoxidation patterns depended on the nature of environmental stress. Thus, oxidative glycosylation of proteins, rather than their glycoxidation or lipid peroxidation, was the main source of AGEs. Dihydroxyacetone phosphate, D-glyceraldehyde and D-ribose demonstrated the highest potential for AGE formation.
DICARBONYL STRESS IN ADIPOSE TISSUE OF MICE FED AN OBESOGENIC DIET

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Main Objectives: To assess the presence of dicarbonyl stress in white adipose tissue (WAT) of mice on an obesogenic high-fat diet (HFD) and the effect of ectopic expression of mitochondrial uncoupling protein-1 (UCP1) in WAT.

Strategy and Methods: Strategy Dicarbonyl stress, the abnormal accumulation of dicarbonyl metabolites such as methylglyoxal (MG) leading to cell and tissue dysfunction, has recently emerged as a possible contributory factor to obesity. MG is metabolised mainly by glyoxalase 1 (Glo1) of the glyoxalase system. We investigated the disturbance of the glyoxalase system in WAT of mice on an obesogenic diet and also in transgenic mice expressing UCP1 gene under the adipocyte lipid-binding protein gene promoter (aP2-Ucp1 mice) in adipocytes which are resistant to obesity.

Methods: At 3 months of age, wild-type (WT) male C57BL/6J mice and their transgenic littermates expressing UCP1 gene under the adipocyte lipid-binding protein gene promoter (aP2-Ucp1 mice) were randomised and fed control chow (3.4% fat) or HFD (ca. 35% fat) for 10 weeks. Mice were sacrificed following overnight fasting. Epididymal WAT was used for experiments. MG was measured by LC-MS/MS and glyoxalase 1 (Glo1) activity by spectrophotometric assay.

Main Results: MG content (pmol/mg wet weight) was increased by HFD in WT mice, with respect to control chow (control, 1.47 ± 0.65 vs HFD, 2.91 ± 0.98, +98%, P<0.01; n = 7 – 8). This increase was prevented in aP2-Ucp1 mice (control, 1.87 ± 0.73 vs HFD, 2.51 ± 0.64; n = 5 - 6). Increased MG was associated with decreased Glo1 activity in WAT of WT mice on a HFD and in aP2-Ucp1 mice but not in the fed state. Glo1 activity (U/mg protein): WT, HFD 13.7 ± 4.9 (-28%, P<0.05) vs control 19.0 ± 2.9; aP2-Ucp1, HFD 15.3 ± 3.7 (-26%, P<0.05) vs control 20.7 ± 2.1 (n = 5 – 8).

Conclusions: Mice on an obesogenic diet suffer dicarbonyl stress in WAT linked to down regulation of Glo1 activity in WAT. Dicarbonyl stress in WAT and the obesity phenotype were prevented in aP2-Ucp1 mice.
DICARBONYL STRESS IN CLINICAL OBESITY AND EFFECT OF A LOW CALORIE DIET

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Main Objectives: To examine markers of dicarbonyl stress in non-obese and obese human subjects and assess the effect in obesity of change to a low calorie diet.

Strategy and Methods: Strategy Dicarbonyl stress, the abnormal accumulation of dicarbonyl metabolites such as methylglyoxal (MG) and related dicarbonyl metabolites leading to cell and tissue dysfunction, has recently emerged as a possible contributory factor to obesity. MG is formed mainly from triosephosphates in glycolysis and also gluconeogenesis and glycerogenesis. 3-Deoxyglucosone (3-DG) is formed mainly by degradation and repair of fructosamine residues. Plasma dicarboxyls are metabolic markers of dicarbonyl stress. It is unknown if dicarbonyl metabolite levels change in clinical obesity and how they respond when subjects are placed on a low calorie diet.

Study groups Non-obese subjects on isocaloric diet (BMI 27.8 ± 1.3 kg/m², n = 18), obese subjects on isocaloric diet (BMI 34.3 ± 3.3 kg/m², n = 29), and obese subjects on a low caloric diet (BMI 34.2 ± 2.3 kg/m², n = 26). Isocaloric diet was 2300 – 2400 kcal/day and low caloric diet 1200 kcal/day (women) and 1500 kcal/day men (men). Subjects were on the dietary regime for 2 weeks prior to sampling.

Sampling Sampling was fasted plasma. Plasma dicarboxyls were measured by stable isotopic dilution analysis LC-MS/MS. Data are mean ± SD, and P and P’ are significance with respect to non-obese and obese subjects on isocaloric diet, respectively.

Main Results: Plasma MG was increased in obese subjects on an isocaloric diet, with respect to non-obese controls and was not corrected by low calorie diet. Plasma MG: non-obese – 181 ± 65 nM, obese – 245 ± 123 nM (P<0.05), and low calorie diet obese – 293 ± 104 nM (P<0.001). Plasma 3-DG was also increased in obese subjects and markedly decreased when changed to a low calorie diet. Plasma 3-DG: non-obese 505 ± 139 nM, obese 692 ± 228 nM (P<0.01), and low calorie diet obese 400 ± 101 nM (P<0.01, P’<0.001). Fasting plasma glucose was 5.1 ± 0.6 mM in non-obese subjects and was unchanged in other study groups.

Conclusions: Clinical obesity is associated with dicarbonyl stress. Increased plasma MG is not corrected by short-term change to a low calorie diet and may be linked to fatty acid/triglyceride cycling not immediately reversed by decreased calorie intake. Plasma 3-DG responded to low calorie diet and may be linked to both insulin resistance and sourced from food intake.
DELAYED INTERVENTION WITH PYRIDOXAMINE IMPROVES METABOLIC FUNCTION AND PREVENTS ADIPOSE TISSUE INFLAMMATION AND INSULIN RESISTANCE IN HIGH-FAT DIET-INDUCED OBESE MICE

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Main Objectives: The development of obesity is one of the largest health problems worldwide and has reached epidemic proportions. Obesity is characterized by a dysregulation of adipokine secretion, which contributes to the development of obesity-associated complications, such as type 2 diabetes and vascular complications. We and others have recently demonstrated that advanced glycation endproducts (AGEs) accumulate in the obese adipose tissue, and that they contribute to complications via an inflammatory reaction. Pyridoxamine (PM), a vitamin B6 analogue, has been identified as an anti-glycating agent. The objective of this study was to investigate the effect of a delayed PM intervention on metabolic and vascular function in high-fat diet (HFD)-induced obese mice.

Strategy and Methods: Three groups (n=15 each) of male C57BL/6J mice were kept for 6 weeks on a low-fat diet (LFD). Then, 2 groups of mice were placed on a HFD (45% kcal% fat) for a period of 18 weeks. In one of these HFD groups, PM (2g/L) was added to the drinking water from 12-18 weeks (HFD+PM). The control group remained on the LFD during the complete study. All groups of mice were subjected to multiple metabolic and vascular measurements during the study protocol.

Main Results: PM-treated, HFD-induced obese mice had reduced body weight gain, hyperglycemia and hypercholesterolemia, as compared to those who were not treated with PM. Furthermore, PM treatment inhibited the expansion of adipose tissue and adipocyte hypertrophy. Adipogenesis of murine 3T3-L1 and human SGBS preadipocytes was dose-dependently reduced by PM treatment, as demonstrated by Oil red O staining and the mRNA expression of several adipogenic markers. The high expression of pro-inflammatory genes in visceral adipose tissue of the HFD group was significantly attenuated by PM. PM also reduced hepatic lipid content of HFD mice. In both HFD-induced and db/db obese mice, impaired glucose metabolism and insulin resistance were prevented by supplementation with PM. Moreover, PM treatment partially prevented HFD-induced mild vascular dysfunction.

Conclusions: In conclusion, we have demonstrated that a delayed intervention with PM is associated with an improvement of several aspects of obesity, including metabolic dysfunction, insulin resistance and adipose tissue inflammation. These findings indicate that PM may be a potential novel intervention strategy for obesity-associated metabolic dysfunction and complications.
ANTI-SRAGE AUTOIMMUNITY IN OBESITY: DOWNTURN AFTER BARIATRIC SURGERY IS INDEPENDENT OF PREVIOUS DIABETIC STATUS.

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Main Objectives: Morbid obesity increases the risk of cardiovascular disease (CVD). The receptor for advanced glycation end-products (RAGE) is implicated in proinflammatory processes that underlie CVD. Its soluble form (sRAGE) has been proposed as a vascular biomarker. Recently, anti-sRAGE autoantibodies were described and found to be increased in diseases where RAGE is overexpressed. This study aimed to investigate serum levels of anti-sRAGE autoantibodies in morbidly obese patients.

Strategy and Methods: After exclusion based on specific criteria, 150 subjects (50 normoglycemics, 50 glucose-intolerants and 50 diabetics) were randomly recruited from a cohort of 750 obese patients (ABOS). Serum sRAGE and anti-sRAGE autoantibodies were measured before bariatric surgery. Sixty-nine patients were followed for up to one year after gastric bypass, and their levels of sRAGE and anti-sRAGE autoantibodies measured. The control group consisted of healthy blood donors.

Main Results: Compared with controls, baseline levels of sRAGE and anti-sRAGE autoantibodies were significantly higher in all obese patients independently of glucose regulation (P<0.001). At one year after gastric bypass, sRAGE and anti-sRAGE were decreased (P<0.001). The decrease in anti-sRAGE autoantibodies was correlated with an increase in high-density lipoprotein (HDL; P=0.02).

Conclusions: Independently of previous diabetic status, morbid obesity increases sRAGE and anti-sRAGE levels. Weight loss after gastric bypass is followed by a decrease in both titers. The decrease in anti-sRAGE correlates with an increase in HDL.
CHARACTERIZATION OF ANTI-INFLAMMATORY ACTIVITY OF AN ADVANCED GLUCOSE-LYSINE MAILLARD REACTION PRODUCT

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Main Objectives: Specific products of the Maillard reaction (MR) have been considered potential dietary sources of pro-inflammatory activity, largely due to involvement with triggering secretion of certain interleukin(s) and an affinity to stimulate excessive nitric oxide (NO) synthesis. In contrast, other studies report anti-inflammatory activity of both intermediate and late-stage MR products; largely by inhibiting inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) activity. Our previous work has identified [5-(5,6-dihydro-4H-pyridin-3-ylidenemethyl)furan-2-yl]-methanol (F3-A), derived from a glucose-lysine MR mixture with a capacity to inhibit NO activity in CaCO₂ cells. These results agree with findings reported by others, that distinct bioactive MRP possess anti-inflammatory activity, manifested through inhibition of iNOS, COX-2 and NF-κB activities in different cell systems. The research objectives of this study were to determine the underlying mechanisms for F3-A-induced NO inhibition in intestinal as well as macrophage cells treated with pro-inflammatory cocktails. We compared the anti-inflammatory activity of F3-A, with two known anti-inflammatory agents, namely aminoguanidine (iNOS inhibitor) and pyrrolidine dithiocarbamate (NF-κB inhibitor).

Strategy and Methods: Glucose and Lysine (2:1 molar ratio) were heated at 100°C; followed by a pH adjustment to 12, using sodium hydroxide, and then extraction with chloroform (2:1 v:v) ratio. A bioactive fraction, termed F3-A was isolated with 95% purity confirmed. Bioactivity was determined using cultured Caco-2 (HTB-37, ATCC) and RAW 264.7 (TIB-71, ATCC). F3-A activity was evaluated on capacity to inhibit intra/extra-cellular NO production, iNOS, and NF-κB in RAW 264.7 macrophage and Caco-2 intestinal cells, respectively.

Main Results: Our results showed that an advanced Maillard reaction product, referred herein as F3-A, possessed activity to inhibit extracellular NO and iNOS protein in Caco-2 cells, while also inhibiting intracellular NO in RAW 264.7 cells.

Conclusions: We have shown a distinct anti-inflammatory capacity of an advanced MRP derived from a model glucose-lysine MR in two cell types, namely macrophage (e.g. RAW 264.7) and intestinal (21 day, differentiated Caco-2) cells. F3-A inhibited NO and iNOS in inflamed Caco-2 cells and its inhibitory effects were relatively greater than those observed with the iNOS inhibitor AG. These results show a potential role for this MRP to attenuate intestinal inflammation.
EFFECTS OF MAILLARD REACTION PRODUCTS ON SALMONELLA ENTERICA UNDER STRESS CONDITIONS

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Main Objectives: Accompanied with an increasing consumer demand for healthy and safe food products, naturally derived antimicrobials are highly desired as alternatives to chemical preservatives in the food industry. Maillard Reaction products (MRPs) have displayed antimicrobial effects on foodborne pathogens in vitro yet the mechanisms for this are not clear. Studies on the antimicrobial effects of MRPs may contribute to development of innovative strategies for the control of foodborne pathogens. Salmonella enterica is the leading cause of global foodborne illnesses and related deaths. The objective of this study was to evaluate the effects of lysine-derived MRPs prepared with one of the four reducing sugars [fructose (FL), glucose (GL), ribose (RL), and xylose (XL)] on the growth of Salmonella enterica serotype Typhimurium (ST) under typical environmental stresses in the food supply system, e.g., acidic and thermal conditions.

Strategy and Methods: Reducing sugars, including xylose, ribose, fructose and glucose were heated with L-lysine (Lys), respectively, with a sugar-Lys (SL) ratio (mole ratio) of 1:1 at 180°C for 60 min in a convection oven. A water-soluble fraction of crude MRPs was used in this study. ST was inoculated in brain heart infusion broth (BHIB) and acidified BHIB (pH 5.5) at 37°C. In the thermal stress assay, bacterial cells in BHIB were subjected to 42°C. All matrices were supplemented with 50 µg/ml crude MRP. The growth of ST was monitored by measuring OD600 for 24 hours with a 96-well plate reader. The ST growth data was fitted with a nonlinear regression model using a data analysis software (R) to obtain the maximum growth rate (µmax).

Main Results: The ranking of the water soluble fraction based on OD420 values was: GL > FL > XL > RL. In BHIB (pH = 7.2, 37°C), all four SL MRPs significantly reduced the µmax of ST compared to the control (p<0.05). A similar effect of MRPs was also observed under acidic (pH 5.5, 37°C) and thermal (pH 7.2, 42°C) stress conditions. Two-way ANOVA demonstrated significant interaction between MRP treatment and acid stress (p=0.003), but not between MRP treatment and thermal stress (p=0.168).

Conclusions: These findings suggest that MRPs may affect the proliferation of ST under acidic and thermal stress conditions through complex mechanisms that require further elucidation. New mitigation strategies aimed at reducing salmonellosis should recognize the importance of MRPs as food constituents in potentially reducing the risk to foodborne illnesses caused by Salmonella.
THE ASSOCIATION BETWEEN DIETARY INTAKE OF ADVANCED GLYcation ENDPRODUCTS AND PLASMA LEVELS OF ADVANCED GLYcation ENDPRODUCTS: THE CODAM STUDY

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Main Objectives: The increased endogenous production of advanced glycation endproducts (AGEs) have been implicated in the pathogenesis of age-related diseases. In addition, exogenous AGEs that are found in foods are believed to be an additional source of exposure to AGEs manifesting similar pathogenic properties to their endogenous counterparts. However, no data are available about intake of AGEs from food. The aim of this study was to determine the concentration of AGEs in food commonly consumed in Western diets and to determine whether and to what extent the concentration of AGEs in diet correlates with the concentration of AGEs in plasma.

Strategy and Methods: The AGEs Nε-(carboxymethyl)lysine (CML) and Nε-(1-carboxyethyl)lysine (CEL) and the hydroimidazolone derived from methylglyoxal (MG-H1) in plasma (free and protein-bound) and in the protein fractions of 172 commonly consumed food items were quantified using a validated ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS). Plasma and selected food items were hydrolysed, butylated and analyzed on a C18-reversed phase column. The dietary intake of AGEs (dAGE) was calculated based on the obtained AGE database and a validated and appropriate food frequency questionnaire (FFQ) as used in the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study, an observational cohort study including 512 individuals (mean (SD) age 59.4 (7.0), 311 men, 125 with type 2 diabetes). We used linear regression analyses to investigate cross-sectional associations between dAGE and plasma AGE. All analyses were adjusted for age, sex, glucose metabolism status, BMI, renal function, physical activity, energy intake, and smoking.

Main Results: Intra- and inter-day accuracy and precision of the analysis of AGEs in food items and in plasma were within the acceptable limits of 2-20%. The calibration curves showed perfect linearity (r²>0.99) in both water and sample matrix. We found the highest AGE concentration in peanut butter, peanuts and high-heat-processed meats. Products with the lowest AGE concentration per 100 g product were fruits, vegetables, butter and coffee. Positive associations were found between dCML and plasma free CML [β=0.23 (95% CI 0.09-0.36)] and between dMG-H1 and plasma free MG-H1 [0.26 (0.13-0.39)]. No statistically significant associations were found between dCML and dMG-H1 and plasma protein-bound CML and MG-H1, respectively, or between dCEL and plasma free or protein-bound CEL.

Conclusions: We successfully quantified CML, CEL and MG-H1 content in 172 selected food items with a UPLC-MS/MS method. Protein-bound CML and MG-H1 intake was positively associated with plasma free CML and MG-H1, respectively.
LONG TERM MODIFICATION OF MICROBIOTA AND ANTIOXIDANT DEFENSES IN COLON BY HIGH FORMULA DERIVED AGES IN IUGR INDIVIDUALS

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Main Objectives: We have shown that Formula-derived dietary advanced glycation end products (F-AGEs) promote programming of inflammation and oxidative stress in kidney of intrauterine growth retardation (IUGR) individuals. In this study we aim to determine whether F-AGEs modify microbial set-up and disturb the antioxidant defenses in colon at adult age.

Strategy and Methods: IUGR piglets received either a low heated formula (LHF, n = 8) or a high heated formula (HHF, n = 8) for 3 weeks. Piglets were fed ad libitum regular diet until postnatal day (PND) 54. Feces and Ceco-colonic microbiota were characterized at PND36 and 54 by CE-SSCP and qPCR analysis. mRNA expression of CAT, INOS, NFkB, GPX and GPX enzyme activity and histological studies were performed in colonic tissue at PND54.

Main Results: Fecal Bacterial diversity and abundance were modified at PND36 in HHF as compared to LHF animals (p<0.05). Importantly, at PND54, an increase of bacterial communities associated with a higher expression of CAT and INOS genes (p<0.05) were observed in HHF as compared to LHF group. GPX expression (p<0.05) and activity (p<0.0001) remained lower in HHF animals. No major structural changes in colon tissue were observed between animals.

Conclusions: F-AGEs alters microbial set-up in IUGR piglets and induce long term disturbance of the antioxidant defenses in the colon at adult age.
Luncheon Seminar
ANTIGLYCATION ACTIVITY OF IRIDOIDS AND THEIR FOOD SOURCES

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Main Objectives: Iridoids are phytochemicals that are available from a few dietary sources, such as Morinda citrifolia (noni), Cornus officinalis (Japanese cornelian cherry), and Olea europaea (olive). Iridoids have a variety of reported biological activities. In vitro tests and human studies were conducted to investigate the antiglycation activity of these iridoids and possible mechanisms of action.

Strategy and Methods: Noni fruit, leaf, and seed, as well as cornelian cherry juice and olive leaf extract, were evaluated for their ability to inhibit the in vitro glycation of collagen, human serum albumin (HAS) and keratin by glucose. Inhibition was determined by comparing fluorescence intensities at 440 nm (370 nm excitation) against controls after incubation. Inhibition of the in vitro glycation of skin samples was also evaluated visually and by fluorescence spectroscopy, following incubation in a glucose solution. The in vitro antiglycation activity of purified iridoids was evaluated in a bovine serum albumin (BSA)/methylglyoxal (MG) incubation model, via fluorescence intensity measurements (360 nm excitation, 460 nm emission). An LC-MS method was developed for the determination of dicarbonyl scavenging activity of iridoids, following incubation of samples with MG. Iridoid samples were evaluated directly and following hydrolysis by β-glucosidase, to mimic the effect of metabolism and absorption via the intestinal wall. An 8 week, open-label clinical trial of an iridoid rich beverage was conducted where glycation stress was measured in the skin with the AGE Reader. A cross-sectional population study was conducted to evaluate the association between iridoid consumption rates and skin AGE levels, as measured by skin autofluorescence. Previous results from an in vitro test of the anti-radical activity of the major iridoid from noni fruit, an in vivo study of the superoxide dismutase inducing action of this iridoid, as well as a clinical trial of noni fruit juice involving cigarette smokers, were reviewed.

Main Results: Iridoid sources, as well as the iridoids themselves, displayed antiglycation activity in the various in vitro models. Noni seed displayed the most activity in limiting collagen, keratin, and HSA glycation. Hydrolysis of iridoids from noni fruit increased their MG scavenging activity in a concentration dependent manner. In the human studies, ingestion of iridoids was associated with lowering of skin autofluorescence, with ingestion rates being correlated to lower than expected AGE levels.

Conclusion: Food sources of iridoids appear to have the potential to limit the formation of AGEs within the body.
NON-INVASIVE MEASUREMENT OF SKIN AGES TO EVALUATE DIABETIC COMPLICATIONS

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Since a full recovery from lifestyle-related diseases, such as atherosclerosis and diabetic complications, is practically difficult, preventive medicine is the most important issue to prevent the progression of these diseases. Therefore, obtaining an early diagnosis, as well as careful evaluation of the therapeutic effects of treatment, is necessary to prevent the progression of these diseases. Although the hemoglobin A1c (HbA1c), an early stage product of the Maillard reaction between the β chain of hemoglobin with glucose, is measured clinically as an index of blood glucose control during the past one to two months, it is difficult to predict the progression of diabetic complications. We have developed a device for the non-invasive measurement of advanced glycation end-products (AGEs) in skin to evaluate diabetic complications. To clarify the association between the duration of hyperglycemia and the accumulation of skin fluorophores, diabetes was induced in mice by streptozotocin. As a result, the accumulation of fluorophores in the auricles of live mice was increased by the induction of diabetes in a time-dependent manner. The change in the accumulation of the fluorophores was also measured in human diabetic patients. The fluorescence intensity was increased by the presence of diabetic complications such as kidney failure, retinopathy and neuropathy. Furthermore, the fluorescence intensity significantly increased in accordance with an increasing number of complications. This study provides the first evidence that the accumulation of fluorophores in the fingertip was increased by the number of complications, demonstrating that the presence of diabetic complications can be evaluated by measuring the fluorophores present in the fingertip.
Poster Presentation
POSSIBILITY OF CELL TRANSPLANTATION THERAPY FOR PERITONEUM INJURED BY GLUCOSE DEGRADATION PRODUCTS.

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Main Objectives:
Long term peritoneal dialysis causes peritoneal fibrosis and decrease of peritoneal function. Glucose degradation products (GDPs), including methylglyoxal (MG), are one of the major causes of peritoneal membrane injury in peritoneal dialysis patients. Cell transplantation is considered one of the possible therapies to recover injured peritoneal membrane. In this study, we evaluated the possibility of peritoneal mesothelium cell transplantation to injured peritoneum caused by GDPs treatment in rats.

Strategy and Methods:
Sprague-Dawley (SD) rats were administered with 0, 1 or 20mM MG intraperitoneally. Three days after administration, injury of peritoneal mesothelial cells (PMCs) were evaluated by measurement of mRNA expression of epithelial mesenchymal transition (EMT) markers in primary cultured PMCs from treated animals by RT-PCR. Primary cultured PMCs derived from GFP transgenic rats were injected intraperitoneally to another SD rats 3 days after 0, 1 or 20 mM MG administration. Attachment of transplanted PMCs was evaluated by fluorescent microscopic observation of primary cultured PMCs or immunohistochemical observation of peritoneum from treated animals on 7th, 14th and 21st day after transplantation.

Main Results:
mRNA expression of mesenchymal markers, α-smooth muscle actin (α-SMA) and matrix metallopeptidase 2 (MMP2), were significantly increased, while mRNA expression of epithelial marker, cytokeratin 18 and ZO-1, was significantly decreased in 20 mM MG treated rats compared to 0 and 1 mM MG treated rats. Fluorescent microscopy detected GFP positive cells in primary cultured PMCs derived from 20 mM MG treated rats on 7th day after transplantation, while no GFP positive cells were detected in those of 0 and 1 mM MG treated rats. Immunohistochemical analysis also detected GFP positive cells in 20 mM MG treated rat on 21st day after transplantation.

Conclusions:
Single administration of 20 mM MG increased EMT in PMCs, while single injection of 1 mM MG could not induce EMT in detectable level. Transplanted PMCs could be attached only to injured peritoneum and survive for at least 3 weeks.
CARBONYL STRESS AND INAPPROPRIATE RENIN ANGIOTENSIN SYSTEM ACTIVITY ASSOCIATE WITH BLOOD PRESSURE ELEVATION

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Main Objectives: Results in the previous study have suggested that carbonyl substances and oxidative stress are associated with clinical parameters of diabetes, hypertension and chronic kidney diseases (CKD). Renin-angiotensin system (RAS) and inflammation are involved in the pathogenesis of hypertension. In various organs, local RAS is independently regulated from circulating RAS. Thus, we hypothesized that the urinary carbonyl/oxidative stress and intrarenal RAS markers could be a predictor for hypertension. In this study, we evaluated that the relationship between carbonyl/oxidative stress, intrarenal RAS and hypertension in young adults and adults.

Strategy and Methods: Urinary carbonyl compounds, monocyte chemotactic protein (MCP)-1, NaCl, thiobarbituric acid reaction substance (TBARS) excretion levels, as markers of carbonyl stress, inflammation, Na intake, and oxidative stress were evaluated using samples from young adults (men; 1794, 18.7±0.4 years old) and adults (men; 161, women; 194, age; 66.6 ± 14.4 years old) obtained at health check-ups. And urinary angiotensinogen level as a marker of intrarenal RAS activity of selected 111 young adult men was evaluated. Correlations were determined among the parameters in the community health checkup. In the young adults, association of carbonyl compounds and elevation of blood pressure were determined by comparison between 2008 and 2010.

Main Results: Urinary carbonyl compounds levels were significantly high in the elevated blood pressure group among 2 years compared to non-elevated blood pressure group in young adults. In obese young adults, blood pressure, urinary oxidative stress marker and inflammation marker level were significantly increased compared to those in non-obese young adults. Urinary carbonyl stress and intrarenal RAS activity marker and NaCl levels, but not oxidative and inflammation markers, were significantly high in hypertension group compared to normotension group in adults. Multiple linear regression analysis results showed that urinary carbonyl stress and intrarenal RAS activity marker levels were significantly associated with blood pressure in adults.

Conclusions: We conclude that urinary excretion of carbonyl compound and angiotensinogen would predict the pathogenesis of hypertension and renal injury.
P3 ANTI-INFLAMMATORY EFFECT OF GLYCATION PRODUCTS DERIVED FROM HIGH HYDROSTATIC PRESSURE ENZYMATIC HYDROLYSATE OF FLATFISH BYPRODUCT AND ITS MECHANISM

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Main Objectives: Flatfish byproducts were hydrolyzed by Protamex at high hydrostatic pressure and glycated with ribose to utilize protein of flatfish byproducts as a nutraceutical.

Strategy and Methods: Flatfish byproducts were hydrolyzed by Protamex at high hydrostatic pressure and glycated with ribose. Anti-inflammatory effects of glycated fish protein hydrolysate (GFPH) were investigated and their anti-inflammatory mechanisms were elucidated in lipopolysaccharide (LPS)-stimulated RAW264.7 mouse macrophage.

Main Results: GFPH suppressed LPS-induced production of nitric oxide (NO) and prostaglandin E2 (PGE2) and expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) with dose dependent manner. Enzyme-linked immunosorbent assay (ELISA) kit clearly demonstrated that GFPH significantly reduced the productions of pro-inflammatory cytokines such as, interleukin (IL)-6, interleukin (IL)-1β and tumor necrosis factor (TNF)-α, and monocyte chemoattractant protein (MCP)-1. Moreover, GFPH reduced nuclear factor κB (NF-κB) and mitogen-activated protein kinases (MAPKs) activation.

Conclusions: These results indicated that the inhibitory effects of GFPH on LPS-induced NO and PGE2 production might be due to the suppression of NF-κB and MAPKs signaling pathway. Therefore, flatfish byproducts is latent bioactive resources and GFPH may have potential as a therapeutic agent for treatment of various inflammatory diseases.
Main Objectives: At hyperglycemia and chronic kidney insufficiency heightened levels of methylglyoxal (MG) are accumulating in blood plasma. MG is also directly forming in red blood cells (RBC) if glucose catabolism is disordered. The damaging MG action is connected with its possibility both to glycate proteins and lipids, and to generate some free-radicals including Schiff bases cation-radical (dialkylimine), MG anion-radical (semidion) and superoxide anion-radical (O$_2^\cdot$). These reactive compounds can influence components of RBC membrane and hemoglobin (Hb). The main mechanism of such modification is the Maillard reaction.

Strategy and Methods: We have earlier shown that NO function in non-enzymatic glycation process is ambiguous. It can both cut reactions leading to advanced glycation end products and initiate forming of free-radical intermediates [1,2]. NO effects depend on its concentration in the system and on the nature of NO donor. The goal of our work was to study influence of main physiological NO donors (nitrosothiols and dinitrosyl iron complexes so as nitrite ions forming during their oxidation) on Hb.

Main Results: Nitrosoglutathione in MG-containing system generated free-radical intermediates (GS-NO$,^\cdot$ ONOO$^-$, and NO$_2^\cdot$). They led to formation of reactive Hb forms (porphyrin$^-\cdot$-Fe(III)-OON, porphyrin$^-\cdot$-Fe(IV)=O, porphyrin-Fe(IV)=O) which participated in nitration and oxidation reactions of cytoskeleton proteins and membrane lipids. These reactions provoked appearance of Hb aggregates and membrane-bound Hb (MBHb). NO$_2^-$ effects were less evident and took place after its reduction to NO.

MG action on RBC suspension led to dose-dependent increasing of MBHb concentration. Correlation of MBHb content and amount of reduced SH-groups of membrane proteins was negative. Addition of NO metabolites to RBC suspension in presence of MG had different results: NO$_2^-$ decreased MBHb concentration by 50%, Cys-NO by 20%, but GS-NO increased it by 9%. Such action of nitrosothiols was connected with formation (in MG-RNH$_2$-RSNO system) of free-radical intermediates promoting Hb binding to RBS membrane.

Conclusions: Influence of NO metabolites (nitrosothiols and in less extent nitrite ions) on processes in RBC under carbonyl stress and destructive Hb modification are conditioned by ability of these metabolites to form reactive free-radical intermediates which can be both initiators and inhibitors of glycation and oxidation reactions.

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**Main Objectives:** Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD). Mesangial cells play a central role in the pathogenesis of diabetic nephropathy. Advanced glycation end products (AGEs) play the pivotal role in the development and progression of diabetic damage and disturbed glomerular homeostasis by inducing human mesangial cell apoptosis. Lots of observations suggested the pathological role for the AGE-RAGE (receptor for AGEs) pathway in glomerular sclerosis and proteinuria. Autophagy is a tightly regulated system in which the endogenous cellular protein aggregates and damaged organelles are degraded via the lysosomal pathway. Despite the emerging evidences suggested that AGEs-induced ER stress, apoptosis, and autophagy participate in podocyte and tubule cell injury in diabetic nephropathy, the involvement of ER stress, apoptosis, and autophagy in AGEs-induced mesangial cell injury in diabetic nephropathy remains unclear. Here, we investigated the effects of AGEs on the activation of autophagy and ER stress, which might influence the growth and function of mesangial cells.

**Strategy and Methods:** Mouse mesangial cells (MMCs) were obtained from Cell Bank in Food Industry Research and Development Institute, Hsinchu, Taiwan. MMCs were treated with 10, 20, 40, 80 and 160 µg/ml AGEs to evaluate the cell viability by MTT assay and flow cytometry. The protein expressions were measured by Western blot assay. Furthermore, MMCs were treated with 3-Methyladenine (3MA), siATG5 and 4-phenylbutyric acid (4PBA) to study the influence of autophagy and ER stress.

**Main Results:** AGEs significantly decreased cell viability in a dose-dependent manner. And the result of flow cytometry indicated that the decreasing of cell viability might due to apoptosis. Further, AGEs induced the protein expressions of LC3-II, p-eIF2α, CHOP and caspase-3 in MMCs in a dose-dependent manner. Also, pre-treatment of 3MA reversed the decreasing of cell viability and treatment with 4PBA reversed the number of apoptotic cells. Finally, treatment with siATG5 reversed the protein expressions of LC3-II, p-eIF2α, CHOP and caspase-3. Therefore, knockdown of autophagy might reverse AGEs-induced ER stress and apoptosis.

**Conclusions:** AGEs impaired cell viability and induced ER stress, apoptosis, and autophagy in MMCs. Inhibition of ER stress reduced apoptosis and autophagy. Inhibition of autophagy increased apoptosis response. AGEs-induced ER stress-regulated autophagy plays a protective role in MMCs.
ADVANCED GLYCATION END PRODUCTS DOWNREGULATED HEAT SHOCK PROTEIN 60 IN ISLET β-CELL HYPERTROPHY AND DYSFUNCTION

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Main Objectives: Islet mitochondria in diabetic patients show large structural and biochemical alterations. Heat shock protein 60 (HSP60), a mitochondrial chaperone, is also located in mature secretory granules and mitochondria of β-cells. Advanced glycation end products (AGEs) have been shown to directly modulate the β-cell function. The interaction between AGEs and HSP60 in the β-cell function and their role in diabetic β-cell dysfunction remain unclear. Here, we investigated the role of HSP60 in AGEs-induced β-cell hypertrophy and dysfunction using a cultured β-cell model.

Strategy and Methods: AGE-BSA was prepared by BSA and glyceraldehyde. Rat pancreatic β-cell line RINm5f cells were used for in vitro study. The cell viability, cell diameter, and cell hypertrophy index were detected. Protein expression was determined by Western blotting. Insulin content and secretion were measured by ELISA kit.

Main Results: Low/non-toxic-concentration AGEs significantly increased the induction of cell hypertrophy and p27^Kip1 expression and decreased the HSP60 expression, insulin secretion, and ATP content in cultured β-cells, which could be reversed by receptor for AGE (RAGE) neutralizing antibody. HSP60-overexpressing β-cells showed cytoprotective effects against AGEs-induced cell hypertrophy, β-cell dysfunction, and reduction in ATP content. Moreover, AGEs significantly increased the reactive oxygen species (ROS) production, which could be reversed by RAGE neutralizing antibody. Antioxidant N-acetyl-L-cysteine could significantly reverse the AGEs-reduced HSP60 expression.

Conclusions: These findings highlight a novel mechanism by which HSP60 is a possible target for AGEs-RAGE axis-induced β-cell hypertrophy and dysfunction under diabetic condition. Moreover, oxidative stress seems to be involved in the AGEs-reduced HSP60 expression.
STABILITY OF ADVANCED GLYcation AND OXIDATION END PRODUCTS (OPS) IN STOREd BLOOD AND URINE SAMPLES FROM DIABETES OUTCOME STUDIES

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Main Objectives: Methods for the collection, long-term storage, and processing of blood and urine samples from diabetes clinical outcome are critical to confirm sample integrity when measuring multiple AGEs and oxidation end products (OPs), since improper handling or storage can lead to artifactual sample modification. Therefore, we tested for sample stability in stored samples from two long-term landmark clinical outcome studies through rigorous assessments of variability and downward or upward trends in analyte profile over time.

Strategy and Methods: Documentation of the stability of AGE and OP profiles in stored plasma and urine samples was evaluated at four time points over 10 years in 60 subjects that were a randomly chosen sub-group of the Diabetes Control and Complications Trial (DCCT/EDIC) study. In addition, serum samples collected at baseline and yearly thereafter over 8-10 years were analyzed from 10 randomly selected subjects (80 samples) in the Pima Indian Nephropathy Study (PINS). DCCT/EDIC samples were collected between 1983 and 1996, and for PINS between 1996 and 2006, and were stored at -80°C. Six AGEs and 4 OPS were measured by LC-MS/MS in samples that were collected and stored by a strictly defined protocol. Various statistical methods were employed to assess the variation in analyte measurements among samples obtained at different points in time.

Main Results: We observed that no significant decay or rise in the levels of products occurred over time in DCCT/EDIC plasma and urine samples or PINS serum by showing that deviation, coefficients of variation (COV), intra-class correlation, regression, and mixed models remained constant for individual analytes and did not vary significantly over time. It was also determined that the levels of AGEs and most oxidative end products were reproducible over time, based on intra-class correlation calculations from repeated measures within subjects. The stability of the stored plasma and urine samples over time was also validated by examining their association with another marker measured over the duration of the DCCT/EDIC (HbA1c). We also found that between subject variability exceeded within subject variability for these timed collections for the DCCT/EDIC and PINS samples.

Conclusions: These studies confirm that the quality and chemical stability of plasma, serum and urine levels of AGEs and OPs stored from long-term diabetes clinical outcome studies samples will yield valid results and correct outcomes if proper complication phenotypes are characterized.
AMADORI-GLYCATED PHOSPHATIDYLETHANOLAMINE INDUCES CELLULAR TELOMERASE ACTIVITY

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Main Objectives: Several lines of data have highlighted a key role of Amadori-glycated phosphatidylethanolamine (Amadori-PE) in the development of diabetic complications. Numerous epidemiologic studies suggest that type 2 diabetes significantly increases overall cancer risk and mortality. A recent meta-analysis estimated that individuals with diabetes have a two-fold greater relative incidence of pancreatic cancer compared with individuals without diabetes. Although there are many epidemiologic researches suggesting the involvement of diabetes in tumor development, little is known regarding the molecular mechanism underlying this phenomenon to date. Hence, we hypothesized that Amadori-PE may participate not only in the pathogenesis of diabetic complications but also in tumor progression. In the present study, this hypothesis was investigated in cell-culture study, with particular emphasis on the effect of Amadori-PE on telomerase activity which contributes to the infinite replicative potential of cancer cells.

Methods and Results: PANC-1 human pancreatic carcinoma cells were cultured in medium containing Amadori-PE, and telomerase activity was then examined by stretch PCR assay. Amadori-PE increased cellular telomerase activity of PANC-1 in time- and dose-dependent manner. On the other hand, intact PE had virtually no effect on telomerase activity, indicating that the telomerase activation was specific to Amadori-PE, but not to intact PE. Amadori-PE elicited telomerase activity by up-regulating hTERT (human telomerase reverse transcriptase) mRNA expression through induction of c-myc. The fluorometric assay for 2',7'-dichlorofluorescein revealed that Amadori-PE significantly induced intracellular reactive oxygen species (ROS) levels in PANC-1. We also found that telomerase activity was decreased by a combination treatment of Amadori-PE and alpha-tocopherol, an antioxidant vitamin, suggesting that Amadori-PE up-regulates telomerase activity through intracellular ROS generation.

Conclusion: These results indicate for the first time that Amadori-PE may be an important compound that promotes tumor progression as a result of telomerase activation and provide experimental evidence for a novel role of lipid glycation in linking diabetes and cancer.
Main Objectives: Non-alcoholic steatohepatitis (NASH) is a liver disease characterized by hepatic lipid accumulation and inflammation. Patients with NASH, but not steatosis alone, were shown to have a higher incidence of cardiovascular disease (CVD)-related death, suggesting that inflammation plays a key role in modulating CVD risk. However, the exact trigger of the inflammation in NASH is unclear. We have previously shown accumulation of advanced glycation endproducts (AGEs) in the fatty liver. We now hypothesize that AGEs play a role in the onset of inflammation in the liver via their receptor, RAGE. Therefore, we investigated the role of immune cell-specific RAGE-deficiency in NASH development.

Strategy and Methods: Bone marrow transplantation (BMT) was performed to produce immune cell-specific wildtype (wt) or RAGE knockout (ko) mice which were fed a chow diet or a western type diet (WTD) for 1 or 3 weeks to induce NASH. Gene expression was measured by qPCR in the liver and immunohistochemistry was performed to identify hepatic immune cells. Immune cell count and cytokine levels in the blood were determined using FACS analysis and ELISA, respectively.

Main Results: On a chow diet, RAGEko-BMT mice showed reduced inflammatory gene expression of hepatic inflammatory markers (TNF, MCP1) and of F4/80, a macrophage marker, while a T-cell marker (CD3) was unaffected and expression of a B-cell marker (CD19) increased. In plasma we observed a reduction of several pro-inflammatory plasma cytokines (mKC (IL-8), IFN-γ, IL-6) in RAGEko-BMT mice in comparison to the RAGEwt-BMT mice on chow. In contrast, inducing NASH with a WTD, induced a stronger hepatic inflammatory response in RAGEko-BMT mice illustrated by higher gene expression of TNF, MCP1, mKC and of the macrophage marker F4/80, but no difference between T- and B-cell markers. In line with hepatic gene expression, circulating CD4+ T-lymphocytes, B-lymphocytes and natural killer cells were increased more in RAGEko-BMT mice than in RAGEwt-BMT mice after 1 week of WTD. Blood monocytes and CD8+ T-cells were unaffected. Hepatic triglyceride accumulation and plasma cholesterol levels did not differ between RAGEwt- and ko-BMT mice.

Conclusions: In control conditions, bone marrow (BM) specific RAGE deletion reduced hepatic inflammation. Surprisingly, BM-specific RAGE deficiency resulted in a stronger inflammatory response in our NASH model.
Main Objectives: Nω-Carboxymethylarginine (CMA), an advanced glycation end-product (AGE), was found in glycated type I collagen (Iijima, 2000, Biochem. J). It was also reported that CMA was detected in human serum protein by using LC-MS/MS and its level was significantly elevated in diabetic patients (Odani, 2001, BBRC). In generally, enzymatic digestion method was used to isolate CMA from tissues because of its unstableness in acid hydrolysis.

Strategy and Methods: We found a small amount of CMA was remained after acid hydrolysis. We have improved quantitative method to measure CMA stably and accurately by acid hydrolysis. We quantified CMA and other AGEs in tissues of STZ mice and compared with control mice.

Main Results and Conclusions: In this report, we proposed the quantification method of CMA in tissues by using acid hydrolysis and LC-MS/MS.
Main Objectives: Nω-(carboxymethyl)arginine (CMA) is identified in glycated collagens, and subsequent study demonstrated that CMA is generated specifically on collagens. Since collagen is the most abundant skeletal proteins which form many organs such as skins, blood vessels and bones, the measurement of CMA would be a suitable marker for glycation of organs. However, since CMA is acid labile and difficult to measure in physiological sample by instrumental analysis, the physiological properties of CMA remains poorly understood. In the present study, CMA content was measured in serum and collagens by liquid chromatography tandem mass spectrometry (LC-MS/MS) to evaluate the physiological significance of CMA.

Strategy and Methods: Arginine or 13C-arginine were incubated with glyoxal in 0.1M NaOH solution, then CMA and 13C-CMA were purified by Dowex 50 and silica gel chromatography. Low molecular weight fraction (LMWF) of serum was obtained by filtration with molecular weight cut-off membrane. Furthermore, the stability of CMA under acid or alkaline conditions were also analyzed by LC-MS/MS. CMA was detected by LC-MS/MS (TSQ Vantage triple stage quadrupole mass spectrometer, Thermo Scientific). LC was conducted on a ZIC®-HILIC Column (150 x 2.1 mm). Parent ion and product ion of m/z CMA were 233 and 116, respectively.

Main Results: CMA content in LMWF of serum in patients with kidney failure was significantly higher than control subjects. CMA was detected by LC-MS/MS in glycated collagen after acid hydrolysis and subsequent alkaline treatment. Furthermore, CMA was also detected in human atherosclerotic lesions.

Conclusions: Although the measurement of CMA by LC-MS/MS in physiological samples has been considered difficult because of its instability, it became possible to analyze not only in serum with molecular weight cut-off membrane but also in organs with acid and subsequent alkaline treatment.
INSTRUMENTAL DETECTION OF GA-PYRIDINE, ONE OF GLYCOLALDEHYDE DERIVED AGES, IN HUMAN ATHEROSCLEROSIS LESIONS

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Main Objectives: Advanced glycation end-products (AGEs) accumulate in various tissues, and is known to be involved in the development of age-related diseases. Our previous studies showed that GA-pyridine, one of AGEs derived from myeloperoxidase system through glycolaldehyde, is accumulated in atherosclerotic plaques, and it may be important role in the progression of pathological condition (Nagai R et. al J. Biol. Chem., 277(50): 48905–48912, 2002). Although GA-pyridine was detected only by immunohistochemical analysis, there is no report to demonstrate the presence of GA-pyridine by an instrumental analysis. In the present study, we studied the suitable procedures for GA-pyridine analyses by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Strategy and Methods: Carotid endarterectomy specimens from human carotid artery stenosis was hydrolyzed by 6N HCl, and GA-pyridine was analyzed by LC-MS/MS using a TSQ Vantage triple stage quadrupole mass spectrometer (Thermo Scientific). LC was conducted on a ZIC®-HILIC Column (150 x 2.1 mm, 5 μm). Parent ion and product ion of \( m/z \) GA-pyridine ware \( m/z \) 255 and 130, respectively.

Main Results: GA-pyridine was detected in human carotid artery and atherosclerotic plaque by LC-MS/MS. In addition, GA-pyridine was also detected in serum in patients with nephropathy.

Conclusions: This study provided the first instrumental evidence that GA-pyridine is accumulated in human carotid artery. Since GA-pyridine was also detected in serum in patient with nephropathy, the multiple AGEs monitoring including GA-pyridine in serum would be an effective means to evaluate a progress of inflammation and atherosclerosis.
MG-H1, WHICH IS DERIVED FROM METHYLGLYOXAL, IS RELATED TO RENAL DYSFUNCTION IN PATIENTS WITH CHRONIC KIDNEY DISEASE INDEPENDENTLY OF DIABETES MELLITUS

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\textbf{Main Objectives:} Intermediate carbonyls and advanced glycation end products (AGEs) are thought to be related to the development of complications of patients with chronic kidney diseases. Especially, N\textsuperscript{\varepsilon}-(carboxymethyl) lysine (CML) has been primarily studied in persons with diabetes or end-stage renal disease as a modifiable risk factor of renal disease. However, each AGE is known to contribute to the different pathology, and the pathophysiology of another AGE is still unclear. The objective is to characterize the relationship between AGEs and renal function, especially in terms of N\textsuperscript{\varepsilon}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) level as a biomarker of carbonyl stress.

\textbf{Strategy and Methods:} The total of 50 patients who admitted in our hospital between April and November 2014 was enrolled in this study (33 men and 17 women, average age: 60.9 years; range 19-88 years). Patients who received corticosteroid and renal replacement therapies were excluded from the analysis. Serum levels of CML and MG-H1, the predominant AGEs, were measured by using liquid chromatography - tandem mass spectrometry, and subjected to a cross-sectional analysis to determine their correlation correlated with renal function.

\textbf{Main Results:} CML and MG-H1 levels were correlated with renal dysfunction status (Spearman rank correlation coefficient: CML vs. estimated glomerular filtration rate [eGFR], \( r = -0.784, p < 0.01 \); MG-H1 vs. eGFR, \( r = -0.691, p < 0.01 \)). In the multivariate logistic regression analysis, advanced renal failure (eGFR < 30mL/min/1.73m\textsuperscript{2}) was associated with higher serum CML levels (higher than the median; odds ratio [OR], 54.43; 95\% confidence interval [CI], 5.83-446.55; \( p < 0.01 \)) after adjusting for age, diabetes mellitus (DM), and systolic blood pressure. Advanced renal failure was also associated with higher serum MG-H1 levels (higher than the median; OR, 20.44; 95\% CI, 3.89-107.49; \( p < 0.01 \)) after adjusting for age, DM, systolic blood pressure, and a history of cardiovascular disease.

\textbf{Conclusions:} Renal dysfunction is strongly associated with serum MG-H1 as well as serum CML. In future, we attempt to clarify what clinical condition MG-H1 is linked to in patients with chronic kidney disease.
PLASMA sRAGE, esRAGE BUT sVAP-1 DECREASE WITH NUMBER OF CARDIOMETABOLIC RISK FACTORS IN APPARENTLY HEALTHY ADOLESCENTS

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Main Objectives: Levels of soluble receptor for advanced glycation end products (sRAGE) decrease with increasing number of cardiometabolic risk factors in apparently healthy subjects. It remains unclear whether changes occur in parallel in sRAGE cleavage versus spliced (endogenous secretory, esRAGE) isoforms. Vascular adhesion protein-1 (VAP-1) presents an activity of semicarbazide-sensitive monoaminooxidase, thus it could contribute to formation of AGE directly (formation of glyoxal, methylglyoxal) and indirectly via H2O2 production. Whether sVAP-1 associates with cardiometabolic risk factors remains unknown. We studied the association of sRAGE, esRAGE, and soluble VAP-1 (sVAP-1) with cardiometabolic risk factors in apparently healthy adolescents.

Strategy and Methods: Into cross-sectional study 1001 female and 954 male non-diabetic adolescents, aged 16-19-years, were included. Elevated blood pressure (BP, systolic BP ≥ 130 mm Hg and/or diastolic BP ≥ 85 mm Hg); insulin resistance (insulin sensitivity check index QUICKI ≤ 0.318); central obesity (waist-to-height ratio ≥ 0.50), and increased atherogenic index of plasma (AIP = log(TAG/HDL-C) ≥ 0.11) were considered as cardiometabolic risk factors. According to their presence adolescents were classified into 4 groups (G): G0: risk factors-free; those presenting one (G1), two (G2), and ≥ 3 (G3) risk factors.

Main Results: 55% of males (M) were risk factors-free, 30% presented one, 9% two, and 6% ≥ 3 risk factors. sRAGE, esRAGE but sVAP-1 levels decreased with the number of cardiometabolic risk factors both in males and females (table). In males sRAGE and esRAGE showed significant inverse relationship with SBP, DBP and waist-to-height ratio. Among females (F) 74% were risk factors-free, 20% presented one, 4% two and 2% ≥3 risk factors. In females sRAGE and esRAGE showed significant inverse relationship with waist-to-height ratio and a direct relationship with QUICKI. esRAGE and sVAP-1 correlated inversely with atherogenicity of plasma.

<table>
<thead>
<tr>
<th>G0</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>M: sRAGE (ng/ml)</td>
<td>1.72±0.58</td>
<td>1.65±0.60</td>
<td>1.54±0.57</td>
<td>1.53±0.47</td>
</tr>
<tr>
<td>M: esRAGE (ng/ml)</td>
<td>0.36±0.15</td>
<td>0.34±0.16</td>
<td>0.32±0.16</td>
<td>0.32±0.15</td>
</tr>
<tr>
<td>M: sVAP-1 (ng/ml)</td>
<td>382±164</td>
<td>375±155</td>
<td>395±200</td>
<td>382±146</td>
</tr>
<tr>
<td>F: sRAGE (ng/ml)</td>
<td>1.66±0.60</td>
<td>1.52±0.52</td>
<td>1.44±0.48</td>
<td>1.23±0.30</td>
</tr>
<tr>
<td>F: esRAGE (ng/ml)</td>
<td>0.35±0.16</td>
<td>0.32±0.15</td>
<td>0.29±0.11</td>
<td>0.26±0.14</td>
</tr>
<tr>
<td>F: sVAP-1 (ng/ml)</td>
<td>383±181</td>
<td>403±226</td>
<td>344±99</td>
<td>415±152</td>
</tr>
</tbody>
</table>

Conclusions: In apparently healthy adolescents both sRAGE and esRAGE decrease with increasing number of cardiometabolic risk factors. Our data suggest that cardiometabolic risk factors associated with this decline differ between the genders.

Supported by APVV grant No.: 0447-12
THE EFFECT OF 1,25-DIHYDROXYVITAMIN D ON GENE AND PROTEIN EXPRESSION OF ENZYMES PROTECTING FROM GLUCOLIPOTOXICITY IN VITRO

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Main Objectives: Beside its classical function as a regulator of calcium and phosphorus homeostasis, vitamin D also affects insulin secretion and its action in target tissues. Number of studies consistently reports inverse relationship between vitamin D deficiency and type 2 diabetes. We hypothesise that activation (including therapeutic augmentation) of certain metabolic pathways and down-stream transcription factors by vitamin D may protect from glucolipotoxicity and the lack of thereof might explain the detrimental role of vitamin D deficiency in diabetes. The aim of the study was to quantify gene and protein expression of selected enzymes involved in protection from glucolipotoxicity, specifically glyoxalase 1 (GLO1), and other enzymes with antioxidant activity — hemoxygenase (HMOX), superoxide dismutase 2 (SOD2), enzyme with glyoxalase activity DJ1, thiamin pyrophosphokinase (TPK1) and transketolase (TKT), in normo- and hyperglycaemic conditions and upon addition of vitamin D in peripheral blood mononuclear cells (PBMCs) in vivo and human umbilical vein endothelial cells (HUVEC) in vitro.

Strategy and Methods: Peripheral blood samples were provided by healthy donors (n = 6). PBMCs separated using Histopaque were pooled and cultured 24 hours in RPMI medium containing 5 or 25mM glucose with or without 100 nM 1,25-dihydroxycholecalciferol or the same amount of solvent (ethanol). HUVEC were cultured in the same conditions. RNA was isolated and reverse transcribed using commercial kits. Total protein was isolated using RIPA buffer. Gene expression of genes of interest was determined using quantitative PCR with predesigned probes (TaqMan™ Assay) with β-actin as a reference gene. Protein expression was determined using immunoblot analysis with specific antibodies and β-actin as a control.

Main Results: In PBMC vitamin D significantly increased gene expression of TKT (by 90 – 100 %, P < 0.01), GLO1 (by 110 – 130 %, P < 0.01) and TPK (90 – 100 %, P < 0.01) in both normo- and hyperglycaemia compared to normo- and hyperglycaemia without vitamin D. Gene expression in HUVEC did not significantly change. Vitamin D did not affect protein levels of targets studied in neither cell type.

Conclusions: The results of our study indicate that the active form of vitamin D regulates gene expression of enzymes opposing harmful effect of glucolipotoxicity whose activities appear suppressed by hyperglycaemia. However, we were unable to confirm this effect on protein level. Enzyme activities will be further studied. Our results support the role of vitamin D in the protection against glucolipotoxicity therefore possibly translating into the prevention of development of diabetic complications.

Acknowledgement: Supported by the grant NT13198 from the Ministry of Health of Czech Republic.
**THE EFFECT OF GLUCOSE VARIABILITY ON THE EXPRESSION OF GENES INVOLVED IN HYPERGLYCAEMIA-INDUCED TISSUE DAMAGE**

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¹Masaryk University, Brno, Czech Republic; ²Charles University, Prague, Czech Republic

**Main Objectives:** Large prospective studies provided evidence of the relationship between long-term diabetes compensation expressed as HbA₁c and the risk of diabetic cardiovascular complications. However, HbA₁c and diabetes duration explain only small portion of the complications risk. Patients with the same HbA₁c differ in amplitudes and duration of glycaemic excursions (so called glucose variability [GV]). Limited number of animal and in vitro studies showed that oscillating glucose may have more deleterious effect on cells than hyperglycaemia itself. Therefore, the aim of our study was to evaluate the effect of GV on the expression of genes whose products are involved in the development and protection against tissue damage in diabetes. Specifically, we measured gene expression of glyoxalase 1 (GLO1), and other enzymes with antioxidant activity – hemoxygenase (HMOX), superoxide dismutase 2 (SOD2) and enzyme with putative glyoxalase activity DJ1 in vitro.

**Strategy and Methods:** Primary human umbilical vein endothelial cells (HUVEC) were cultured 24 hours in conditions mimicking real situation in humans (derived from 24hrs continuous glucose monitoring curves both in diabetics and healthy) and in commonly used in vitro hyperglycaemia model: (A) high GV [SD>5], (B) low GV [SD<3], (C) physiological GV of non-diabetics [SD=1], (D) continuous normoglycaemia (5 mmol/l) and (E) continuous hyperglycaemia (25 mmol/l). After 24 hours all cells were harvested, RNA isolated and reverse transcribed using commercial kits (Roche). Gene expression was determined using quantitative PCR with predesigned probes (TaqMan™ Assay) with β-actin as a reference gene.

**Main Results:** HMOX expression was significantly lower in both low and high GV compared to GV of healthy subjects (P=0.04, Mann-Whitney). Similar pattern was found in SOD2, GLO1 and DJ-1 although statistically not significant (all P>0.05, Mann-Whitney). Expression of all genes with exception of DJ-1 was significantly higher in continuous normoglycaemia when compared to low and high GV (all P<0.04, Mann-Whitney). Finally, the effect of continuous hyperglycaemia was similar to low and high GV (all P>0.05, Mann-Whitney).

**Conclusions:** Results of our pilot study indicate that oscillating glucose (GV clinically observed in diabetic patients) can have equally as deleterious effect as continuous hyperglycaemia of extreme value commonly used in experimental settings. GV can be therefore considered additional risk factor of glucotoxicity.

**Acknowledgement:** Supported by the grant NT13198 from the Ministry of Health of Czech Republic.
**P17** DICARBOXYL STRESS IMPAIRS FUNCTION OF PERIODONTAL LIGAMENT FIBROBLASTS IN MODEL HYPERGLYCAEMIA

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**Background and aim:** Periodontal ligament inflammation (periodontitis) is a common disease characterized by gradual destruction of connective tissue fibres that attach a tooth to the alveolar bone within which it sits. Diabetes and inflammation enhance periodontal bone loss through enhanced resorption and diminished bone formation. PDL fibroblast attachment to type 1 collagen and function was impaired by methylglyoxal (MG) modification in vitro. We hypothesise that increased PDL detachment and dysfunction may be exacerbated by increased endogenous MG in hyperglycaemia associated with diabetes. The aim of this study was to evaluate the effects of high and low glucose concentrations on MG metabolism in human periodontal ligament fibroblasts (hPDLFs) with and without resveratrol (RSV) treatment.

**Materials and methods:** Primary hPDLFs were cultured for three days in medium containing low glucose (LG, 8 mM) and high glucose concentration (HG, 25 mM) to model hyperglycaemic conditions with and without RSV (10 µM). The activity of Glo1, D-glucose consumption and D-lactate formation were measured. The concentrations of MG and MG-derived AGE, MG-H1, were quantified by stable isotopic dilution analysis using LC-MS/MS. The efficiency of hPDLF adhesion to collagen type 1 was tested by colorimetric cell adhesion assay.

**Results:** In hPDLFs incubated with HG there was a significant decrease in Glo1 activity, increase in D-lactate flux, increase in cellular concentration of MG and increase in MG-H1 residue content of cell protein, compared to LG control. Decrease of Glo1 activity and increase in MG and MG-G1 residue content of cell protein was corrected with RSV treatment. The results are summarised in the table below. The efficiency of hPDLF adhesion to collagen type 1 was decreased by 37% in HG and was corrected by RSV.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Low glucose (8 mM)</th>
<th>High glucose (25 mM)</th>
<th>High glucose + RSV (10 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glo1 activity (mU/mg protein)</td>
<td>928 ± 84</td>
<td>529 ± 109**</td>
<td>964 ± 87***</td>
</tr>
<tr>
<td>D-Lactate (nmol per million cells/day)</td>
<td>7.00 ± 0.36</td>
<td>9.90 ± 1.08**</td>
<td>9.78 ± 0.78 ***</td>
</tr>
<tr>
<td>MG (pmol/million cells)</td>
<td>3.98 ± 1.00</td>
<td>10.6 ± 1.07</td>
<td>6.98 ± 0.89**</td>
</tr>
<tr>
<td>MG-H1 (mmol/mol arg)</td>
<td>0.431 ± 0.061</td>
<td>0.806 ± 0.184 *</td>
<td>0.560 ± 0.088”</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n = 4. Significance: *, ** and ***, P<0.05, P<0.01 and P<0.001, respectively, compared to LG; ** and *** P<0.01 and P<0.001 compared to HG.

The efficiency of hPDLF adhesion to collagen type 1 was decreased by 37% in HG and was corrected by RSV.

**Conclusion:** We conclude that hPDLFs suffer dicarbonyl stress and functional impairment in model hyperglycaemia in vitro which is corrected by RSV treatment.
Main Objectives: Glyoxalase1 is the key enzyme in the metabolism of reactive dicarbonyl metabolites, glyoxal and methylglyoxal, to less reactive products and prevention of formation of dicarbonyl-mediated advanced glycation endproducts (AGEs). Glo1 deficient mice and transgenic mice overexpressing Glo1 provide valuable models to study control of change in extent of dicarbonyl glycation in mammalian systems. The aim of this study was to characterise the genotype and phenotype of the Lexicon Glo1 mutant mouse.

Strategy and Methods: The Glo1 mutant mice (+/- breeding pair) were produced by Lexicon Pharmaceuticals Inc., USA. In a C57BL/6 genetic background, mutation was produced by retroviral insertion of a DNA cassette between coding exons 1 and 2 (LEXKO-1493). We maintained a colony of Glo1Lex heterozygotes and sibling wild-type controls (WT).

Genotyping Forty-four offspring were genotyped. For PCR, three pairs of primers were used to discriminate between WT, heterozygote and homozygote mutant mice. Mouse genome CNV microarray was used to identify the genomic duplicated region.

Phenotyping Nineteen mice, 12 Glo1Lex (+/-) and 7 WTs were sacrificed at 7 months old. Aliquots of tissues (brain, heart, liver, spleen, kidney, pancreas and skeletal muscle) were homogenised and Glo1 enzymatic activity was determined in cytosolic extracts by spectrophotometric assay, protein by Western blot and mRNA by RT-PCR. Protein damage markers were analysed in 10 Glo1Lex (+/-) and 10 WTs liver homogenate using LC/MS/MS and normalized to the corresponding protein.

Main Results: In the genotyping of Glo1Lex mutant mice we found only Glo1Lex (+/-) heterozygote and WTs. No homozygous Glo1Lex (-/-) mice have been born to date. No significant impairment in fertility was found for Glo1Lex (+/-) mice. In Glo1Lex mutant mice, at least one copy of duplicated area in the mouse genome react with 300 probes covering an area of 473,479 bp and includes a complete duplication of Glo1. Consequently the activity of Glo1 was not significantly different between WTs and Glo1Lex (+/-) mice (P>0.05, Mann-Whitney U). The expression of Glo1, as judged by protein and mRNA, in Glo1Lex mice was not significantly different from WT (P>0.05, Mann-Whitney U). Levels of methylglyoxal-derived AGEs were not significantly different between WTs and Glo1Lex (+/-) mice (P>0.05, Mann-Whitney U).

Conclusions: Glo1Lex mutant mouse appears embryonically lethal for homozygous inheritance. It has compensatory increased Glo1 copies, copy number alternation, and expression with normal fertility. The mutant mouse appears to have been incorrectly genotyped in preliminary characterisation by the originator.
ENHANCED UPTAKE OF A GLYCATED FOOD ALLERGEN
BY IL-4-TREATED HUMAN CULTURED MACROPHAGES

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Main Objectives: As the Maillard reaction occurs during thermal processing and storage of foods, a possible involvement of advanced glycation end products (AGEs) in the pathology of food allergy is of great concern. In this study, we aimed to investigate the interaction of AGEs of a food allergen with macrophages, which are crucial antigen presenting cells in initiating and maintaining immune responses in allergic diseases.

Strategy and Methods: AGEs of ovalbumin (OVA, a model food allergen) was prepared by thermal processing of OVA in the presence of glucose. Macrophages were generated by culturing peripheral blood derived monocytes from healthy donors in the presence of rGM-CSF for 8 days. During the last 2 days of the culture, the cells were treated with rIL-4, a cytokine playing a pivotal role in induction of allergic responses. The uptake mechanisms of macrophages for AGE-OVA were investigated using inhibitors of putative cell surface receptors for AGEs. The activation of macrophages by AGE-OVA was assessed by measuring production of cytokines (e.g. TNF-alpha and IL-6) and expression levels of maturation markers (e.g. CD80, CD86 and HLA molecules) in the cells.

Main Results: The uptake of AGE-OVA by macrophages was significantly higher than that of the controls, native OVA and thermally processed OVA. We identified scavenger receptor class A and the mannose receptor as the mediators of the AGE-OVA uptake, whereas galectin-3 and scavenger receptor class B were not responsible. Importantly, IL-4 enhanced the expression of the mannose receptor on the cell surface of macrophages, and promoted the AGE-OVA uptake. However, AGE-OVA itself did not induce cell activation. Stimulation of macrophages with AGE-OVA did not induce detectable levels of cytokine production and expression of maturation marker.

Conclusions: The uptake of AGEs of food allergens by macrophages could be promoted in allergic status, and thereby enhance allergen-specific immune responses. Our results indicate the significance of glycation products as a pathogenesis-related factor in food allergy.
RAGE PROMOTER METHYLATION IN VITRO AND IN VIVO

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¹Masaryk University, Brno, Czech Republic

Main Objectives: Sustained over-activation of the Receptor for Advanced Glycation End-products (RAGE) in diabetes is considered one of the pathways involved in hyperglycemia induced damage and chronic inflammation. One of the possible explanations might be epigenetic changes induced by high glucose, which were described to be responsible for the “metabolic memory” phenomenon. The aim of this study was to determine RAGE promoter methylation in cytosin-guanine dinucleotides (CpGs) in vitro in endothelial (HUVEC) and embryonic kidney (HEK-293) cell lines in hyper- vs. normoglycemic conditions and in vivo (peripheral blood cells, PBCs) in patients with T2DM with established diabetic kidney disease (DKD, n = 12) compared to healthy counterparts (n = 12).

Strategy and Methods: HEK-293 and HUVEC cells were cultivated in media mimicking normoglycemia (5.5 mM glucose) and hyperglycemia (25 mM glucose). DNA and RNA isolation and subsequent DNA modification by bisulphite conversion was performed using commercial kits. DNA and RNA of all participants were isolated from EDTA-blood using phenol-chloroform extraction and commercial kits. RAGE promoter sequence was divided into 3 parts containing 9 of total 12 CpGs and analyzed by DNA sequencing. RAGE promoter methylation was expressed as percentages of methylated CpGs on each position and overall. RAGE gene expression was analysed using commercial TaqMan® assays with beta-actin as a reference gene.

Main Results: RAGE promoter region was highly methylated in PBCs of both T2DM patients (99.1 %) and healthy subjects (100 %) and also in HEK-293 cells in both normo- (82.5 %) and hyperglycemic conditions (85.2 %) (both P > 0.05, Fisher exact test). The promoter region was completely methylated in HUVEC cells cultivated in normoglycemic conditions (100 %), however CpG position -286 bp was demethylated in hyperglycemia (33.3 %, putative binding site of IL-6). RAGE expression was higher in PBCs of T2DM patients compared to controls and HEK-293 cells in hyperglycemic conditions (P < 1×10⁻⁶ and P = 0.0002 respectively, one sample t-test), meanwhile RAGE gene expression did not change in HUVEC cells cultivated in hyperglycemic conditions (P > 0.05, one sample t-test).

Conclusions: We ascertained statistically significant differences in RAGE gene expression in T2DM patients with concomitant DKD. RAGE gene expression was increased in HEK-293 but not HUVEC cells in hyperglycemic conditions however this was not correlated to significant changes in CpG methylation in RAGE promoter region.

Acknowledgement: Supported by the grant NT11405 from the Ministry of Health of Czech Republic.
SERUM CARBOXYMETHYL-LYSINE, A DOMINANT ADVANCED GLYCATION END PRODUCT, IS INCREASED IN WOMEN WITH GESTATIONAL DIABETES MELLITUS

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2Institute of Molecular BioMedicine, Medical Faculty, Comenius University, Slovakia
3Diabetes Centre of the Dept. of Internal Medicine - Gastroenterology, University Hospital Brno, Czech Republic

Main Objectives: Gestational diabetes mellitus (GDM) is a common complication of pregnancy whose incidence is rising worldwide. Advanced Glycation End products (AGEs), are traditionally considered as a result of long-term dysregulation of glucose homeostasis, however detailed kinetic studies indicate a prompt reflexion of glucose metabolism. From this point of view - GDM represents a highly relevant phenotype to study acute changes of AGEs dynamics and its relationship to glucose metabolism. The objective of the study was to measure circulating AGEs - namely Nε-(carboxymethyl)lysine (CML) - in case-control study (n=307) of pregnant women with GDM and physiological pregnancies and to ascertain the factors contributing to CML levels and the potential relevance of CML for selected perinatal and postpartum outcomes.

Strategy and Methods: All subjects were Caucasians, Czech nationality. GDM screening was carried out using oral glucose tolerance test (oGTT) with 75g of glucose performed between 24th and 30th week of gestation and GDM was diagnosed according to WHO criteria. 222 women had GDM and 85 had physiologic pregnancy. CML was determined by ELISA using commercial kit.

Main Results: Unadjusted and plasma protein adjusted CML levels were significantly higher in women with GDM compared to healthy controls (P= 0.00043 and P=1x10⁻⁵, respectively, Mann-Whitney). CML was significantly inversely correlated with both pre- and mid-gestational BMI, however, differences between GDM and control group remained significant even after adjustment for BMI. CML levels correlated with 1-hr and 2-r post-load glycaemia during oGTT. Plasma CML or plasma protein adjusted CML levels did not significantly correlated with offspring birth weight or pregnancy weight gain (P=NS, Spearman).

Conclusions: In conclusion, we found statistically significantly higher protein- and BMI normalised CML levels measured during 24-30th week of gestation in women with GDM compared to healthy pregnant women. Further studies are warranted to more comprehensively asses the spectrum of AGEs in GDM and their relevance to future metabolic health of mother and baby.
P22

STRUCTURAL STUDIES ON THE SUGAR MOIETY OF COLLAGEN FLUOROPHORE LW-1 IMPLICATES THE PRESENCE OF GLUCURONIC ACID

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Main Objectives: We identified a major acid-labile molecule purified from an enzymatic digest of human insoluble skin collagen, named LW-1 (Arch. Biochem. Biophys. 493:192-206, 2010). It has a mass of 623 Da and fluorescent maxima at ex/em 348/463 nm. In autopsied skin, levels are significantly elevated by age, diabetes, and especially end-stage renal disease (ESRD). Although its full chemical structure is still elusive, NMR analysis showed that it has a lysine residue in an aromatic ring coupled to a sugar molecule that is reminiscent of AGEs. Further evidence by mass spectrometry (MS) shows a transition of m/z 623 (parent) → 447 (product) with elimination of 176 amu; i.e., a glycosidic cleavage amu frequently observed in MS studies on the metabolism of drugs by glucuronidation. The purpose of this study was to investigate the nature of the sugar moiety.

Strategy and Methods: LW-1 was purified from ~100 g insoluble collagen prepared from human skin from patients with diabetes/ESRD. The collagen was sequentially digested with five different proteolytic enzymes. LW-1 was purified by HPLC (C18 and Hypercarb) and analyzed by NMR: 1H-NMR, 13C-NMR, 1H-1H-TOCSY, 1H-13C-HSQC (single bond direct couplings), 1H,13C HMBC (long range couplings). In order to cleave the sugar from the molecule, purified LW-1 was separately incubated with glycosidases: β-glucosidase (I), β-galactosidase (II) and β-glucuronidase (III) followed by assaying levels by HPLC-MS and total fluorescence at ex/em 348/463 nm.

Main Results: HSQC showed four (1H,13C) signals with chemical shifts (ppm) at range (3.6-3.8, 73-78) and one anomeric proton-carbon signal at (5, 106) consistent with a sugar. TOSCY confirmed protons at 5 ppm were correlated with those at 3.6-3.8 ppm. The 13C spectrum showed four signals between 72-77 ppm (sugar carbons), an anomeric carbon signal at 106 ppm and a carboxylic acid signal at 175 ppm. HMBC linked a signal at (3.77, 106) with one at (3.7, 175) ppm. Only glucuronidase was able to cleave the sugar. Yield recoveries of LW-1 after digestion with I, II, III were 93, 95, 4% (based on MS) and 100, 98 and 15% (based on fluorescence), respectively.

Conclusions: The surprising results suggest glucuronic acid as the sugar moiety present in the LW-1 structure. Because of the high levels of toxins that accumulate during ESRD, a precursor for LW-1 molecule may originate as a glucuronide present in plasma which readily reacts with collagen suggesting an association between detoxification and protein modifications.
THE COMPARISON OF SKIN AUTOFLUORESCENT MARKERS FOR FUTURE RISK IN THE DEVELOPMENT AND PROGRESSION OF SUBCLINICAL CARDIOVASCULAR DISEASE IN TYPE 1 DIABETES

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Departments of 1Pathology, 3Medicine and 4Biochemistry, Case Western Reserve University, Cleveland, Ohio, USA; 2 Biostatistics Center, George Washington University, Rockville, Maryland, USA

Main Objectives: Skin collagen long wave fluorescence (LWF) is widely used as a surrogate marker for the accumulation of advanced glycation end-products (AGEs). Here we compared three different autofluorescent markers for the future risk in the development of subclinical cardiovascular disease in 216 patients/participants of the DCCT (1983-1993) and subsequent EDIC (1993-present) studies. The following markers were evaluated: collagen-linked fluorescence (CLF), LW-1 (a specific fluorescent skin collagen-linked molecule of partially known structure) and skin intrinsic fluorescence (SIF) measured by the SCOUT DS.

Strategy and Methods: CLF was determined by measuring total nonspecific fluorescence at excitation (ex)/emission (emission) 370/440 nm in collagenase digests of insoluble collagen prepared from skin biopsies obtained at the end of the DCCT study at years 1993-1994. LW-1 was measured as a specific peak in chromatograms using HPLC-fluorescence monitored at ex/em 348/463 nm in the same insoluble collagen preparations after exhaustive proteolytic digestion. SIF was determined noninvasively on forearm skin of patients in EDIC years 16-17 by the SCOUT DS at ex/em 375/435-655 nm. Cardiovascular outcomes included: coronary artery calcification (CAC) measured at EDIC years 7-9 by computed tomography; intima-media thickness (IMT) of the common carotid artery at EDIC years 6 and 12 by ultrasonography & image analysis; and left ventricular mass (LVM) at EDIC years 14-16 by cardiac magnetic resonance imaging (cMRI).

Main Results: The association of CLF and LW-1 with SIF was positive and strongly significant even though SIF was determined 16-17 years after the skin biopsy used to measure CLF and LW-1. SIF significantly (P<0.0001) correlated with both CLF (r=0.31) and LW-1 (r=0.26). After the correction for age, SIF significantly correlated with LW-1 (r=0.15, P=0.046), but not CLF (r=0.11, P=0.16). CAC was significantly (P<0.05) associated with CLF and SIF after adjustment for age, diabetes duration and HbA1c. Using similar adjustments, LW-1 (P<0.02) and SIF (P<0.01) were significantly associated with IMT progression while LW-1 (P=0.008) and CLF (P=0.035) were significantly associated with LVM.

Conclusions: All three fluorescent markers showed some degree of correlation among each other. However, LW-1 surprisingly emerged as an important long-term risk factor for the development of IMT and LVM associated with cardiovascular disease. This risk factor remained significant after further adjustment for HbA1c. Conversely, HbA1c was not significantly associated with either IMT or LVM.
SPECIFIC DETECTIONS OF THE FRUCTATED PROTEINS IN DIABETIC RAT LENS

Emi Ito1; Ryoji Nagai2 and Naoyuki Taniguchi3
1RIKEN, Saitama, Japan; 2Tokai University, Kumamoto, Japan

Main Objectives: Accumulating evidence indicated that the Maillard reaction is related to diabetic complications as well as the development of age-related diseases such as arteriosclerosis, Alzheimer’s disease and cancer. Glucose is a major sugar in blood, therefore, glucose and its metabolites are thought to be a major glycating agent in the body. The contribution of glycation reaction by fructose is, however, less considered than that by glucose, because of the low concentration of fructose in blood. However, the glycation reactivity of fructose is much higher than that of glucose. Fructose is generated from glucose through the polyol pathway in the body. In some tissues such as lens, kidney and peripheral nerves in diabetic patients, the polyol pathway is active, therefore, fructose-induced glycation may also play a significant role in such tissues. In this study, the glycation reaction by fructose in control and diabetic rat lens was analyzed by using newly developed antibody which specifically recognizes fructated lysine residue with fructose.

Strategy and Methods: A polyclonal antibody was raised by immunizing fructated lysine-conjugated bovine serum albumin (BSA) in rabbits. Fructated-BSA was generated under the dry and thermal conditions and then used as an immunogen. BSA (3mg) and fructose (6mg) dissolved in 300 μl water were mixed and lyophilized. Reaction of the mixture was conducted at 90 °C for 30 min. After the reaction, aqueous solution of fructated BSA and non-glycated BSA were analyzed by Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. The number of sugar residues bound to BSA were estimated to exhibit approximately 40 % of lysine residues of BSA.

Main Results: Glucose and galactose adduct BSA samples were generated with the same protocol and analyzed by immunoblotting with a newly developed antibody. As the result, this antibody specifically recognized fructated-BSA but not glucated-BSA and galactated-BSA. The soluble lens proteins prepared from normal and streptozocin-induced diabetic rats were analyzed by this antibody. Immunoblot analysis indicated that several lense proteins from diabetic rats markedly enhanced their immunoreactivity against anti-(fructated lysine) antibody.

Conclusions: We could demonstrate that fructated-proteins but not glucated-proteins were generated in diabetic conditions, especially in rat lens.
KYNOXAZINE: A NOVEL FLUORESCENT COMPOUND IN HUMAN LENS DERIVED FROM THE MAILLARD REACTION OF 3-HYDROXYKYNURENINE AND ERYTHRULOSE

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Main Objectives: Cataractogenesis is one of the major causes for blindness worldwide. The opacification of the lens is a result of protein aggregation and modification. Various small molecules have been identified to play a role in cataract formation. Kynurenines, naturally occurring UV filters in human lenses are known for lens protein modification. Another important pathway is the modification of lens proteins by ascorbate degradation products. Our objective was to study the reactions of kynurenines with ascorbate degradation products and their capability of lens protein modification.

Strategy and Methods: Kynurenine, 3-hydroxykynurenine and 3-hydroxykynurenine-O-glucoside were incubated in the presence of erythrulose at physiological conditions. The reaction mixtures were analyzed by UPLC. The product arising from 3-hydroxykynurenine/erythrulose incubations was isolated and characterized by mass spectrometry and NMR. Extracts from non-cataractous lenses were analyzed by UPLC/MS. RNase A (type IIIA) and recombinant human αB-crystallin were incubated with purified product at physiological conditions and analyzed by SDS-PAGE.

Main Results: The reaction of 3-hydroxykynurenine with erythrulose yields a fluorescent product. This conversion was very fast and limited to 3-hydroxykynurenine. The product was identified as 2-amino-4-[2-hydroxy-3-(2-hydroxyethyl)-2H-1,4-benzoxazin-5-yl]-4-oxobutanoic acid, henceforth called kynoxazine. Neither kynurenine nor 3-hydroxykynurenine-O-glucoside showed a similar reaction. The presence of free kynoxazine in human lenses was established by UPLC/MS. In vitro studies showed that kynoxazine modifies and crosslinks RNase A (type IIIA) as well as recombinant human αB-crystallin.

Conclusions: For the first time we identified a novel fluorescent kynurenine-like compound formed from the reaction of 3-hydroxykynurenine and the ascorbate degradation product erythrulose. The aminophenol functionality of 3-hydroxykynurenine is essential for this reaction. Kynoxazine is relatively unstable at physiological conditions and reacts with proteins. Due to this high reactivity and its presence in human lenses we hypothesize that kynoxazine is also bound to lens proteins in vivo. Through the modification and crosslinking by kynoxazine lens protein functionality may be compromised ultimately leading to the formation of cataracts. The nature of those modifications is currently under investigation.
SOD1 plays a protective role in cells by catalyzing the conversion of the superoxide anion into molecular oxygen and hydrogen peroxide. SOD1 is highly expressed in the liver. It has been reported that the concentration of glucose in blood is high in SOD1 KO mouse. We measured expression level of RAGE and found that RAGE was highly expressed in SOD1 KO mouse. The concentration of NADH was also high in KO mouse. These results suggest that both hyperglycemia and oxidative stress accelerated the Maillard reaction.

In addition, SOD1 KO mouse are fatty liver, resulting in liver cancer finally through fibrosis, NASH, cirrhosis of the liver with further progression. It has been reported that SOD1 KO mouse increases lipid accumulation on liver. Histological analysis revealed that collagen productions were increased in SOD1 KO mouse. Collagen, which is one of the typical long-life proteins, is hard to digest by matrix metalloproteinase (MMP) by AGE modification. We measured carboxymethylargine (CMA), which is a specific AGE for glycated collagen, and identified CMA in SOD1 KO mouse liver. CMA formation may also one of the key factor why collagen were accumulated in the liver.
CO-LOCALIZATION OF AGES AND RECEPTOR FOR AGE IN PTERYGIUM.

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Purpose: Pterygium is one of the vision-threatening disorders of the eye. Histologically, proliferative fibrovascular tissue with thickened epithelium extends toward the central part of the cornea. In addition, abnormal aggregation of proteins are seen in the center of the proliferative fibrovascular tissue. However, the pathogenesis of pterygium and its association with abnormal aggregation of proteins are still unclear. In the present study, we investigated the immunohistochemical localization of AGEs and receptor for AGE to reveal the pathogenesis of pterygium.

Materials and methods: Immunohistochemical localization of AGEs (CML, pyrraline, and pentosidine) and RAGE (receptor for AGE) was investigated in surgical specimens with pterygium from 20 patients and 4 control specimens without pterygium.

Results: In the control conjunctiva, AGEs were not detected and weak immunoreactivity to RAGE was detected in the wall of capillary vessels. In contrast, AGEs (CML, pyrraline, and pentosidine) was detected in the aggregated proteins in the pterygium. In addition, the expression level of RAGE was moderate in the wall of capillary vessels surrounding the aggregated abnormal proteins.

Discussion: Abnormally-aggregated proteins in pterygium contained AGE componets. Continuous interaction of AGEs and RAGE is speculated to be the development of proliferative fibrovascular tissue in pterygium.
Localisation of Advanced Glycation End Products in Exfoliation Syndrome

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Main Objectives: Exfoliation syndrome (XFS) is an age-related ocular disease whose mechanism is still unknown. Abnormal deposits on anterior lens surface and the pupillary border are observed in XFS. XFS is one of the main factors of secondary glaucoma because the abnormal proteins obstruct the drainage system of eyes. However, the process of how abnormal proteins accumulates in XFS remains unclear. The aim of this study is apparent to immunohistochemical localization of advanced glycation end products (AGEs) in the lens capsule with XFS.

Strategy and Methods: The anterior lens capsules from 10 eyes of 10 patients with XFS, and 10 eyes of 10 control patients were excised in the routine cataract surgery. In the sections of the anterior lens capsules, immunohistochemical localization of AGEs was investigated using monoclonal antibodies to pyrraline, imidazolone and pentosidine.

Main Results: Abnormal protein accumulation was detected the lens capsule surface in XFS. This accumulation was positive for pyrraline and imidazolone, and negative for pentosidine. Whether it was XFS or not, pyrraline, imidazolone and pentosidine were not detected in lens capsule in themselves. On the other hand, all the AGE components were detected in the lens cortex, which was attached on the lens capsule.

Conclusions: Accumulation of abnormal protein with XFS contained abundant AGEs. It is possible that protein modified with AGEs leads to resistance to proteases, which will be a mechanism of the accumulation of abnormal proteins. Suppression of AGE formation will be a promising target to treat glaucoma secondary to XFS.
MOLECULAR BASIS FOR LENS CRYSTALLIN DENATURATION AND PRECIPITATION BY VITAMIN C OXIDATION PRODUCTS IN AGE-RELATED CATARACTS

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Purpose: Research by Ortwerth and colleagues (1988) showed that incubation of bovine lens protein fractions with vitamin C, or dehydroascorbic acid itself, was associated with advanced glycation/ascorbylation product formation, protein aggregation, crosslinking, insolubilization and opacification as observed in age-related human cataracts. The goals of this research was to test the hypothesis that the reported protein precipitation was associated with modification of amino acid residues that were selective for precipitated but not soluble proteins.

Methods: To test this hypothesis, calf lens crystallin homogenate was separated into α-, βH-,βL- and γ-crystallin rich fractions by Sephadex G-200 gel filtration chromatography, and fractions were incubated with 20 mM ascorbic acid until precipitation occurred. Proteomic analysis with mass spectrometry was used to reveal and identify protein modification sites that are selectively present in precipitated vs. soluble proteins, focusing on the AGE hydroimidazolones from xylosone (XYH), methylglyoxal (MG-H1), CML, CEL, and tryptophan oxidation to N-formyl kynurenine (NFK) and kynurenine(KYN).

Results: Broad formation of AGEs and oxidation was observed in both precipitated and soluble crystallins. However, modification of selective lysine (K) and arginine (R) residues, and oxidation of embedded tryptophan (W) residues occurred in precipitated but not soluble fractions rich in CRYBB1, CRYBB2, CRYBA4 and CRYBA1 crystallins: Major sites include CRYBB1 (K61CML, K119CML, R93XYH, W102NFK), CRYBB2(K68CML, R98XYH, K101CML, K168CML) CRYBA1 (K15CML, K114CML, R78XYH, R194XYH,W79NFK, W181NFK) and CRYBA4 (W31, R39, R40, R59XYH, K62CML). Surprisingly, although CRYGS (B,C, and Z) were detected and CRYGS was oxidized at W46 and W137, no relationship with precipitation was found.

Conclusions: This study is the first to provide specific molecular insights into glycation and oxidation in relationship to protein precipitation, whereby beta-crystallins emerge as major targets and constituents in the precipitate. Molecular modeling studies are in progress to find out if ascorbylation favors protein unfolding and exposure of the protected tryptophan residues.
**Main Objectives:** Skin aging results from intrinsic and extrinsic phenomena. Among the factors involved in skin aging, the glycation reaction and the formation of advanced glycation end-products (AGEs) is an important mechanism (1). AGEs such as pentosidine are formed by an oxidative process, and often referred to as a glycoxidation product. AGEs have been reported to accumulate during chronological aging but sun exposure has also been shown to contribute to this accumulation through inducing an oxidative environment. Recently we demonstrated *in vivo* that the damaging effects of UV radiation might be more detrimental in aged skin than in young skin due, in part, to an increased accumulation of pentosidine and, in turn, to the exacerbation of alterations related to chronological aging (2). The purpose of this work was to investigate the biological effect of pentosidine *in vitro* systems (monolayer culture and reconstructed skin).

**Strategy and Methods:** Fibroblasts in monolayer were cultured in the presence of solubled pentosidine. Expression of mRNA for IL8, and MMP12 and tropo-elastin protein levels were determined at various time points. In addition, the effects of pentosidine were determined in reconstructed skin containing a collagen chemically modified with pentosidine. Analysis of several mRNAs and expression of MCP1 and VEGF were performed.

**Main results:** In fibroblasts grown in monolayers, soluble pentosidine induced up-regulation of IL8 mRNA and MMP12 mRNA. Tropo-elastin expression also increased. In contrast, pentosidine bound to collagen in reconstructed skin was associated with a down-regulation of every mRNA studied. At the same time, in the medium of reconstructed skin, MCP1 and VEGF expression was increased and decreased, respectively.

**Conclusion:** These results demonstrate that, depending on whether pentosidine is in solubled or protein-bound form, it could possibly contribute to the inflammatory process and the imbalance of extracellular matrix in the aging skin. In addition, pentosidine appears to take part in the solar elastosis environment, possibly by lessening of the surrounding vascular network.

**Keywords:** pentosidine, glycation, glycoxidation, skin aging, ultraviolet exposure.

2. Pageon H, Pournès-Ballihaut C, Zucchi H, Bastien P, Tancrede E and Asselineau D. Aged Human Skin is More Susceptible than Young Skin to Accumulate Advanced Glycoxidation Products Induced by Sun Exposure Journal of Aging Science 2013, 1:3
EFFECT OF A TOPICAL DICARBONYL SCAVENGING GEL ON SKIN AUTOFLUORESCENCE AND ELASTICITY, MARKERS OF ADVANCED GLYCATION END PRODUCT LOAD IN HUMAN SKIN

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Main Objectives: Advanced glycation end products (AGEs) result from nonenzymatic reactions between reducing sugars and protein amino groups, with reactive dicarbonyls being prominent intermediates. AGE accumulation tends to increase with chronological age, but may also be accelerated under certain conditions such as increase blood glucose levels, increased exposure to UV light, and exposure to tobacco smoke. Among the many potentially adverse biological effects of AGEs is their ability to cross-link proteins and thereby disrupt normal protein conformation. Skin collagen is a susceptible target of AGE damage, resulting in modifications to skin properties, such as elasticity and firmness. The main objective of the current study was to evaluate the anti-AGE activity of a hydrogel containing dicarbonyl scavengers, specifically carnosine and iridoid sources (TruAge AGE Therapy Gel), when repeatedly applied to human skin.

Strategy and Methods: A 12-week open-label clinical trial was conducted with 39 women, ages 49-59 years, in which the volunteers applied the hydrogel daily. Many AGEs have fluorescent properties which are useful for their noninvasive detection in human skin. Skin deformation measurements may also be useful in detecting AGE associated changes in skin properties. Therefore, pre and post-trial skin autofluorescence was measured with the TruAge Scanner (Morinda, Inc.) and reported as AGE score, while pre and post-trial skin elasticity and firmness were measured with the Cutometer (Courage + Khazaka Electronic, GmbH). AGE scores were controlled for increased UV-light exposure that occurred during the summer months by adjusting for changes in melanin content.

Main Results: Data analysis revealed that the adjusted average 12-week AGE score (223.23) was significantly less ($p < 0.05$) than the baseline score (245.11). This equates to an approximate decline of nine AGE-associated years. Skin firmness of the crow’s feet area of the face, as measured by the Cutometer, improved by an average of 33.82%, with improvement seen in 89.7% of the participants. An even greater average increase of 83.87% was measured for skin elasticity, with 82.1% of the women experiencing improvement. Both firmness and elasticity improvements were statistically significant ($p < 0.001$).

Conclusions: Repeated topical application of the dicarbonyl scavenging hydrogel appears to be associated with reduction and/or mitigation of AGE accumulation in the skin.
MEASUREMENTS OF FLUOROPHORE IN SKIN FOR NON-INVASIVE EVALUATION OF AGES ACCUMULATION

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Main Objectives: Although hemoglobin A1c (HbA1c) is measured clinically as an index of blood glucose control of the past 1-2 months, it is difficult to predict the progress of diabetic complications. Recent studies demonstrate that advanced glycation end products (AGEs) accumulate in our bodies in accordance with aging, and enhanced by the pathogenesis of diabetic complications. Therefore, since the measurement of AGEs is expected as a marker for diabetic complications, development of a rapid and reliable method to measure AGEs is also expected. The aim of our study is to develop a simple and non-invasive detection device for AGEs.

Strategy and Methods: The wavelength for measurement of AGE-like fluorophore was selected by comparison of fluorescence wavelength of serum between control subjects and patients with kidney failures. Diabetes was induced in 8-week-old male mice by abdominal injection of streptozotocin (STZ) in 200 µL of 0.05 M saline-citrate buffer (pH 4.5). Age-matched control mice were given abdominal injections of 0.05 M saline-citrate buffer alone. Similarly, diabetes was also induced in 8-week-old male rats. AGE accumulation in patients with diabetes in the skin was estimated by measuring fingertip autofluorescence. Furthermore, fluorophore in serum in patients with kidney failures was purified by high performance liquid chromatography (HPLC).

Main Results: Wavelength scanning showed that both serum gave a fluorescent spectrum with an excitation maximum at 340 nm and emission maximum at 440 nm, whereas emission intensity in patients with renal disease was significantly higher than that of control subjects. Fluorescence intensity in auricle in diabetic mice and diabetic rats increased in time-dependent manner. Furthermore, a positive correlation was found between the intimal thickening (IMT) and the level of fluorescence intensity of the skin in diabetic patients. The fluorophore in serum was purified by HPLC and structure was analyzed by NMR.

Conclusions: The measurement of fluorophore by the device may be used as an important marker for the progression of diabetic complications.
MANGOSTEEN PERICARP EXTRACT INHIBITS THE FORMATION OF AGES AND IMPROVES SKIN CONDITIONS

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Main Objectives: The inhibition of advanced glycation end-products (AGEs) by daily meals is believed to become an effective prevention for lifestyle-related diseases. In the present study, the inhibitory effect of hot water extracts of mangosteen (Garcinia mangostana L.) pericarp (WEM) on the formation of pentosidine, one of AGEs, in vitro and in vivo and the remedial effect on skin conditions were measured.

Strategy and Methods: Hot water was added to dried mangosteen pericarp, filtered, concentrated, added 33% dextrin for solid content of extract, and spray-dried to obtain the WEM. Then, the compounds were isolated from WEM. To examine the inhibition of pentosidine formation, geratin and rebose incubated with WEM and its compounds, and determined of the pentosidine content by HPLC. Furthermore, the tablet contained 100 mg WEM, once a day, was administrated to 11 healthy women (32-48 years old) for 12 weeks. Routine blood and skin function testing were evaluated every 4 weeks. AGEs accumulation in the skin was estimated by measuring fingertip autofluorescence, skin elasticity was measured with Cutometer (MPA580), and skin moisture content was measured with Robo Skin Analyzer (RSA50).

Main Results: 0.2 μg/ml of WEM significantly inhibited pentosidine formation during gelatin incubation with ribose. Therefore, 0.1 nM of purified compounds from WEM, such as garcinomangosone D, eucryphin, rhodanthenone B, and epicatechin inhibited pentosidine formation by 38%, 38%, 45%, and 35%, respectively. Oral administration of WEM at 100 mg/day to volunteer subjects for 12 weeks reduced the serum pentosidine contents, although fasting the blood glucose (mg/dl) and HbA1c (%) levels do not change. Because obtaining skin biopsies from healthy volunteers is ethically difficult, AGE accumulation in the skin was estimated by a fluorescence detector. The oral administration of WEM significantly reduced the skin autofluorescence intensity, demonstrating that WEM also reduced AGE accumulation in the skin. The decrease of the skin autofluorescence levels by the WEM administration was associated with the decrease of the serum pentosidine levels. Furthermore, the elasticity of the skin was also improved comparing with before WEM administration. The average moisture content of the skin in the subject before WEM administration increased in a time-development manner.

Conclusions: These results demonstrate that intakes of WEM reduced the glycation stress results in the improvement of skin condition.
SKIN AUTOFLUORESCENCE AS A SURROGATE BIOMARKER TO PREDICT RETINAL RE-DETACHMENT AFTER VITRECTOMY

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Main Objectives: Retinal re-detachment is the main cause of a poor visual outcome after surgical treatment in rhegmatogenous retinal detachment (RRD). Advanced glycation endproducts (AGEs) have been suggested to play a role in vitreo-retinal interface disease by disturbing retinal integrity and promoting neovascularization. In a pilot study (n= 33 RRD patients), we showed that vitreous pentosidine and skin autofluorescence, as a measure of tissue AGEs deposition, were elevated in patients with a severe retinal detachment, a risk factor of retinal re-detachment. The current study aims to address whether skin autofluorescence can be used as a surrogate biomarker to prospectively identify patients with increased risk of retinal re-detachment after vitrectomy.

Strategy and Methods: In this prospective cohort study, 500 patients diagnosed with primary RRD and scheduled for vitrectomy were included. Before surgery, skin autofluorescence was determined with the AGE reader using lower arm measurements, and clinical risk factors of retinal re-detachment were collected by questionnaire and general practitioner data. During surgery, local disease features were registered by the surgeon. The main outcome measure was retinal re-detachment within three months after surgery. Using regression analyses, the relation of skin autofluorescence with retinal re-detachment was explored.

Main Results: Interim analysis was performed on 265 patients (aged 60 ± 9.7 years, 69% male), of whom 38 patients (aged 65 ± 9.9 years, 66% male) developed a retinal re-detachment. Skin autofluorescence ranged from 1.3 - 4.1 arbitrary units. There was a weak correlation between skin autofluorescence and retinal re-detachment, r = .14 (p < .05). However, this relation was not significant after correction for age. Regression analysis showed that age (OR 1.05 per year, CI 1.01 – 1.09), and intra-operative area of retinal detachment ≥ 50% (OR 2.47, CI 1.21 – 5.04) were the most important variables in the prediction of retinal re-detachment. However, the overall fit of this model was poor, R² = .092.

Conclusions: These provisional results suggest that skin autofluorescence will not contribute to identifying patients with increased risk of retinal re-detachment. Furthermore, retinal re-detachment could only be poorly predicted by other, conventional, risk factors.
Main Objectives: Type 2 diabetes mellitus (T2DM) is accompanied by premature arterial stiffness. The underlying mechanisms are unclear. We investigated the possible relation between subclinical arterial inflammation, calcification, atherosclerotic risk factors, skin advanced glycation end products (AGEs), and arterial stiffness in T2DM.

Strategy and Methods: 34 patients with recently diagnosed T2DM, without a history of cardiovascular disease and not using glucose lowering drugs, were studied (age 63 (55-66) yrs, 65% male, HbA1c 46±4.3 mmol/mol (6.4±0.4%), 18% smokers). Arterial stiffness was non-invasively assessed by aortic systolic blood pressure (aSBP, mmHg) and aortic (carotid–femoral) pulse wave velocity (aPWV) using applanation tonometry. A whole body 18-Fluorodeoxyglucose positron emission tomography-(low dose) computed tomography (18F-FDG-PET-CT) scan was performed to assess arterial inflammation (standard uptake value (SUV)max (according to EARL guidelines)) and CT-scored arterial calcification (AC): visual score: 0 (no) to 4 (>50% calcified plaque). SUVmax and AC scores were calculated in 4 individual segments (carotid arteries, ascending aorta and aortic arch, descending and abdominal aorta, and iliac and femoral arteries) and for the total aortic tree (aSUVmax, mean of 4 segments). Skin AGEs were measured as skin autofluorescence (SAF).

Main Results: aSUVmax and AC were not associated. AC was associated with male gender (r=0.48, P=0.004), age (r=0.51, P=0.002), BMI (r=0.43, P=0.011), and SAF (r=0.37, P=0.036) and not with HbA1c, smoking, waist circumference, and lipids. None of AC scores correlated with aPWV, aSBP, or any of the SUVmax segments. After multivariate adjustment (r²=0.39, P=0.001) only male gender (β=0.49, P=0.003) and SAF (β=0.34, P=0.033) remained significant.

aSUVmax was associated with atherosclerotic risk factors: BMI (r=0.64, P<0.001), HbA1c (r=0.59, P<0.001), waist circumference (r=0.55, P<0.001) but not with, age, gender, smoking, lipids, and SAF. aSUVmax was associated with non-invasive markers of arterial stiffness: aSBP (r=0.55, P=0.001) and tended to correlate with aPWV (r=0.33, P=0.059). After multivariate adjustment (r²=0.79, P<0.001), only HbA1c (β=0.36, P<0.001), waist circumference (β=0.49, P<0.001), aSBP (β=0.49, P<0.001), and aPWV (β=0.24, P=0.014) remained significant.

Conclusions: These results show that in recently diagnosed T2DM patients 18F-FDG-PET-CT revealed subclinical arterial inflammation but not calcification, and is positively associated with determinants of arterial stiffness, glycemic control and central obesity. Interestingly, while the extent of arterial calcification did not show any relation with arterial inflammation, it was independently associated with skin AGEs, suggesting discordant pathways at different stages of atherosclerotic disease.
INSUFFICIENT LEPTIN ACTION INDUCES RAGE EXPRESSION AND TRIGGERS PANCREATIC BETA-CELL FAILURE IN TYPE 2 DIABETES

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Objectives: Glucolipotoxicity, which is exerted by free fatty acids (FFA) and prolonged hyperglycemia, is considered to play a critical role in the pathogenesis of the β-cell failure in the late phase of type 2 diabetes. Pattern-recognition receptors such as receptor for advanced glycation end-products (RAGE) could mediate danger signals in β-cells. However, the underlying molecular and cellular mechanisms of glucolipotoxicity remain to be defined. To reveal the functional roles of the AGE-RAGE axis, lipotoxicity and leptin signaling in β-cell failure, we examined whether RAGE expressed on β-cells and whether RAGE could contribute to pancreatic β-cell failure in vitro and in vivo.

Methods and Results: Pancreatic islets were isolated from ob/ob (leptin-deficient), db/db (leptin receptor-mutant), diet-induced obesity (DIO), RAGE-null (RAGE−/−), and RAGE+/+ wild-type (WT) mice and dispersed into single cells for flow cytometry. RAGE expression was detected in insulin-positive β-cells of ob/ob and db/db mice, but not of WT, DIO or RAGE−/− mice. RAGE−/− db/db mice were protected from β-cell apoptosis and impaired insulin secretion, when compared to RAGE+/+ db/db mice, with similar serum levels of FFA and adiponectin. To clarify the links of RAGE expression to leptin action, glucolipotoxicity, insulin secretion, and apoptosis in β-cells, we employed the mouse pancreatic β-cell line MIN6 in culture. The pretreatment of FFA such as palmitate or oleate at 0.2 mM significantly combined with a leptin antagonist (D23L/L39A/D4A/F41A) induced RAGE expression, AGE-elicited apoptosis and impaired glucose-induced insulin secretion by AGE in MIN6 cells.

Conclusions: Glycolipotoxicity characterized by FFA elevation concomitant with AGE formation caused by prolonged hyperglycemia could induce pancreatic β-cell failure through the up-regulation of RAGE expression on β-cells due to insufficient leptin action in type 2 diabetes. The annulment of the leptin resistant state or supplementation of sufficient leptin action may be protective against glycolipotoxicity in β-cells in type 2 diabetes.
Objectives: The cell surface receptor for advanced glycation end-products (RAGE) is implicated in neurodegenerative disorders and the development of neuronal cell death during brain ischemia. In contrast, RAGE has a decoy-type receptor generated from alternative mRNA splicing, which is named endogenous secretory RAGE (esRAGE). The esRAGE has been considered to work as a decoy against RAGE ligands and to attenuate the ischemic neuronal damages. However, its neuroprotective mechanisms remain to be clarified. In this study, we hypothesize that esRAGE can be closely interacted with heparan sulphate proteoglycans (HSPG) of brain endothelial cell surface and will play a protective role in the ischemic brain diseases.

Methods and Results: We first employed cell free assays and found the direct association of human recombinant esRAGE proteins with heparin. The treatment of 0.1 IU/ml heparin or 1.0 mU/ml heparitinase significantly decreased cell surface esRAGE signals and concomitantly increased esRAGE levels in the cell culture supernatant of human brain endothelial cells (HBEC). An intravenous heparin injection revealed an elevation of circulating esRAGE concentrations in human esRAGE transgenic (Tg) mice. In addition, we used parabiotic mouse models among Tg, RAGE knockout (KO) and wild-type (WT) mice. Immunofluorescent studies showed esRAGE signals on the brain endothelial cells of WT mice after surgical parabiosis between Tg and WT (Tg/WT) mice. esRAGE is thus considered to associate with HSPG of the cell surface of brain endothelial cells. Using a bilateral common carotid artery occlusion (BCCAO) model, less neuronal cell death was observed in the brain CA1 region of WT mice of the parabiotic Tg/WT pairs, when compared to the WT/WT pairs. Moreover, esRAGE signals were observed around neurons in WT mice of the parabiotic Tg/WT, but not in KO mice of the Tg/KO pairs, suggesting the esRAGE transition from circulation into the brain through blood brain barrier (BBB). To resolve this issue, we used an in vitro model of blood brain barrier (BBB) (PharmaCo-Cell) constituted of brain capillary endothelial cells, pericytes, and astrocytes. We found luminal esRAGE was transferred to an abluminal (brain) site through BBB in an endothelial RAGE-dependent manner.

Conclusions: Circulating esRAGE is closely associated with HSPG of the cell surface of brain endothelial cells and can be transferred to brain through BBB by endothelial RAGE. Collectively, esRAGE may work as neuroprotective decoys in ischemic cerebrovascular diseases.
**Main Objectives:** Pancreatic islet transplantation (tx) is an attractive procedure for the treatment of insulin-dependent diabetes mellitus. Currently, however, its low efficiency due to early loss of transplanted islets requiring repeated txs with the use of 2-3 donors for a single recipient hampers its clinical application. In the present study, we hypothesized that RAGE of donor islets plays an essential role in early loss of tx islets since RAGE has been reported to be involved in beta cell death.

**Strategy and Methods:** In order to examine the role of RAGE in beta cell death after tx, isolated syngenic islets from wild-type (WT) vs RAGE-deficient (ko) mice were used as donors and transplanted beneath the kidney capsule of STZ-diabetic WT mice (C57BL/6).

**Main Results:** Hyperglycemia of diabetic recipient mice was ameliorated after tx when the number of donor islets from WT mice was >100 but not when it was reduced to 50, equivalent to 20-25% of whole islets in the mouse pancreas. Thus, 50 islets were used as donors per each tx for the following study. In marked contrast to mice receiving WT islets (n=7), hyperglycemia of all mice (n=10) receiving RAGE ko islets was ameliorated after tx. Morphologically (LM), WT and RAGE ko islet grafts at 14 day after tx appeared degenerated and intact, respectively. Of note, EM showed that degranulation and vacuolization of mitochondria were seen in WT beta cells, while in contrast, intact beta granules and mitochondria were observed in RAGE ko beta cells. When WT islets were pretreated with soluble RAGE (100µg/ml, 24h) in vitro to block RAGE signaling prior to tx, all recipient mice (n=5) became normoglycemic after tx. DNA array of isolated WT vs RAGE ko islets yielded the differences in profiles of gene expression including GLP-1R which was upregulated in RAGE ko islets (7 folds vs WT islets). When RAGE ko islets pretreated with excendin9-37 (1µM), GLP-1 antagonist, were used as donors, all recipient mice (n=3) remained hyperglycemic after tx. When WT islets were pretreated in vitro with liraglutide (10µM, 24h) and liraglutide (0.3mg/kg) was administered recipient mice (sc, 1/day) for 60 days after tx, all diabetic mice (n=6) became normoglycemic. Neither treatment alone was effective on amelioration of recipient hyperglycemia after tx.

**Conclusions:** These findings clearly demonstrate that RAGE of donor islets plays an essential role in early loss of tx islets in association with GLP-1R expression and indicate that targeting donor RAGE may be a novel strategy to improve the efficiency of clinical islet tx.
Main objectives: Deleterious renal effects of Advanced Glycation End-products (AGEs) as potent inducer to inflammation and fibrosis are well described in diabetes, thus contributing to the glomerulosclerosis pathogenesis. Conversely, few studies investigated the renal effects of dietary AGEs. Our preliminary data showed a high renal accumulation of dietary carboxymethyllysine (CML), though. This work thus aimed to analyze the effects of long-term CML-enriched diet on kidney and the implication of RAGE.

Strategy and methods: Wild-type (WT) or RAGE-/- male C57BL/6 mice were exposed to control diet or CML-enriched diet (200µgCML/g of food) during 18 months. After the follow-up, renal function markers (urine albumine/creatinine ratio and uremia) were measured. CML deposits were analyzed by immunohistochemistry. Inflammation (VCAM-1, TNFα), fibrosis (collagen I and IV) and endothelial to mesenchymal transition (vimentin) markers were measured by RT-PCR in kidneys.

Main results: We confirmed higher CML deposits in kidneys of CML-diet mice compared to normal-diet, with a predominant endothelial localization. A proteinuria was found in each group without significant difference. VCAM-1 and TNFα expressions were lower in RAGE-/- mice than in WT mice, without effect of the diet type. Expression of Collagen IV was statistically increased in the WT mice with the CML-diet compared to the normal-diet (p=0.03), while there was not difference in RAGE-/- mice. A similar inclination was observed with collagen I. Vimentin expression was significantly increased in the CML-diet RAGE-/- mice compared to normal diet (p=0.015), while no difference in WT mice was found.

Conclusions: Dietary CML may accumulate within the kidneys, but we observed no difference of renal function maybe due to age-related renal alterations of the majority of mice (proteinuria). However, we reported that dietary CML may induce RAGE-dependant fibrosis in mice kidneys, favoring thus renal aging. Surprisingly, dietary CML may also promote EMT in RAGE-/- mice. Further studies are needed to confirm these data and to determine specific signaling pathways.
Main objectives: Antiphospholipid antibodies (aPLs) are not only diagnostic biomarkers but also play a central pathogenic role in antiphospholipid syndrome (APS). aPLs are able to activate endothelial cells and to induce endothelial dysfunction (ED), one of the main pathologic event in APS which allows thrombotic events. The receptor for advanced glycation end products (RAGE) is known to be involved in ED in patients with diabetes or renal failure. Recent works have shown that RAGE was involved in ED of other autoimmune diseases. The aim of our work was to evaluate the implication of RAGE in the aPL-induced ED in the mouse model.

Strategy and Methods: Total IgGs were purified from primary APS or healthy control subjects’ sera (aPL-IgG and Ctl-IgG, respectively). IgGs have been intraperitonealy injected to wild-type (WT) or to RAGE-deficient (RAGE-KO) C57BL/6 male mice. One week after injection, ED was studied: arterial endothelium-dependent relaxation was measured in isolated-organ chamber, plasma levels of VCAM-1 were measured by ELISA and tissue factor (TF) expression was analyzed in aortic wall by immunohistochemistry. RAGE expression in aortic wall was also measured.

Main Results: aPL-IgGs, but not Ctl-IgGs, induced ED in WT mice. Endothelium-dependent relaxation of aortic and mesenteric ring was altered in a dose-dependent manner. This alteration was statistically significant on aortic ring relaxation for a dose of 6 mg of aPL-IgG ($p<0.01$ vs controls WT mice non injected; $p=0.028$ vs Ctl-IgG injected WT mice). This alteration was not observed in RAGE-KO mice. RAGE and TF expression was increased in WT aortic wall after aPL infusion but not in RAGE-KO aortic wall.

Conclusions: We studied a murine model of ED induced by aPL. In this model, RAGE-KO mice are protected from this ED. RAGE is probably involved in APS physiopathology. Further studies are needed to confirm and elucidate the underlying mechanism.
Main Objectives: *Osteomeles schwerinae* (OSSC) is a medicinal plant traditionally used to treat various diseases in Asia and chemical constitutes of OSSC have the inhibitory effect on aldose reductase activity, has been implicated in the pathogenesis of diabetic complications such as cataracts. To explore the pharmacological activity and potential mechanisms of OSSC in the treatment of diabetic nephropathy, OSSC examined the AGEs-receptor of AGEs (RAGE) binding activity and signal mechanisms in mouse glomerular mesangial cells (GMCs).

Methods: In this study, we determined the effects of OSSC on AGEs and receptor for AGEs (RAGE) binding and studied the mechanism of OSSC in GMCs. Human RAGE overexpressed GMCs were cultured in AGEs-BSA labeled with Alexa 488 and OSSC and AGE/RAGE binding was measured using fluorescence (Ex. 485 nm/Em. 528 nm).

Main Results: Inhibition of AGEs/RAGE binding by OSSC gradually increased in a dose-dependent manner. OSSC reduced AGE-induced ROS formation and nuclear translocalization of transcription factor NF-κB in GMCs. OSSC also reduced the increased TGF-β1 and VEGF expressions and inhibited the phosphorylation of ERK1/2, p38MAPK, IkB, PI3K, and Akt. Moreover, hyperoside, one of single compounds of OSSC, also inhibited AGE/RAGE binding and ROS formation. It also reduced TGF-β1 and VEGF expressions and IkB phosphorylation.

Conclusions: Taking together these results, OSSC may provide a potential therapeutic approach for prevention of diabetic nephropathy. Hyperoside would be one of effective compounds of OSSC.
LYSINE-GALACTOSE MAILLARD REACTION PRODUCTS (MRPs)-INDUCED ANTI-INFLAMMATORY EFFECT IN A CO-CULTURE SYSTEM

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Main Objectives: In the present study, we employed an in vitro model of intestinal inflammation by using a co-culture between intestinal cells (Caco-2 cells) and macrophage cells (RAW264.7 or THP-1 cells), and investigated the effect of lysine-galactose (Lys-Gal) Maillard reaction products (MRPs) on the anti-inflammation of intestine using a co-culture system.

Strategy and Methods: Several MRPs were made by reacting amino acids (lysine, arginine and glycine), and sugars (glucose, fructose and galactose) for 1 h at 121°C. Treatments with the MRPs on RAW264.7 cells stimulated with lipopolysaccharide decrease nitric oxide (NO) expression compared to control.

Main Results: Lys-Gal MRPs had the most significant inhibitory effect on NO expression. We also observed that permeate of Lys-Gal MRPs (PLGM) processed through ultrafiltration membranes with molecular cut-off of 3 kDa to 10 kDa suppressed more NO expression than its original Lys-Gal MRPs. Furthermore, we evaluated anti-inflammatory effect of PLGM with a co-culture system consisting of Caco-2 (apical side) and RAW264.7 or THP-1 (basolateral side) cells to investigate the intestinal inflammatory reaction by stimulating macrophage cells. PLGM prevented decrease in transepithelial electrical resistance, and decreased both TNF-α productions in macrophage cells and IL-8 and IL-1β mRNA expression in Caco-2 cells.

Conclusions: These results suggest that the intake of PLGM may provide anti-inflammatory effect in the intestinal epithelial cells by reducing IL-8 and IL-1β secretion along with suppressing TNF-α expression from the inflammatory macrophage cells.
FORMULA MILK DERIVED CML INDUCES RAGE ACTIVATION, LONG TERM INFLAMMATION AND OXIDATIVE STRESS IN IUGR PIGLETS

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Main Objectives: Formula-derived dietary-advanced-glycation end products (AGEs) may promote programming of inflammation and oxidative stress in the kidney of intrauterine growth retardation (IUGR) piglets.

Strategy and Methods: IUGR piglets received either a low heated formula (LHF, n = 8), a high heated formula (HHF: n = 8) or suckled naturally for 3 wk postnatally. Then they were fed with normal ad libitum regular diet. N(ε)-carboxymethyllysine (CML) was measured in plasma, feces, and formula by HPLC/MS-MS.

Main Results: CML was detected by immunofluorescence in kidney cells. Target renin-angiotensin-apoptotic, pro-inflammatory genes p62-NF-κB, and soluble receptor of AGE (sRAGE) levels were quantified. Compared with that in controls, free CML and plasma urea increased significantly in the HHF-fed group at PND36 (p < 0.05). CML was detected in the nuclei of renal tubular cells of formula-fed piglets but not in suckled ones. Furthermore, the activation of sRAGE was increased in HHF group (p < 0.01). AT1, AT2, caspase 3 and 8, p62-NF-κB, and total protein oxidation in kidney were higher in HHF-fed group as compared to LHF-fed group (p < 0.05).

Conclusions: Food processes aimed at reducing the concentration of AGEs in infant formula are urgently needed and may be therapeutically relevant for premature and/or IUGR babies.
RAGE MEDIATES MYOCARDIAL DYSFUNCTION AND DEFECTS IN MITOCHONDRIAL ENERGETICS IN HIGH FAT FED MICE

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Main Objectives: Cardiovascular diseases are the most common causes of death in people with diabetes and metabolic syndrome. Qualitative, quantitative and functional perturbations in mitochondria have been identified as one of the major cause of myocardial dysfunction in both diabetic and obese patients. Advanced glycation endproducts (AGEs) have been implicated in diabetic cardiovascular complications through direct protein glycation and activation of the specific receptor for AGEs (RAGE). Although glycation of mitochondrial protein has been reported in vitro, whether cardiac formation of AGEs and RAGE activation would mediate impaired mitochondrial bioenergetics and cardiac contractile performance is unknown. This work investigated the functional effects of RAGE activation on cardiac performance and mitochondrial bioenergetics in high fat fed mice.

Strategy and Methods: After 16 weeks of control normal diet (ND) or high fat diet (HFD), weight, visceral fat evaluated by magnetic resonance imaging scan, AGEs accumulation and RAGE expression, cardiac function evaluated in isolated-perfused heart, and mitochondrial respiration performed in permeabilized heart fibers were measured in wild-type and RAGE−/− male C57BL/6 mice. Variables are mean±SEM and analyzed using one-way ANOVA with Bonferroni post hoc tests.

Main Results: Compared to ND mice, weight and visceral fat were increased in HFD mice. Whereas measurement of AGEs content was higher in chow diet compared to the high fat diet, HFD in mice elicited increased AGEs deposits and RAGE activation in myocardial tissue. Compared to ND mice, mitochondrial respiratory control ratio (RCR) with L-glutamate/L-malate and ADP as substrates (n= 12 mice per group) and left ventricular isometric force (LV) in heart Langendorff preparation (n= 10 mice per group) were reduced in HFD mice, RCR: 3.87±0.23 vs 2.92±0.24, p<0.05 and LV: 1.28±0.20 vs 0.66±0.16 gram-force, p<0.05, respectively. Despite higher weight gain and visceral fat, mitochondrial and cardiac contractile performance were prevented in RAGE−/− high fat fed mice compared to wild-type HFD mice.

Conclusions: High fat fed mice developed cardiac contractile and mitochondrial dysfunction that were prevented in RAGE−/− male C57BL/6 mice.
Main Objectives: Protein is modified by carbonyl compounds in the Maillard reaction. It is also called glycation. Lysine and arginine residues of protein are modified into adduct or cross-linked compounds in the reaction. These compounds are known as advanced glycation end products (AGE). The glyceraldehyde-modified proteins have cytotoxic effects. This study revealed the glyceraldehyde-derived pyridinium-type glycation product [1-3] as cytotoxic epitope in the glyceraldehyde-modified protein.

Strategy and Methods: PC12 cells were cultured in medium at 37°C. (1) The cells were exposed to glyceraldehyde-modified albumin-containing medium with or without anti-glyceraldehyde-derived pyridinium antibody. (2) The cells were exposed to glyceraldehyde-derived pyridinium-containing medium with or without anti-glyceraldehyde-derived pyridinium antibody. (3) The cells were exposed to glyceraldehyde-derived pyridinium-containing medium with or without anti-RAGE antibody. (4) PC12 cells were exposed to glyceraldehyde-derived pyridinium-containing medium with or without NADPH oxidase inhibitor (acetovanillone), NO synthase inhibitor (L-NAME), or p38 MAPK inhibitor (SB203580). Cell numbers were assessed by trypan blue exclusion method.

Main Results: Glyceraldehyde-modified albumin and glyceraldehyde-derived pyridinium showed cytotoxicity in PC-12 cells. The cytotoxic effect was dose-dependent. The cytotoxicity was inhibited by anti-glyceraldehyde-derived pyridinium antibody, anti-RAGE antibody, NADPH oxidase inhibitor, NO synthase inhibitor, and p38 MAPK inhibitor. It was suggested that NADPH oxidase and MAPK were activated by the interaction of glyceraldehyde-derived pyridinium with RAGE, and then superoxide and NO were produced in the cells.

Conclusions: The glyceraldehyde-derived pyridinium is the cytotoxic epitope in the glyceraldehyde-modified protein. It is recognized by RAGE, and cause reactive oxidation species (ROS)-related cell injury.

IMMUNOCHEMICAL AND INSTRUMENTAL EVIDENCE OF THE INCREASE OF Nω-CARBOXYETHYL ARGININE (CEA) IN ACCORDANCE WITH KIDNEY FAILURES

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Main Objectives: One of alpha-Oxoaldehydes, methylglyoxal (MG), is a reactive dicarbonyl derived from glycolytic pathway and known to be a precursor of advanced glycation end products (AGEs), such as Nε-(carboxyethyl) lysine (CEL), methylglyoxal hydroimidazolone (MG-H1). It is reported that AGEs including CEL and MG-H1 are correlated with some pathological conditions including diabetes, cataract, nephropathy and Alzheimer disease. Nω-(carboxyethyl) arginine (CEA) was recently identified as a new AGE structure which is also derived from MG, and monoclonal antibody against CEA (3A7) was obtained. In this study, we validated the specificity of this antibody and compared the CEA content in MG-modified BSA measured by ELISA and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Furthermore, serum CEA content was also analyzed in order to clarify the correlation of CEA and some pathologies.

Strategy and Methods: Monoclonal antibody (3A7) was purified from ascitic fluid by protein G column. MG-modified bovine serum albumin (MG-BSA) was prepared by incubating MG (0.1-10 mM) with BSA for up to 1 week. CEA and CEL contents in MG-BSA were measured by two methods, ELISA and LC-MS/MS. In addition, CEA levels in human serum with several diseases were also measured by competitive ELISA and LC-MS/MS and compared.

Main Results: The result showed that 3A7 reacted with CEA but not cross-reacted with CEL, CML and CMA. During incubation of MG with BSA, production rate of CEA was significantly higher than that of CEL. The reactivity of 3A7 to MG-BSA was correlated with CEA content. CEA was also identified in vivo by competitive ELISA and LC-MS/MS. Furthermore, LC-MS/MS analysis demonstrated that levels of CEA elevated as progressing CKD stage, whereas its increase with diabetes was not significant.

Conclusions: Since production rate of CEA in MG-modified proteins is significantly higher than that of CEL, CEA could be more detectable in early stage of the abnormality of carbohydrate metabolism, and measurement of CEA could be a useful measure to predict those diseases. LC-MS/MS analysis of human serum demonstrated that CEA level was highly associated with CKD stage. These data suggested that CEA potentially becomes a biomarker or could be the cause of deterioration of CKD.
P47 MULTIPLE AGES MONITORING BY LC-MS/MS IS AN EFFECTIVE MEANS TO EVALUATE A METABOLIC ENVIRONMENT IN VIVO

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Main Objectives: Recent studies demonstrate that accumulation of Advanced Glycation End-products (AGEs) in our bodies increases in accordance with aging, and is enhanced by the pathogenesis of life style-related diseases such as diabetes. Since there are many pathways for AGEs formation such as glycolysis and inflammation, multiple measurement of AGEs could evaluate metabolic disorders in vivo. However, most reports measure a single AGE structure because of difficulty of multiple AGES analysis. In the present study, several AGEs are measured simultaneously in order to conduct early diagnosis of metabolic disorders in vivo.

Strategy and Methods: Serum was hydrolyzed by 6N HCl and AGEs such as Nε-(carboxymethyl) lysine (CML), Nε-(carboxyethyl) lysine (CEL), Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1), Nω-carboxymethylarginine (CMA) and Nω-(carboxyethyl) arginine (CEA) in patients with diabetes or kidney failure were determined by tandem mass liquid chromatography (LC-MS/MS). Furthermore, low molecular weight fractions (<3,000) (LM method) of serum were obtained by cut off filter, then AGEs contents were also determined. Similarly, AGEs content in serum of animal models such as Streptozotocin-induced type 1 diabetic rats and Watanabe heritable hyperlipidemic (WHHL) rabbits were also measured.

Main Results: LC-MS/MS analysis demonstrated that AGEs are detectable in hydrolysates of 5 µL of serum, and 50 µL of serum filtrates. The difference of AGEs contents in serum between normal subjects and patients were higher in LM method among all experiments. Streptozotocin-induced type 1 diabetic rats showed high serum CML levels compared to control group, whereas serum MG-H1 increased more prominently than other AGEs in patients with diabetes. Furthermore, the concentrations of all AGEs in the serum such as CML, MG-H1, CEL, CMA and CEA increased in accordance with the stages of kidney failures. Especially, MG-H1 showed an increase from the initial stage compared to other AGEs. Increased AGEs in serum is different by AGE structures and diseases.

Conclusions: Since AGEs contents in blood is able to measure by 5 µL serum, metabolic disorder could be estimated by the blood collection from the fingertip. Furthermore, the multiple AGES monitoring is an effective means to evaluate a metabolic environment in vivo.
ESTABLISHMENT OF METHOD FOR THE DETERMINATION OF ADVANCED GLYCATION END PRODUCTS (AGES) IN HUMAN BONE AND CARTILAGE USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

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Main Objectives: Connective tissues like bone and cartilage are the targets for AGEs formation considering large proportions of collagen fibers and relatively low turnover. To date, the determination of pentosidine using HPLC with fluorescence detector was utilized for the measurement of AGEs accumulation in animal and human bone. We previously reported the method for the determination of Nε-(carboxymethyl) lysine (CML) in normal rat using LC-MS/MS, and clarified the highest CML accumulation in femur bone compared with lens, muscle and serum (24th JMARS). In the present study, we established the accurate and precise method for the determination of AGEs in relatively hard tissues such as human bone and cartilage using LC-MS/MS.

Strategy and Methods: Bone and cartilage samples that were otherwise discarded during orthopedic surgery were obtained from one healthy human subject. After pulverization by liquid nitrogen, defatting and demineralization, samples were reduced, hydrolyzed and loaded onto positive cation-exchange column. Then CML, Nε-(carboxyethyl) lysine (CEL), Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1), and Nω-(carboxyethyl) arginine (CEA) were determined by LC-MS/MS. Furthermore, we also evaluated the effect of defatting, demineralization of sample and sample size on the reproducibility of analysis.

Main Results: AGEs such as CML, CEL, MG-H1 and CEA were determined in human bone and cartilage by our detection system. The minimum sample requirement was 1 µg and the limit of detection was 0.5 pmol. Additional studies showed that defatting and demineralization procedure improved the accuracy of detection. Incomplete pulverization decreased the precision of AGEs analysis. Among AGEs determined in the present study, MG-H1 accumulated in the largest quantities both in human bone and cartilage. Surprisingly, MG-H1 content in human bone and cartilage were 6-folds and 12-folds, respectively, higher than that in rats.

Conclusions: Our present study clearly demonstrates that human bone and cartilage are the predominant targets for the accumulation of AGEs in vivo. Conventionally, the AGEs content in bone was estimated by measuring pentosidine. Most AGEs contents determined in the present study were higher than pentosidine. Taken together, these results demonstrates that the determination of various AGEs using LC-MS/MS will be beneficial to understand the mechanism by which the quality of connective tissues is decreased because of unfavorable metabolisms.
The metabolic syndrome is a cluster of risk factors for diabetes mellitus and high dietary AGEs intake could be associated to this syndrome.

Main Objectives: To estimate the intake of dietary AGEs (dAGEs) in Mexican young adults and to study the association between dAGEs and metabolic syndrome (MS).

Strategy and Methods: Healthy volunteers (n=126) 18 to 35 years old were recruited in North-Central Mexico for a cross-sectional study. Dietary records for 3 days were used to estimate usual dAGEs intake from a published food database (Uribarri 2010). Reporting cooking methods and food brands was emphasized. MS was evaluated with the following 5 components: waist circumference (men ≥90cm, women ≥80cm), blood pressure (systolic ≥130mmHg, diastolic ≥85mmHg), HDL-Cholesterol (men<40mg/dL, women<50mg/dL), triglycerides (≥150mg/dL), and glucose (≥100mg/dL). MS was diagnosed when at least 3 components were altered. Weight and height was evaluated to calculate body mass index and a medical history obtained. Spearman correlation was used to evaluate association between dAGEs and individual components of MS and logistic regression to evaluate the relationship between MS and dietary AGEs.

Main Results: dAGEs intake among participants was 11996±6287 KUAGE and energy intake 1927±655 Kcal. A positive correlation between dAGEs and waist circumference (0.24) (p<0.05) was found and a negative correlation with HDL-Cholesterol (-0.2) (p<0.05). The prevalence for MS was 20.6%, the combined prevalence for overweight and obesity 30.5%, and for altered components were 55%, 36.5%, 22.2%, 16.7% and 15.9% for waist circumference, HDL-Cholesterol, triglycerides, glucose and blood pressure respectively. Subjects were classified in 2 groups according to dAGEs intake (>10337 KUAGE: high-intake), and the MS prevalence was higher for the high-intake group (31.7% vs 9.5%) (chi-square=9.9 p<.01). The association between level of dAGE intake and MS prevalence was maintained after adjusting for family history of diabetes and age (R²=0.2, chi-square=17.4, p=0.001), the odds ratio for the high-intake was 4.7 (1.6-13.6). Therefore, we find that the high-intake group was associated with a 370% increase in the predicted odds for having MS. It is important to notice that no association with other dietary variable was found.

Conclusions: This study shows a significant association between dAGEs and the prevalence of MS, subjects with high dAGEs intake were 4.7 more likely to have MS. This association should be explored in future research.
Main Objectives: Glycation induced modification of high density lipoprotein (HDL) could result in the loss of anti-inflammatory/anti-oxidative properties of HDL contributing to complications in patients affected by diseases associated with ageing/oxidative stress. Isoferulic acid is a major active ingredient of a herbal medicine Cimicifuga heracleifolia, popular in oriental countries which shows anti-inflammatory, anti-viral, anti-oxidative and anti-diabetic properties. Recent studies support protective role of isoferulic acid against diabetic complications, via inhibition of AGE formation and oxidative-dependent protein damage. Thus, aim of the study was to examine possible anti-glycation effect of isoferulic acid in comparison to aminoguanidine; an already well-documented anti-glycation agent against compositional modifications and loss of biological activity of HDL-paraoxonase induced on incubation with different sugars.

Strategy and Methods: Purified HDL (1 mg/ml) from human plasma was incubated with glucose, glycoaldehyde or ribose at a final concentration of 15 mM at 37 °C for 10 days. Where indicated 8 mM isoferulic acid or aminoguanidine were included. HDL incubated with 30 µM EDTA under the same conditions served as control. HDL alone was also incubated with 8 mM isoferulic acid or aminoguanidine to detect any possible effect of the inhibitors on HDL, independent of sugars. The physicochemical and functional changes were evaluated in samples by monitoring the decrease in free amino groups and intrinsic fluorescence of tryptophan, development of AGE fluorescence and decrease in HDL-paraoxonase activity.

Main Results: Glycation induced changes in sugar-modified HDL were obtained in the decreasing order as glycoaldehyde > ribose > glucose, as seen by significant decrease in free amino groups, tryptophan fluorescence and paraoxonase-1 activity whereas increase in development of AGE fluorescence. Isoferulic acid showed efficient protection of HDL against sugar induced modification as values for different parameters measured were comparable to that of HDL incubated with aminoguanidine and both the inhibitors were able to retain 80% of paraoxonase activity even in HDL samples incubated with glycoaldehyde, the most reactive glycating agent of the study. HDL incubated with the inhibitors alone showed no change in their structural and functional attributes with results similar to that of native HDL.

Conclusions: Our findings demonstrate effective protection against glycation-induced modification in HDL by isoferulic acid, even in the presence of highly reactive carbonyl, glycolaldehyde. This signifies therapeutic potential of isoferulic acid for patients with diabetes especially those with micro- and macrovascular complications, who tend to be in a proinflammatory state and thus at a higher risk for cardiovascular disease.
MAILLARD REACTION PRODUCTS IN FOLLOW-UP FORMULA FOR INFANTS AND TODDLERS

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Main Objectives: The aim of the study was to investigate the formation of Maillard reaction products (MRPs) in the two most consumed brands of follow-up formula (gruel) products on the Swedish market, brand 1 (B1) and brand 2 (B2) from the moment that the package is opened to 28 days after storage at room temperature in the dark.

Strategy and Methods: The gruel products, B1 and B2, were purchased from a local supermarket. On the experimental starting date (day 0), three gruel packages of B1 and three of B2 were opened and samples were collected for analysis. To investigate the effect of storage on MRPs content, new samples from the opened packages were collected after two weeks (day 14) and four weeks (day 28), respectively. To exclude any effect associated to differences between batches of gruel powder, triplicate of three different batches of both brands were analyzed. The MRPs: furfural, 5-hydroxymethyl furfural (HMF), N-(1-Carboxyethyl)-L-Lysine (CEL), N-(1-Carboxymethyl)-L-Lysine (CML), fluorescent advanced glycation end products (AGEs) and melanoidins (brown colour) were selected for analysis.

Main Results: Gruel of brand B1 had consistently lower MRPs compared to B2. The initial MRPs content in B1 and B2 respectively was as follows: 4.39 and 13.74 µg/g of furfural; 1.11 and 1.47 µg/g of HMF; 73.64 and 134.3 µg/g of total CML; 19.79 and 30.42 µg/g of total CEL; 51.11 and 73.01 AU/g of fluorescent AGEs; 0.52 and 1.45 AU/g of MRPs that absorb UV-light at 420 nm and 1.40 and 3.22 AU/g of MRPs that absorb UV-light at 360 nm. Moreover during storage, a more pronounced increased was observed in B2 compared B1. No batch to batch difference was apparent.

Conclusions: This study revealed that gruel products of different brands vary in their content of MRPs, with B2 having higher levels compared to B1 at the time of opening the packages. In addition, significant amounts of MRP were formed during the recommended shelf life for gruel powder. Based on the results children consuming gruel from B2 are exposed to 1.3-3.1 times more MRPs compared to gruel from B1. Several studies have linked CML and CEL to inflammation and associated pathological conditions such as Diabetes. Bearing in mind that gruel intake constitutes a relatively large and consistent proportion of food intake in Swedish infants and toddlers, the effects of relatively high dose/kg body weight of process and storage-induced MPRs/AGEs on inflammatory markers in children need to be evaluated.

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IS FAT OR PROCESS-INDUCED CHEMICALS THE CULPRIT?

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**Main Objectives:** A high-fat diet as well as intake of heat-induced toxic chemicals such as advanced glycation/lipid-oxidation end products (AGEs/ALEs) has been linked to inflammation-mediated pathological conditions such as cardiovascular disease, Alzheimer’s disease and diabetes including associated complications. However, since intake of fat is usually accompanied by heat processing, the specific effect of high fat intake versus the effect of AGEs/ALEs has not been clearly distinguished. Thus, collective measures to decrease intake of fat without specific consideration of the effect of processing may not be optimal in development of effective prevention policies. The aim of the study was therefore to investigate the effects of different diets (low-fat control diet, high-fat diet, heat-treated high-fat diet) on body and organ weights, glucose tolerance, cholesterol levels, gut microbiota composition, and atherosclerosis in mice.

**Strategy and Methods:** The study was performed in weight-matched male hypercholesterolemic Apoe\textsuperscript{-/-} mice (Jaxson Laboratories). At 8 weeks of age the mice were administered the test diets: control diet (LF), high fat diet containing 40 E\% saturated fat (HF) and heat treated (200 °C for 10 min) HF diet. The diets were given ad libitum during 8 weeks. Body and organ weights, blood cholesterol levels, oral glucose tolerance, gut microbiota composition and atherosclerosis in the aortic root region of the heart were analyzed.

**Main Results:** Effects of heated HF diet were distinct from effects of HF diet alone. On organ weights we observed that the heat-treated HF-fed mice had on average 1.8 times higher spleen weight compared to mice fed the same high-fat diet that was not heated. Furthermore, the epididymal fat pads of mice fed heat-treated HF diet weighed significantly less than fat pads in mice fed un-heated high-fat diet. The study also showed that the heated high fat diet affected the gut microbiota and lipid profile in a different way compared to the high fat diet. The study however showed no differences in glucose tolerance, atherosclerosis or body weight between mice fed the different high fat diets.

**Conclusions:** Results from the study suggest that heating of high fat diet have specific adverse effects that are distinct from the adverse health effects associated to intake of high fat diet. Such information need to be considered for effective preventive policies. Moreover, the study indicates that heat-induced chemicals in heated high fat diet may have a vital role in the initiation of immune-mediated pathological conditions.

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Formation of Amino Sugars in the Maillard Reaction

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Main Objectives: The different modifications that sugars undergo during the Maillard reaction such as oxidation into glucosone or β-elimination into different deoxy-sugar derivatives are well known and documented. However their conversion into amino sugars such as glucosamine or fructosamine during the Maillard reaction is not studied in detail. Amino sugars are typically generated through synthetic preparations and they are found to be more reactive in enhancing color and aroma formation during the Maillard reaction. The objective of this study was to explore the role of metal ions in the formation of such amino-sugars since transition metals are highly effective in inducing oxidative decarboxylation that may lead to amino sugar formation.

Strategy and Methods: Aqueous model systems containing synthetic amino acid-copper complexes or CuCl₂ and glucose or fructose were heated in open vials at 110°C for 2 hours and the residues were analyzed by ESI/qTOF/MS. Commercial standards, isotopically labeled precursors and tandem mass spectrometry were used for confirmation of findings. Also dry model systems comprising of amino sugars, glucose, fructose and glycine with and without metal salts were prepared and their volatile profile analyzed by Py/GC-MS.

Main Results: The results indicated that the addition of copper, either as (Gly)₂Cu or CuCl₂ salt, to glycine/glucose or glycine/fructose model systems enhanced the formation of amino sugars during heating. The formation of mono- and di-conjugated sugar complexes as Amadori/Heyns products were considered to be the key intermediates undergoing oxidative decarboxylation through an intramolecular redox reaction leading to the formation of the amino sugars. Isotope labelling studies confirmed the presence of 6 labelled glucose atoms and 1 labelled glycine nitrogen. The MS/MS fragments of the target ion also matched those of the commercial standards. The enhanced reactivity of the amino sugars was demonstrated through detection of their further reaction products with glucose/fructose and glycine and the formation of several ions which along with their dehydration products constituted the major ions observed in the reaction mixture.

Conclusions: The generation of amino sugars during the Maillard reaction can be enhanced through oxidation and the use of transition metals.
INHIBITORY EFFECT OF RHODIOLA ROSEA EXTRACTS ON AGES FORMATION

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Main Objectives: Advanced glycation end products (AGEs) is known to accumulate in our bodies in accordance with aging, and enhanced by the pathogenesis of diabetic complications. It is known that preventive medicine is the most important approach to prevent the life-style related diseases such as atherosclerosis and type 2 diabetes, and improvement of daily nutritional intake is thought to prevent the pathogenesis of these diseases. To discover the effective inhibitors for AGEs formation from natural products, we focused on *Rhodiola rosea* (RR) and measured the inhibitory effects on the formation of N-ω-carboxymethylarginine (CMA), a collagen-specific AGE, and N-(carboxymethyl)lysine (CML), a oxidation dependent AGE since it possesses a strong anti-oxidative and anti-inflammatory activities.

Strategy and Methods: Rhizome of RR was extracted with 60% ethanol and further separated into 3 fractions such as ethyl acetate fraction, water fraction, and butanol fraction by separating funnel. Ethyl acetate fraction was further purified with silica-gel chromatography into 19 fractions. Gelatin and ribose was incubated with or without those fractions at 37°C for 7 day. CML and CMA contents were determined by ELISA using the monoclonal antibodies against CML and CMA.

Main Results: All extracts such as Rhizome 60% ethanol, ethyl acetate fraction, water fraction, and butanol fraction showed inhibitory effects on CML and CMA formation. Among those fractions, 18-19th fractions in 19 fractions separated from the ethyl acetate fraction showed the highest inhibitory effect on CML and CMA formation. Thus, 18-19th fractions inhibited CML and CMA formation by less than 10 μg/ml.

Conclusions: Inhibitory effect of 18-19th fractions on CML and CMA formation was higher than that of reported AGE inhibitors such as aminoguanidine and pyridoxamine. Further study is required to identify the structure of active compound(s) by NMR. Daily intake of RR would be beneficial to prevent the life-style related diseases by inhibiting AGEs formation.
ANTI-GLYCATION ACTIVITY OF KAEMPFERIA PARVIFLORA EXTRACT (SIRTMAX®) AND ITS EVALUATION IN CLINICAL TRIALS

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Main Objectives: The accumulations of advanced glycation end products (AGEs) in skin and in blood vessels causes the browning of proteins and dulling of skin, and arteriosclerosis. The biochemical process of advanced glycation is markedly accelerated in diabetes, and AGEs play a significant role in the formation and progression of many diabetes complications. The rhizome of Kaempferia parviflora (KP) (Zingiberaceae) has been used as a folk medicine to lower blood glucose level. In this presentation, anti-glycation activities of KP ethanol extract (SIRTMAX®) and its characteristic ingredient, polymethoxyflavonoids, were assayed in vitro. Furthermore, the efficacy of SIRTMAX® was also evaluated in a clinical trial, by the determination of AGEs level in blood and arterial stiffness.

Strategy and Methods: The inhibition rate for the glycation reaction of SIRTMAX® and polymethoxyflavonoids was determined by adding the samples to the reaction solution of protein and glucose and then comparing the AGE fluorescent intensity to the control. The inhibition activities on both proteins of human serum and collagen, were assayed respectively. In a double-blind, placebo-controlled crossover clinical study, 27 healthy volunteers were given a test product containing 100 mg of SIRTMAX® or a placebo by oral administration for 7 weeks. The safety and efficacy of SIRTMAX® on obesity, glucose and lipid metabolism, AGEs production and arterial stiffness were evaluated.

Main Results: In the assay by human serum protein, the IC50 value of SIRTMAX® was 25.15 mg/ml, which was seven times more effective than aminoguanidine (IC50 = 165.49 mg/ml), a clinically used anti-diabetes drug. Among polymethoxyflavonoids tested, 3,5,7,4'-tetramethoxy-, 3,5,7,3',4'-pentamethoxy-, and 5,7,4'-trimethoxyflavones showed the highest anti-glycation activities. Almost the same activity was also observed with SIRTMAX® in the assay by collagen. In the clinical trial, fasting blood glucose declined significantly in the SIRTMAX® group. For the subjects with mild hyperglycemia (HbA1c ≥ 5.4%), improvement trends in the cardio-ankle vascular index (CAVI, an index for arterial stiffness) were observed in the SIRTMAX® group. Furthermore, only a slight increase in the production of AGEs was observed in the SIRTMAX® group, compared with a significant increase in the placebo group (p = 0.0150). No abnormal values were observed during the test.

Conclusions: SIRTMAX® showed strong anti-glycation activity in vitro, and polymethoxyflavonoids were responsible for this activity. In addition, SIRTMAX® had ameliorative effects on blood glucose level, improved vascular function and inhibited AGEs production for the subjects with mild hyperglycemia (HbA1c ≥ 5.4%). No severe adverse effects caused by SIRTMAX® were found.
Main Objectives: Maillard reaction is one of the significant factors that form the redox properties of processed food. Many reductones, aminoreductones as well as active methylene furanones and pyranones are believed to be behind the redox status shift to lower voltages and thus the potential improvement of the antioxidant capacity of foods. Redox-active Maillard intermediates and products may be detected and quantified by a variety of electrochemical techniques (1). In addition to other methods, electrochemical behaviour of aqueous Maillard systems was measured with a redox electrode (2). We assess the effect of various factors (pH, reaction time, temperature, etc.), kind of Maillard reactants and the presence of other compounds, such as phenolic acids, on the increase of negative redox potential during the Maillard reaction. We also compare potentiometric (galvanic cell measurements) and amperometric (electrochemical detector) methods for the assessment of the redox-active MRPs’ formation.

Strategy and Methods: To accomplish the objectives, we used ORP (Oxidation-Reduction Potential) electrodes, HPLC with diode-array and electrochemical (+0.8 V) detectors and GC-MS systems for the determination of redox potential changes, redox-active compounds incl. phenolic acids, colour development, sugars and volatiles, respectively.

Main Results and Conclusions: Factors including temperature, reaction time, pH and kind and concentration of reactants were found as determining in the development of Maillard-derived reductants. The kinetics of sugar transformation was measured; the extent of reducing power development depends particularly on the transformation rate of particular sugar. The decrease of redox potential may be significantly suppressed by the presence of the compounds being able to enter the Maillard reaction (phenolic acids in our case) due to the reactions with the Maillard reductants and their intermediates. Close correlations were found between the decrease in redox potential, determined potentiometrically by a redox electrode, and the increase in the concentration of electrochemically active substances, in particular active methylene compounds determined by HPLC with amperometric detection (+0.8 V). Relatively stable norfuraneol formed from pentoses and 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP) from hexoses were found to contribute significantly to the overall electrochemical capacity of reaction mixtures.

References:
Main Objectives: Advanced glycation end-products (AGEs) and receptor for AGEs (RAGE) are implicated in the development of endothelial dysfunction and progression of atherosclerosis. We investigate the effect of AGEs-RAGE axis on endothelium, and affect vascular smooth muscle cell (VSMC) proliferation and activation of matrix metalloproteinase (MMP)-2 and 9 in monocyte, HUVEC, and VSMC co-cultivation system.

Strategy and Methods: For co-cultivation system, VSMC were seeded on the bottom of the 6-well plates, and HUVEC cells were seeded onto the 0.4 µm transwell inserts and allowed to grow overnight. The next day the inserts were placed into the 6-well plates containing the VSMC, and THP-1 were seeded on inserts, and co-cultured for 24 hr. The production of intracellular ROS was determined by 2',7'-dichlorofluorescin diacetate (DCF-DA) by fluorescence spectrophotometry. The phosphorylation levels of mitogen-activated protein kinases (MAPK) family proteins were measured by western blot. We monitored nuclear factor (NF)-κB p65 nuclear translocation using immunofluorescence staining. The mRNA expression was determined with quantitative real-time reverse transcriptase-polymerase chain reaction. Also, VSMC proliferation were assessed by Ki-67 immunofluorescence staining, and MMP-2 and MMP-9 activities were detected by gelatin zymography.

Main Results: RAGE deletion markedly inhibited ROS generation and production of pro-inflammatory mediators such as tumor necrosis factor-α and interleukin-1β via extracellular-signal-regulated kinases phosphorylation and nuclear factor (NF)-κB activation on HUVEC. We also observed that AGEs affect VSMC proliferation and MMP-2 and 9 activation in a co-culture system with monocyte, HUVEC and VSMC.

Conclusions: AGEs activates several signals transduction in endothelium, which affect VSMC proliferation and vascular remodeling. These may give an advanced understanding of AGEs effects on vascular endothelial dysfunction, by playing a linker role in the crosstalk among monocytes, EC and VSMC. In addition, the present study suggest that the employing co-culture system can be an effective way in atherosclerosis research involved in AGEs via creating an in vivo-like environment.
**Main Objectives:** Photo-aging and glycation stress are major causes of skin deterioration. Glycation causes advanced glycative endproducts (AGEs) to accumulate in skin, especially in long-lived proteins such as dermal elastin and collagen during skin aging. Oxidative stress caused by UVB irradiation can mediate induction of matrix metalloprotease-1 (MMP-1), a major enzyme responsible for collagen damage. Plantamajoside (PM), phenylpropanoid glycoside isolated from Plantago major, have been shown to have various biological functions such as anti-hepatotoxic, anti-inflammatory, and antioxidant activity. However, anti-skin photoaging effects of PM have not yet been reported. In this study, we investigated the protective effects of PM against AGE-induced glycative stress and UVB damage on immortalized human keratinocytes (HaCaT). We explored the inhibitory effects of PM on AGEs and UVB-induced MMP-1 and investigated the molecular mechanism underlying those effects. Those effects of PM was also examined in the aspect of MMP expression, extracellular signal-regulated kinase (ERK), P38 mitogen-activated protein kinases (p38) / NF-κB pathway, reactive oxygen species (ROS) generation.

**Strategy and Methods:** HaCaT cells were pretreated with PM for 24 h and then irradiated with UVB (20mJ/ cm2). After irradiation, the cells were further incubated in fresh serum-free culture medium. Expression of MMP-1, mitogen-activated protein kinases (MAPKs), phosphorylation of MAPKs, NF-κB were assayed by western analysis. Also, MMP-1 expression on mRNA was determined by qRT-PCR. Production of ROS was measured by 2′,7′-dichlorofluorescein diacetate (DCF-DA) assay.

**Main Results:** We have found that PM inhibited UVB and AGEs-induced MMP-1 mRNA and protein expression levels in HaCaT cells through inhibition of UVB and AGEs-induced activation of NF-κB. Inhibitors of NF-κB (Bay11-7082), and MAPKs such as ERK (PD98059), and p38 (SB203580) suppressed expression of MMP-1, but not c-Jun N-terminal kinase (SP600125). Among the upstream events of MMP expression, PM attenuated ROS production induced by UVB and AGEs.

**Conclusions:** Our study demonstrates that PM inhibits UVB and AGEs-induced MMP-1 expression in HaCaT cells through the inhibition of ROS and the inactivation of ERK, p38/NF-κB signaling pathway. Therefore, PM might be used as a potential agent for prevention and treatment of UV and AGEs-induced skin aging.
THE PROTECTIVE EFFECT OF MAILLARD REACTION PRODUCTS OF FISH PROTEIN HYDROLYSATE WITH RIBOSE AGAINST OXIDATIVE LIVER DAMAGE

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Main Objectives: Halibut (Hippoglossus hippoglossus) is often used in sushi and slices of raw fish in the world. Previous study showed that the utilization of fish byproduct by enzymatic hydrolysis for the recovery of various valuable components and fish protein hydrolysate (FPH) have been shown to possess antioxidant activity. Also recently studies demonstrated that Maillard reaction products (MRPs) play an important role in functional activities such as antioxidant, anti-inflammation. Therefore, we investigated the MRPs prepared from the reaction between FPH (MFPH) and ribose for enhancing hepatic protective effect.

Strategy and Methods: FPH was kindly provided by Dr. Kim from Korea Food Research Institute. Ribose were mixed in a 1:0.28 weight ratio with fish protein hydrolysate and heat processed at 121 °C for 38 min (pH 8.26). Human hepatoma, HepG2 cells were obtained from the ATCC, and were cultured using general methods. Cell viability was measured by MTT assay and mRNA expression of γ-glutamylcysteine ligase (γ-GCL) and Heme oxygenase-1 (HO-1) were measured by RT-PCR and quantitative PCR. Expression of MAPK proteins and phosphorylated MAPK proteins were detected using western blot assay. Also synthesis of MFPH was measured using SDS-PAGE and mass shift of FPH was analyzed by TOF-MS.

Main Results: In our results, MFPH had approximately 9 kDa molecular size in SDS-PAGE using Coomassie blue and silver staining. MFPH increased cell viability against t-BHP-induced oxidative stress. And 100 µg/mL of MFPH increased the glutathione content, γ-GCL and HO-1 mRNA expression compared with non-maillard reaction product in HepG2. Also, MFPH phosphorylated ERK and JNK MAPK proteins and increased nucleic translocation of Nrf2. And 15 types of molecular mass shift with FPH were analyzed and new molar mass produced GFPH compared with FPH.

Conclusions: Therefore, we concluded that MFPH, which had enrich hepatic protective activity against t-BHP-induced oxidative damage than non-Maillard reaction fish protein, can be used as a functional dietary source. Based on these findings, further investigation of the characterization of GFPH about ribose binding ratio or site of FPH peptides.
HEPATOPROTECTIVE EFFECT OF MAILLARD REACTION PRODUCTS OF WHEY PROTEIN CONCENTRATE AGAINST OXIDATIVE STRESS THROUGH THE NRF2-DEPENDENT ANTIOXIDANT PATHWAY IN HEPG2 CELLS

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Main Objectives: Whey proteins which contain α-lactalbumin and β-lactoglobulin, lactoperoxidase and lactoferrin, are utilized widely in food industry. Maillard reaction is a complicated reaction that produces the so-called Maillard reaction products (MRPs). The MRPs have been associated with the formation of compounds with strong antioxidant activity.

Strategy and Methods: This study examined the hepatoprotective activity of MRPs of WPC against oxidative stress through the Nrf2-dependent antioxidant pathway in human hepatoma HepG2 cells. Whey protein concentrate was glycated through Maillard reaction with glucose, at 1:5 ratios in 0.1 M sodium phosphate buffer (pH 7.4). The glycation reaction occurred at 55°C, 60 rpm in shaking water bath for 7 days. The confirmation of formed MRPs was performed by using fluorescence intensity and SDS-PAGE. The cytotoxicity of MRPs was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The hepatoprotective effects of the MRPs were evaluated by measuring the glutathione levels in the HepG2 cells. The intracellular reactive oxygen species (ROS) generation was determined using the 2′,7′-dichlorofluorescein diacetate (DCFA-DA). Glutathione levels were spectrophotometrically measured by conversion of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to its colored product upon reduction by GSH-dependant glutathione reductase. Expression of Nrf2 translocation was assayed by western blot. Gene expression of glutamate-cysteine ligase subunit was assayed by RT-PCR.

Main Results: The fluorescence intensity of glucose-WPC increased after 7 days compared to WPC. The result of SDS-PAGE showed that high molecular weight compounds by the maillard reaction was formed. The treatment of glucose-WPC increased the cell viability against oxidative stress induced by t-BHP. Glucose-WPC inhibited the generation of intracellular reactive oxygen species upon t-BHP, and increased glutathione level in HepG2 cells. The treatment of glucose-WPC induces Nrf2 translocation and gene expression of glutamate-cysteine ligase modifier subunits.

Conclusions: The results of this study demonstrate that the Nrf2 pathway plays an important role in the regulation of glucose-WPC-mediated antioxidant effects in HepG2 cells.
PLANTAMAJOSIDE ATTENUATED ADVANCED GLYCATION END-PRODUCTS-INDUCED ADHESION MOLECULES AND RAGE VIA NF-κB TRANSLOCATION MODULATING

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Main Objectives: In hyperglycemia condition, advanced glycation end-products (AGEs) are considered as a risk factor, which are multiple simultaneously formed between sugar and proteins. The receptor for AGEs (RAGE) provokes intracellular reactive oxygen species (ROS) to progress inflammatory cascades. Extensive inflammation responses were regarded to cause various pathogenic diseases, including diabetes. NF-κB is a transcription factor to induce inflammatory cytokines by translocating from cytosol to nucleus. AGEs are inducible sources for generating ROS as well as triggering NF-κB translocation that express RAGE and adhesion molecules. Atherosclerosis, major inflammatory disease, is initiated with endothelial dysfunction that mediated with monocytes interaction through adhesion molecules. Plantamajoside (PM), is isolated from Plantago asiatica, is natural compound what it was reported to enhance anti-oxidant effects. We investigated how PM inhibits AGEs-induced steps of endothelial dysfunction.

Strategy and Methods: Intracellular ROS quantified with 2’, 7’-dichlorofluorescin diacetate (DCF-DA) and monocyte adhesion assay with 2’,7’-bis(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxyethyl ester (BCECF-AM) labeling on monocyte, THP-1, and detected by using a fluorescence multi-plate reader and microscopy. Cellular total nuclear factor-like 2 (Nrf2), RAGE, adhesion molecules were detected by western blotting and NF-κB also detected by same methods after manual nuclear fraction steps. Cell were co-treated with sample and lysates supernatant used for research.

Main Results: We confirmed that PM decreased in revelation of RAGE and monocytes adhesion by decreasing adhesion molecules. In addition, PM attenuated the generation of intracellular ROS induced by AGEs in human umbilical vein endothelial cells (HUVEC). Moreover, PM not only repressed trans-localization of NF-κB but also up-regulated total Nrf2 for transcription anti-oxidant proteins, especially γ-GCS.

Conclusions: Natural compound from Plantago asiatica, PM, enhanced anti-oxidant defense system and prevents inflammation response. This study suggested that PM is a potentially nature source for preventing AGEs-induced endothelial dysfunction.
IDENTIFICATION OF IMMUNOMODULATORY ACTIVE COMPOUND FROM GLYCATED WHEY PROTEIN CONCENTRATE BY MALDI-TOF/TOF MS/MS

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Main Objectives: The Maillard reaction, or non-enzymatic browning, occurs between amino groups of protein and carbonyl groups of sugar during the cooking or storage of foods. On the previous study, glycated whey protein concentrate (G-WPC) was determined to have the stimulatory effects on the pro-inflammatory cytokines expression and phagocytosis activity on RAW 264.7 cells. The aim of this study was to identify the active components having immune-enhancing effect in G-WPC with lactose via Maillard reaction.

Strategy and Methods: G-WPC was prepared during a glycation between WPC and the lactose contained in the WPC under the condition of 55°C for 24 h. To confirm and characterize the active compound of G-WPC, the formations of high molecular weight complex (HMW) were separated using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the HMW was digested using in gel trypsin digestion. Active compounds of G-WPC proposed a 3D structure based on the results of a matrix-assisted laser desorption / ionization coupled with time-of-flight tandem mass spectrometer analysis (MALDI-TOF/TOF MS/MS).

Main Results: The formations of high molecular weight complex (HMW) of G-WPC were found in the SDS-PAGE profiles. The result of MALDI-TOF/TOF MS/MS was shown that the peptide mass of HMW was similar to the peptide mass of original proteins such as lactoferrin (LF), β-lactoglobulin (β-LG), α-lactalbumin (α-LA), and bovine serum albumin (BSA). The mass matching rate of these original proteins on HMW was followed by LF, BSA, β-LG, and α-LA.

Conclusions: On the result of this study, we found the several active components of immune-enhancing activity in G-WPC. The immunomodulatory effects of G-WPC were observed in active components obtained from LF, BSA, β-LG, and α-LA via Maillard reaction. The glycated LF with lactose was the major component having immunomodulatory activity in G-WPC. Therefore, we suggested that glycated LF could be a potential material as an immune enhancer in the pharmaceutics and foods.
ACRYLAMIDE AND HMF FORMATIONS IN CHITOSAN CONTAINING MODEL SYSTEMS DURING HEATING

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Main Objectives: Chitosan is the collective name for a group of partially and fully deacetylated chitins. Chitin, a major component of the shells of crustacea, is a linear polysaccharide composed of 2-acetamido-2-deoxy-D-glucose, while chitosan is the copolymer of 2-acetamido-2-deoxy-D-glucose (acylated unit) and D-glucosamine (deacetylated unit). Chitosan has been widely applied in numerous fields including food. It has wide spectrum of antimicrobial activity and has therefore taken attention as a potential food preservative of natural source in fresh fruits and seafood. Chitosan was incorporated into thermally processed foods like bread, to enhance its technological properties and to delay staling.[1] On the other hand, increased acrylamide content was reported when chitosan was incorporated to the biscuit recipe. In this context, the objective of this study was to investigate the formation of acrylamide, as well as HMF, in chitosan containing dry model systems during heating.

Strategy and Methods: Four aqueous model systems, (i) asparagine, (ii) asparagine-chitosan, (iii) asparagine-glucose, (iv) glucose-asparagine-chitosan were prepared in test tubes. They were lyophilized and heated at 180°C for 5, 10 and 15min. After heating, the reaction products were extracted with 10mM formic acid. For acrylamide analysis, extract was cleaned-up with Oasis-MCX SPE and analyzed by Waters UPLC system coupled to MS/MS. HMF content was analyzed using Shimadzu UFLC system after filtering the extract through 0.45µm nylon-filter.

Main Results: Asparagine either alone or in the presence of glucose formed acrylamide as repeatedly reported in previous studies. However, presence of chitosan increased acrylamide formation from asparagine in dry model systems. Such that, acrylamide content increased by 3.6 and 5.4 times when chitosan was heated with asparagine for 5 and 10min, respectively. Besides, when chitosan was heated with asparagine-glucose system for 10 and 15min, acrylamide content was increased by 1.3 and 1.5 times, respectively. Presence of chitosan in asparagine-glucose model system also led to increase in HMF content by 14 times. The results revealed that carbonyl group in the acetylated unit of chitosan might be a potential carbonyl source to react with asparagine and to form acrylamide. Besides, HMF formed during heating can be a potent carbonyl source to form acrylamide as reported previously.[2]

Conclusions: The results revealed that chitosan causes an increase in the formations of acrylamide and HMF during heating under certain conditions. Therefore, its use in foods should be evaluated carefully from a food safety point of view, especially for foods processed at elevated temperatures like bakery products.


EFFECT OF IN VITRO GASTROINTESTINAL DIGESTION ON 
α-DICARBONYL COMPOUNDS IN BISCUITS

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Main Objectives: α-dicarbonyl compounds are the intermediates formed in thermally processed foods such as wine, carbonated soft drinks, fruit juices, coffee, high-fructose corn syrup, baked cookies, manuka honey and baby foods. Since α-dicarbonyls react irreversibly with the amino and sulphhydril residues of proteins and peptides leading to formation of advanced glycation end products (AGEs) that are known as being involved in some chronic-degenerative diseases in humans, amount of ingested dicarbonyl compounds with foods is of importance. As total amount of dicarbonyl compounds in ingested food does not reflect the available amount for the body owing to their highly reactive nature, fate of α-dicarbonyl compounds during digestive process is also another important issue for human health. This study aimed to investigate the effect of gastrointestinal conditions on α-dicarbonyl content of biscuits.

Strategy and Methods: An in vitro multi-step enzymatic digestion system simulating gastric, duodenal and colon phases was used to evaluate the fate of dicarbonyl compounds during digestion. Besides twice baked, regular and baby biscuits, different model systems were also subjected to in vitro digestion to explain the interactions between α-dicarbonyl compounds and enzymatic or food proteins. Moreover, furosine analyses were performed in model systems subjected to digestion aiming to understand if early protein glycation is possible under the gastrointestinal conditions.

Main Results: The levels of MGO decreased during gastric, duodenal and colon phases and finally MGO reduction was found to be around %64 at the end of digestion in both regular and twice-baked biscuits. In contrast, MGO levels increased significantly in baby biscuits indicating that intermediates accumulated during baking are converted to MGO under gastrointestinal conditions. Model systems, composed of cysteine and MGO, were used to understand the interaction mechanism and the results confirmed that the disappearance is due to reactions of MGO with free amino acids. Besides, glucose-protein model systems were subjected to digestion in order to evaluate the interactions between α-dicarbonyl compounds and protein during digestion. During digestive process, 3-DG decreased whereas furosine concentration increased.

Conclusions: The results reveal that gastrointestinal conditions favor the reactions of dicarbonyl compounds with free amine and thiol groups of proteins. This leads to decreased amounts of available dicarbonyl compounds in the gastrointestinal system. On the other hand, early glycation of proteins is also favored under the same conditions that mean at least the loss of lysine, an essential amino acid. These facts should be taken into account to design both the formulation and processing conditions of bakery products.
HEAT-INDUCED TOXICANTS AND ODORANTS IN COOKED MEAT

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Main Objectives: Cooking conditions highly influence meat properties by producing compounds responsible for cooked meat aroma and heat-induced toxicants. This paper investigates the consequences of main cooking processes on beef meat profile in odorants such as pyrazines and toxicants with the example of polycyclic aromatic hydrocarbons (PAHs).

Strategy and Methods: Common cooking modes including conduction (pan cooking), convection (oven and grilling), and radiation (microwave) were selected. Toxicants were identified and quantified in the cooked meat samples by comprehensive two-dimensional gas chromatography - time-of-flight mass spectrometry (GC×GC-TOF/MS) after extraction by accelerated solvent extraction (ASE), centrifuge evaporation and clean-up.

Odorants were extracted by Dynamic Headspace and identified by “high resolution olfactometry” coupling dynamic headspace-GC-eightbooth olfactometry (GC-MS/8O) and heart-cutting multidimensional GC hyphenated with olfactometry and mass spectrometry (GC-GC-MS/O) and validated by using retention index, odour and mass spectra databases. Compounds whose level was affected by cooking mode were selected by One-way-ANOVAs. Principal Component Analysis (PCA), processed on the normalized datasets, permitted to investigate the influence of meat cooking conditions based on PAHs content or odorants.

Main Results: GC-8O/MS enabled to identify 53 odorants. To resolve certain coelution zones, a GC-GC-O-MS was run and 15 additional odorants were found. PCA results confirm that the more intense cooking conditions are, the more odorant pyrazines, sulfur and carbonyls compounds are formed.

A GC×GC-TOF/MS method was developed to achieve a multiresidue separation of 17 PAHs in complex meat matrix and to solve common coelutions. Recoveries and sensibility were satisfactory and the level of benzo[a]pyren was compatible with concentrations potentially met in food. PCA results confirm that intense cooking mode promotes the generation of benzo[a]pyren.

Conclusions: This paper demonstrated that high resolution olfactometry was relevant to determine the key odour-active compounds in cooked meat. Therefore, this approach should be applied more systematically to investigate the aroma of complex processed food. Similarly, GC×GC-TOF/MS was relevant for multiresidue determination of toxicants like PAHs. It was demonstrated that severely cooked meats are characterized by significant presence of the most carcinogenic PAH, benzo[a]pyrene and at the same time, by aromatic pyrazines and carbonyl compounds which can drive the acceptance of some consumers. Those results could be used for multi-objective optimization of cooking process of meat to find proper balances between flavor acceptability and food safety for different consumer targets.
INFLUENCE OF VARIETY AND AGRONOMIC FACTORS ON THE LYSINE CONTENT IN CHICORY ROOTS, AND ON $N^\varepsilon$-CARBOXYMETHYL-LYSINE FORMATION DURING ROASTING

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Main Objectives: During the heat treatment of the coffee and its substitutes many neoformed compounds are synthesized by the Maillard reaction. This reaction between reducing sugars and amino acids leads to the formation of compounds potentially deleterious to health. Among these the $N^\varepsilon$-carboxymethyl-lysine (CML) was detected at high levels in coffee substitutes. The objective of this study was to evaluate the impact of changes in agricultural practice over the total lysine content present in chicory roots and try to limit the formation of CML during roasting without modifying the process.

Strategy and Methods: The levels of total lysine and protein were quantified in 24 varieties of chicory and in the course of three years of cultivation the contents of total lysine and protein were compared among five commercial cultivars with different levels of nitrogen fertilization and varying harvesting dates. The chemical degradation of lysine during the drying and roasting of the chicory relative to the formation of CML was also studied. Both lysine and CML were quantified using stable isotope dilution liquid chromatography/tandem mass spectrometry methods.

Main Results: Of the 24 varieties of chicory analyzed, small variations in total lysine content and proteins were observed, 213 ± 8 mg and 4.8 ± 0.2 g/100 g dry matter (DM), respectively. The formations of lysine and proteins tested in 5 commercial varieties were affected by the nitrogen treatment with mean levels of 176 ± 2 mg and 3.9 ± 0.1 g/100g DM, respectively, when no fertilizer was added , and 217 ± 7 mg and 5.1 ± 0.2 g/100g DM, respectively, with a nitrogen supply of 120 kg/ha. The varying lengths of time taken in the chicory cultivation and the harvest year had uncertain impacts on the level of lysine and proteins in roots. In accord with the low levels of lysine in roots cultivated without nitrogen fertilizer, their roasting led to the formation of low levels of CML (490 ± 51 µg/100g DM) compared to higher levels in roasted roots initially cultivated with a nitrogen supply above 120 kg/ha. The total lysine content of fresh roots was significantly correlated to the concentration of CML formed in roasted roots (r = 0.51; p <0.0001; n=76).

Conclusions: Roasted foods such as coffee substitutes which are not consumed as a major source of protein and lysine should be prepared from raw materials low in these nutrients in order to lower CML levels.
**Main Objectives:** This study aimed to examine the impact of odor from the glycine/glucose Maillard reaction on human mood and brainwaves. The odorants possibly affecting mood and brainwaves were identified.

**Strategy and Methods:** An equimolar solution of glucose and glycine (1 mol/l) was prepared in sodium carbonate buffer and the pH was adjusted to 7. The solution was heated at 90°C for 30 min in an oil bath. Potent odorants in the solution were determined by aroma extract dilution analysis (AEDA) and the concentrations of each odorant were quantified. Based on the obtained results, a “model solution” mimicking the Maillard solution was prepared. Next, 10 healthy subjects aged 21-24 were asked to inhale the Maillard reaction solution, the model solution comprising the potent odorants, the two strongest odorants detected by AEDA and distilled water (control). The change in subjects’ mood was scored and alpha and beta brainwaves were recorded before and after inhalation.

**Main Results:** Subjects felt less tense after inhalation of the Maillard reaction solution than from the control. This finding was supported by an increase in proportion of alpha-brainwaves and decrease in proportion of beta-brainwaves. As detected by AEDA, the potent odorants in the pH-7 sample were 2, 3-dimethylpyrazine; 2, 5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF); trimethylpyrazine and octanoic acid. The model solution comprised these four odorants. Subjects showed an increase in alpha-brainwaves after inhalation of the model solution. The two strongest odorants, 2, 3-dimethylpyrazine and DMHF, were prepared at their concentrations in the Maillard reaction, and the effects of the individual potent odorants on alpha-brainwaves were investigated. An increase in alpha-brainwaves was observed, suggesting that these two odorants play an important role in increasing alpha-brainwaves.

**Conclusions:** The odor arising from the glycine/glucose Maillard reaction has relaxing effects in humans; these effects could be attributed to 2, 3-dimethylpyrazine and DMHF.
THE FORMATION AND BROWNING MECHANISMS OF BLUE PIGMENTS, MELANOIN INTERMEDIATES

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Main Objectives: We proposed that yellow, blue, and red pigments involved in pyrrolopyrrole ring would be key intermediate in melanoidin formation from reducing sugar and amine reaction systems. We studied on the formation and browning mechanisms of blue pigments produced from D-xylose and glycine reaction system. Furthermore, we tried the detection of pyrrolopyrrole compounds in model melanoidin and browned foods by specific antibody.

Strategy and Methods: D- Xylose (1.0 M) and glycine or β-alanine (0.1 M) were incubated at 26.5 °C for 24h in 0.1 M sodium bicarbonate containing 60% ethanol solution. Precursors of blue pigment were isolated and purified from the reaction solution using xylose-glycine and xylose-β-alanine by various chromatographies. When these precursors were incubated at 26.5 °C for 15h, blue pigments were formed. Major blue-pigment named Blue-M1 was incubated at 26.5 °C for 7 days. The purified reaction products were analyzed by LC-ESI-Q-TOF-MS and NMR measurement. An immunogen consisting of pyrrolopyrrole aldehyde was linked onto KLH and used to raise polyclonal antibodies in the mice. The antibodies cross-reacted with pyrrolopyrrole aldehyde and Blue-M1.

Main Results: Blue compounds were detected in the D-xylose-β-alanine reaction system. The major blue pigment named Blue-B1 was identified as having a similar structure to Blue-M1. Blue-B1 had four carboxyethyl groups instead of carboxymethyl groups of Blue-M1. From the results using 13C1-D-xylose-glycine reaction system, it is confirmed that the carbon at the 1-position of D-xylose has been taken in two adjacent carbons of pyrrolopyrrole ring. Blue-M1 and Blue-B1 were also formed from labile precursors called PM and PB, respectively. Our studies revealed that novel blue compounds with both glycine and β-alanine residues were generated by the incubation of mixture of PM and PB. We assume that PM might be formed by the reaction among pyrrolidone, glycine, and 3-deoxygenolose or xylosone. When the Blue-M1 was incubated, colour of Blue-M1 changed to brown. The reversed phased HPLC pattern after the incubation of Blue-M1 indicated that yellow pyrrolopyrrole aldehyde compounds and blue degradation compounds of side chain of Blue-M1 were generated. These findings suggest that the methine bridge and side chain of Blue-M1 is cleaved to form pyrrolopyrrole aldehydes and blue compound with methyl group. Anti-pyrrolopyrrole antibody cross-reacted with model melanoidins and browned soy paste.

Conclusions: Our studies indicate that precursor compounds of blue pigments might be formed by the reaction among pyrrolidone, glycine, and 3-deoxygenolose or xylosone. Moreover, blue pigments are polymerized with degradation to form melanoidins. We obtained anti-pyrrolopyrrole-antibody recognized model melanoidin and browned foods, indicating that pyrrolopyrrole structure contain in melanoidin molecules.
QUANTITATIVE ANALYSIS OF D-AMINO ACIDS IN SWEET RICE WINE (MIRIN) AND STUDIES ON THE MECHANISM OF THEIR FORMATION DURING MATURATION

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Introduction and Objectives: Sweet rice wines (MIRIN) are alcoholic condiments used as seasoning in Japanese cuisine, and those matured for a long period are considered especially as key ingredients for top-level cuisine. Mirin is produced from glutinous rice, Koji malt (non-glutinous rice malted by Aspergillus oryzae), and distilled spirit, and is fermented at room temperature for about two months. Then, after filtration some Mirins are immediately made into seasoning products, but others are stored for long periods to ripen in the presence of high concentration alcohol and glucose, in order to produce well balanced sweet flavor and “mouthfullness”. This study was conducted to investigate the influence of the Maillard reaction on the amino acid racemization of Mirin during maturation.

Strategy and Methods: The content of thirteen L, D-AAs (ALA, ARG, ASP, GLU, ILE, LEU, LYS, PHE, SER, THR, TRP, TYR, and VAL) in eleven Mirins (three non-ripened and eight ripened) were analyzed by RP-HPLC using derivatization reagents (o-phthalaldehyde /N-isobutyryl-l-cysteine). Fru-Ala was synthesized by the method of Anet et al. (1958), and the Fru-Ala content in the eleven Mirin samples was analyzed by cation-exchange HPLC. The relative quantities of fructose-amino acids (ALA, ARG, ASP, GLU, PHE, SER, TYR, and VAL) in the ripened and non-ripened Mirins were calculated from peak areas acquired by ESI-TOF-MS.

Main Results and Conclusions: The proportions of D-amino acid forms (%D) of ALA, ARG, ASP, GLU, PHE, and SER in ripened Mirins were higher than in non-ripened Mirins. Fru-Ala was detected in all Mirins, ranging from 0.55-2.43mM and 0.01-0.10mM in ripened and non-ripened Mirins, respectively. ASP and PHE had the highest correlations (R²) between %D and the amount of fructose-amino acids in ripened Mirins. Bruckner et al. (2001, 2005) proposed a mechanism for the formation of D-amino acids via Amadori rearrangement products, and reported that %D in ASP, ALA and PHE were increased by addition of glucose under neutral conditions. Therefore, our study suggests that ASP and PHE were racemized via Amadori rearrangement products during the maturation of ripened Mirin, but that racemization of ALA appeared to be by a different process, possibly by an enzymatic reaction.
VITAMIN C DEGRADATION PRODUCTS INDUCE NON-ENZYMATIC PROTEIN GLYCATION

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Main Objectives: L-threo-ascorbic acid (AsA) and its degradation products participate in chemical modifications of proteins in vivo through non-enzymatic glycation (Maillard reaction) and formation of Advanced glycation end products (AGEs). 2,3-diketogulonic acid (DKG) has a characteristic α-dicarbonyl structure, and α-dicarbonyl compounds are known as central intermediates in AGEs formation. In the present work, characterization of DKG modified protein was investigated.

Strategy and Methods: Bovine serum albumin (BSA) -AGEs were prepared with 0.5M Glucose, AsA, 0.05M methylglyoxal (MGO) or DKG during incubation at 37°C from 0 to 4 weeks. After incubation, protein solutions were extensively dialyzed for 4°C for 24 hours. The BSA-AGEs were characterized by absorbance, carbonyl content, and extent of side chain modifications (lysine and arginine). Also, the molecular mass information of the obtained AGEs were determined using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Main Results: The fluorescence spectra recorded at an excitation wavelength of 370nm increased as time progressed. Modification of BSA with DKG for 24 hours yielded a carbonyl content of roughly 130%, while modification for 1 week yielded approximately 200%. DKG seems to modify lysine side chains to a higher degree than arginine. It was estimated to be roughly 50% for lysine, and 0% for arginine during incubation for 24 hours.

The molecular weight of non-modified BSA was found to be about 66400Da. On the other hand, BSA modified with DKG for 24 hours was found to be 67496Da. This suggested that approximately 7 molecules of DKG might be involved in the modification of BSA molecule in 24 hours.

Conclusions: Our findings suggest that DKG modified BSA is considered to exhibit the characteristic of AGEs. It is clear that degradation of L-threo-ascorbic acid into DKG is a significant part of glycation pathway of AsA.
Main Objectives: Cheese turns brown during storage. Although there are a lot of studies on the browning of milk, the study on the browning of cheese are a few. As the browning of milk occurs by the Maillard reaction, the browning of cheese is generally attributed to the Maillard reaction. But cheese contains far less lactose than milk and there is no chemically clear evidence on the relationship between the browning of cheese and the Maillard reaction. The aim of this study was to identify the precursor of browning of cheese and clarify the relationship between the browning of cheese and the Maillard reaction.

Strategy and Methods: Cheddar cheese was extracted with hexane, ethyl acetate, ethanol, and methanol, successively. Each fraction and residue was incubated at 70°C for a day, before the browning being spectrophotometrically or visually estimated. Methanol extract was dissolved in water and applied to ODS column. No-adsorbed fraction was further applied to a cation exchanger (Amberlite IR-120[H+]). After non-adsorbed fraction was concentrated, excessive acetone was added, before the formed precipitate being removed. The supernatant was concentrated and purified with silica gel chromatography with a developmental solvent of n-BuOH/ acetic acid / water (12:1:1). The fractions turning brown were collected and evaporated. This isolated compound was applied to instrumental analyses such as NMR and MS, and analysed with HILIC-HPLC for sugars.

Main Results: The methanol extract of a Cheddar cheese showed the most intensive browning. The methanol extract was then purified with chromatographical procedures. Each fraction was incubated at 70°C, and a fraction turning brown was further purified. As a result, an isolated fraction was identified with galactose using instrumental analyses. This result clearly showed that galactose was the major precursor of the brown color of the cheese during storage and that the Maillard reaction contributed to the discoloration of cheese. The similar analysis was done for two other brands of Cheddar cheese. As a result, galactose was not detected but lactose was detected in a cheese turning brown during storage. Both sugars were not detected in a cheese turning little brown. These results suggest that residual lactose or galactose after lactic acid bacteria fermentation during cheese-making play an essential role of browning of cheese during storage.

Conclusions: We identified galactose and lactose as major precursors of browning of cheese, showing these residual sugars mainly contributed to the browning of cheese by the Maillard reaction.
Main Objectives: In general, cysteine, a sulfur containing amino acid, represses the browning reaction between sugars and amino acids. However, our group identified 2,4-dihydroxy-2,5-dimethyl-3(2H)-thiophenone as a low-molecular weight pigment from soy sauce. As this compound contained sulfur, it was considered to be a Maillard reaction product derived from cysteine. This finding stimulated us to find a Maillard pigment derived of cysteine. The aim of this present study was to find a low-molecular-weight Maillard pigment formed from cysteine and glucose. Here we describe isolation and identification of a novel Maillard yellowish pigment, named pyrrolothiazolate, as well as its formation mechanism.

Strategy and Methods: A solution containing 0.1 M L-Cys, 0.03 M L-Lys, 0.5 M D-Glc and 0.5 M acetate buffer (pH 5) was autoclaved at 120°C for 3 h. After being washed with EtOAc, the solution was adjusted to pH 2 and a yellow pigment was extracted with EtOAc. The extract was purified with a silica gel and preparative HPLC. As a result, a pale yellow powder was obtained. The powder was dissolved in a little amount of MeOH and placed on refrigerator. A colorless prism crystal was obtained.

Main Results: The isolated compound showed two absorption maxima at 300 and 360 nm under neutral and acidic conditions, while did at 320 and 400 nm under alkaline conditions. Yellow color of the solution turned dense in alkaline region. This compound was optically active and identified as 6-hydroxy-3[R],7a[S]-dimethyl-7-oxo-2,3-dihydropyrrolo[2,1-b]thiazole-3-carboxylic acid by such instrumental analyses as MS, NMR and X-ray. As this compound was a novel pyrrolothiazole derivative carrying a carboxy group, we named it pyrrolothiazolate. The detection limit for pyrrolothiazolate visually estimated was about 0.6 mg/ml. In a model system containing cysteine, lysine, and glucose, 1-2 mg/mL of this compound was formed. Pyrrolothiazole was considered to be formed through 1-deoxyglucosone. Pyrrolothiazole showed anti-oxidative activity.

Conclusions: A novel Maillard yellowish pigment formed from cysteine and glucose was identified.
HYDROTHERMAL AGGREGATION BEHAVIOR OF BOVINE SERUM ALBUMIN REGULATED BY GLOXAL-DERIVED GLYCATION

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Main Objectives: Even though the extensive application of glycation in food technology, it remains a problem for the effect of each stage of glycation on thermal aggregation of protein. This work aims to explore the mechanism underlying the effect of the final stage of glycation on the thermal aggregation behavior of protein.

Strategy and Methods: A model system consists of bovine serum albumin (BSA) and glyoxal (GO) was established at 100 °C to simulate the final stage of glycation. Aggregation behavior of both glycated and controlled samples was studied using fluorescence spectrum, gel permeation chromatography (GPC) and dynamic light scattering (DLS). Structural analysis of both unglycated and glycated aggregates was obtained by using transmission electron microscope (TEM) and small angle x-ray scattering (SAXS).

Main Results: Extrinsic 1-anilinonaphthalene-8-sulfonate (ANS) fluorescence results revealed a sharp decline of surface hydrophobicity (from 1247.21 to 142.73) for glycated sample compared with the controlled one (from 1247.21 to 983.32) during the initial 30 min incubation. Furthermore, formation of intermolecular disulfide bond was inhibited along with the formation of cystine-derived AGEs in initial 15 min in glycated samples. In line with the declining surface hydrophobicity and intermolecular disulfide bond, hydrothermal aggregation of glycated samples was markedly suppressed. Majority of glycated aggregates were restricted to dimmer, trimmer, tetramer with a diameter less than 15 nm when GO concentration was elevated to 10 mM, as illustrated in GPC and DSL results. In addition, conformation of aggregates were shown to transform from solid globular granules with surface fractals into loose and short branched ones which belong to mass fractals when glycation was intensified gradually according to TEM and SAXS results.

Conclusions: Declined surface hydrophobicity, decreased intermolecular disulfide bond and elevated steric hindrance are concluded to play crucial role in the dramatic change of aggregation behavior of BSA as well as conformational change of glycated aggregates.
CHANGES IN N- (CARBOXYMETHYL) LYSINE CONTENT OF SOY SAUCE DURING STORAGE

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Main objective: Soy sauce is supposed to be an abundant source of advanced glycation end products (AGEs), and more than 98% of its AGEs are in free form which is more bioavailable and harmful to human beings and plays a critical role in the progress of many chronic diseases. In addition, soy sauce also has plenty of monosaccharide and amino acid which may continue to generate more AGEs during storage. The purpose of this study is to investigate the AGEs content changes of soy sauce during storage.

Strategy and methods: In this study, 6 different brands of soy sauce were stored at 0 °C, 4 °C, 35 °C and room temperature from 0 d to 57 d, and high performance liquid chromatography-mass spectrometry (HPLC-MS) was used for the quantitative determination of free N- (carboxymethyl) lysine (CML, a major AGEs) in soy sauce.

Main Results: Within 57 days, the CML contents in all 6 soy sauces were almost kept the same at 0 °C and 4 °C (their CML content growth percentages were both lower than 5%). However, when storing soy sauce in a higher temperature, its CML content could be increased rapidly. In 35 °C, the average increased percentage of 6 different soy sauces was 66.57%, while in room temperature it would be 76.11%.

Conclusions: The temperature fluctuation might account for the highest increase of CML in room temperature. Therefore, the soy sauce should be kept in low temperature to prevent the generation of new AGEs.
DISCOVERY OF ANTI-GLYcation AGENTS: STUDIES TOWARDS THE MOLECULAR TREATMENT OF DIABETES

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Main Objectives: Diabetes mellitus is a metabolic and endocrine disorder. Hyperglycemia is the hallmark of diabetes. Glycation is a spontaneous non-enzymatic reaction between proteins and reducing sugars, and results in the formation of AGEs. Continuous hyperglycemic stage creates glycation of many proteins and ultimately diabetic complications. One of the strategies for relief from diabetes is to prevent glycation of biomolecules by safe and effective anti-glycation agents.

Strategy and Methods: The present study encompasses the discovery of anti-glycation agents by using appropriate and mechanism based high throughput screening techniques. Over 2,000 fully characterized natural and synthetic compounds were systematically evaluated (on primary screening assay i.e. in vitro BSA-Methylglyoxal model) for their anti-glycation activity. In second stage of the study, potent compounds were selected for further investigation. In this stage, proteins such as elastin (long lived structural protein), insulin (short lived signalling protein), and hemoglobin (circulatory transport protein) were employed in the assay. In the 3rd stage of the study, selected compounds were subjected to α-glucosidase inhibition assay. Finally these dual inhibitors of glycation and α-glucosidase activity were subjected to cytotoxicity evaluation against 3T3 (mouse fibroblast) cell line.

In the present study, we report here for the first time impaired biological activity of MG modified insulin in in vivo mice model system and then glycation inhibitory activity of different classes of synthetic compound by using an in vitro insulin-MG glycation system.

Main Results: This study led to the discovery of potent and highly active anti-glycation agents of polyphenols, cyclopeptide alkaloids, flavonoids, oxindole derivatives, benzohydrazide Schiff bases, urea derivatives and organomatallic class of compounds with no cytotoxicity. Additionally some of these compounds were found to possess inhibitory activity against α-glucosidase enzyme.

<table>
<thead>
<tr>
<th>Activity</th>
<th>IC50 ± SEM (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA-Methylglyoxal</td>
<td>936.39 ± 0.3</td>
</tr>
<tr>
<td>Hemoglobin- Methylglyoxal</td>
<td>420.15 ± 2.6</td>
</tr>
<tr>
<td>Elastin- Methylglyoxal</td>
<td>3.05 ± 0.1</td>
</tr>
<tr>
<td>Insulin-Methylglyoxal</td>
<td>156.22 ± 0.41</td>
</tr>
<tr>
<td>α-glucosidase</td>
<td>936.39 ± 0.3</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>Non cytotoxic</td>
</tr>
</tbody>
</table>

Conclusions: On the basis of results obtained during this study, our work represents an example of systematic and comprehensive investigation of chemical and biochemical aspects of inhibition of protein glycation. A number of potent and novel “helper molecules” were discovered and structure activity relationship studies were conducted. Further research is, however, needed in order to evaluate the therapeutic potential of compounds, identified during this study, as drugs.
CAPSAICIN, AN ACTIVE INGREDIENT FROM CHILI PEPPERS, 
ATTENUATES GLYCATIVE STRESS AND RESTORES SRAGE LEVELS IN 
DIABETIC RATS

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This study assessed the effects of capsaicin (CAPS) on protein glycation and the subsequent 
formation of advanced glycation endproducts (AGEs). In vitro glycation assays showed that 
CAPS retards the late stages of glycation and crosslinking. Dual mechanisms of action 
involve oxidant and dicarbonyl trapping activities may contribute to the antiglycation 
effects. In a rat model of streptozotocin-induced permanent hyperglycaemia, 12 weeks of 
CAPS administration resulted in a reduction of the levels of circulating and tissue AGEs, as 
well as activation of the receptor for AGEs (RAGE). The levels of oxidative biomarkers, 
evaluated by assessing 8-isoprostane and lymphocyte DNA damage, were also decreased in 
the CAPS-treated groups compared with the diabetic group. Intriguingly, the reduction of 
serum and renal soluble RAGE (sRAGE) observed in diabetic rats was restored by CAPS 
administration. This is the first study to demonstrate that CAPS can reduce the burden of 
AGEs, relieving glycatve stress in diabetics.
Main Objectives: Accumulation of advanced glycation end products (AGEs) is one of the causes of diabetic complications. As short chain aldehydes have higher reactivity to amino group than glucose, much attention has been focused on these aldehydes in aspect of AGE formation. Methylglyoxal has been analyzed as a biomarker for diabetic nephropathy. There are no reports for glyceraldehyde content in diabetic animals and patients, while glyceraldehyde-derived AGEs have been demonstrated to accumulate in the atherosclerosis region of diabetic patients. The objective of this study was to develop a quantification method for glyceraldehyde in plasma and organ.

Strategy and Methods: Glyceraldehyde was determined by pre-column derivatization with 2, 4-dinitrophenylhydrazine (DNPH) or 3-methyl-1-phenyl-5-pyrazolone (PMP) followed by LC-MS/MS analysis. Plasma from diabetic patients and rat were deproteinized by mixing 3 vol. of ethanol. Rat liver was homogenized with 0.75 vol. of PBS and then deproteinized by mixing 2.25 vol. of ethanol. The supernatants were used for determination of glyceraldehyde. Rhamnose was used as an internal standard.

Main Results: DNPH-glyceraldehyde could be ionized by electrospray ionization (ESI) mass spectrometer by negative mode, but it gave multi-peaks by LC-MS/MS possibly due to keto-enol tautomerism. On the other hand, PMP-glyceraldehyde can be ionized in positive mode and gave a symmetric and single peak, which can be resolved from the peak of its isomer; PMP-dihydroxyacetone. Plasma glyceraldehyde levels were distributed between 0.24 and 50 µM in diabetic patients, which are generally higher than those of methylglyoxal. Rat plasma and liver glyceraldehyde were also detected without interfering peaks.

Conclusions: This quantification method using pre-column derivatization with PMP and LC-MS/MS would be a powerful tool for elucidating the relation of glyceraldehyde formation and progression of diabetic complications.
MICROBIOLOGICAL SCREENING TO DEGRADE MODEL MELANOIDIN
AND DETECTION OF THE ENZYMES INVOLVED

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Background: Maillard reaction to show yellow to brown color is a quite popular reaction generally
found in food with heating process or long period of storage. The occurrence of Maillard reaction in
food is considered as a symbolic step for the advances in civilization of human kind. The reaction also
occurs in vivo, due to diabetes. The purpose of this work is to know chemical structure and molecular
size of repeating unit of melanoidin.

Materials and Methods:

Model melanoidin: The authors are interested in degradation mechanisms of melanoidin in nature as
speculated by P.L. Maillard. Microbiological screening to degrade model melanoidin was made in
the islands of Okinawa. The model melanoidin used as the substrate was prepared by the reaction
between glucose(2M) and glycine(2M) for 48 hr heating at 60C, pH 7 followed by the dialysis with
visking tube against water. The nondialyzable fraction was condensed in vacuo, being stored under
freezing or at 4C, was used as the substrate of the fermentation and enzymatic detection.

DAD profile of the medium: The collected soil samples were allowed to stand still at 29C in the GYP
medium added with the model melanoidin (final conc: 0.1 mg/ml). Many enrichment cultures
showed apparent color change compared with the control. The supernatants of these cultures were
analyzed by 3D-HPLC equipped with a column of gel permeation type TSK-G3000 PW.

The amount of degraded model melanoidin was firstly judged by the reduction of peak size on
3D chromatographic profile compared with the control: that is, shifts of the peak top in the range of
void-volume to down-sizing direction, changes in peak height and width, and shoulders.

Results: About 50 soil samples, and stems and leaf of mangrove were collected at Miyakojima
Island in the Okinawa area. More than 2000 cultures were subjected to screening with 3D-HPLC.
Among them 10 cultures were screened as the candidates, and major microbial flora of 2 cultures
were characterized as Bacillus.

The Bacillus sp. M4-3 was selected. The enzymatic activity was shown in the cell free extract
prepared by supersonic treatment from the insoluble material of the medium. This extract was
subjected to the ammonium sulfate fractionation, and the fraction with 30~70% saturation was
found to be active in the degradation of the melanoidin. A novel enzyme to degrade the model
melanoidin was found to be formed in the fermentation. The specificity of the enzymes to other model
melanoidin is under investigation.
Main Objectives: Maillard reaction is nonenzymatic glycation between reducing sugars and free amino group in amino acids or proteins. The reaction is important in baking, frying or otherwise heating of nearly all foods, and responsible for many colors and flavors in foods. In addition to these reactions, we and other researchers have proposed that the free amino groups of aminophospholipids (e.g., phosphatidylethanolamine, PE) are also targets for the glycation\(^1\), which expands the concept of the Maillard reaction. Indeed, we analyzed various processed foods such as milk powder, chocolate, and soybean milk, and confirmed that these foods, especially milk powder, contains a significant amount of PE-linked Amadori product (Amadori-PE; Fig. 1A). Hence, various Amadori-PE species having different sugars and fatty acyl groups may exist in foodstuffs, which contribute to the value of foods. However, Amadori-PE analysis at molecular species levels is not still sufficient. In this study, we therefore tried to identify major Amadori-PE species in milk powder by using LC-MS/MS.

Strategy and Methods: Phospholipids extracted from milk powder were analyzed by using LC-MS/MS.

Main Results: As shown in Fig. 1B, Amadori-PE could be clearly detected in single ion-chromatograms of the extract from milk powder. LC-MS/MS analysis revealed that there were 2 types of Amadori-PE: Glc-PE, Amadori-compound derived from glucose and PE; Lac-PE, Amadori-compound derived from lactose and PE. Major molecular species (fatty acids) of both Glc-PE and Lac-PE were found to be 16:0/18:1, 18:0/18:1 and 18:0/18:2. Based on the peak intensities of Amadori-PE species, it was hypothesized that more than 50% of PE in milk powder would be glycated. The high glycation rate may contribute the value of the products.

Conclusions: To evaluate above hypothesis, we are now determining Amadori-PE species by using reaction of Amadori-PE and UV-chromogenic reagent, 3-methyl-2-benzothiazolinone hydrazone (MBTH). Furthermore, we are developing LC-MS/MS method to quantify Amadori-PE via promotion of sodium adduct formation.

Reference:
Main Objectives: Vitamins containing amino group, such as pyridoxamine, is effective in inhibiting the progress of the Maillard reaction. Although these vitamins are expected to react with carbonyl compounds, the structural data on the Maillard reaction products formed from these vitamins has been very few.

As a result of the search for the Maillard reaction products derived from vitamins and carbonyl compounds, the major reaction product derived from pyridoxaime and xylose was found and named as XP-1. In this study, we isolated XP-1 and clarified the chemical structure of this compound. Next, we developed a method for determination of XP-1 using LC-MS/MS, and clarified the factors affecting the formation of XP-1.

Strategy and Methods: XP-1 was isolated from the reaction mixture of 60 mM xylose and 60 mM pyridoxamine (pH 7.4, 90°C for 5 h) using preparative HPLC with a multimode ODS column. Structural analysis of XP-1 was performed using MS and NMR. Next, the MS/MS parameters were optimized with standard XP-1 under positive ion electrospay ionization, and then determination method for XP-1 was developed using LC-MS/MS with MRM mode. Using this method, we investigated the effect of pH, temperature, DTPA and kinds of sugars on the formation of XP-1.

Main Results: The molecular formula of XP-1 was determined as C_{13}H_{13}N_{1}O_{5} by ESI-MS, corresponding to the addition of one molecule of xylose to one molecule of pyridoxamine, and the losses of two molecule of water and each one molecule of ammonia and hydrogen. Instrumental analysis showed that XP-1 was a novel compound.

We observed that XP-1 was formed under weakly acidic and neutral conditions and that XP-1 production was markedly increased when heated at the temperature above 50°C. These findings suggested that XP-1 was likely to be produced in food processed at the high temperature. In addition, XP-1 could be degraded in the presence of metal ion because the addition of DTPA, a strong metal chelator, resulted in the increase of XP-1 production. Moreover, we found that XP-1 was formed from not only pentose but also hexose.

Conclusions: XP-1 was a novel Maillard reaction product derived from pyridoxamine and sugars (pentose or hexose). XP-1 was likely to be produced in food system.
**EFFECT OF RUTIN SUPPLEMENTATION IN RYE-BUCKWHEAT GINGER CAKES ON MAILLARD REACTION DEVELOPMENT AND ANTIOXIDATIVE PROPERTIES**

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**Main Objectives:** The objective of this study was to find out the effect of rutin (quercetin-3-O-rutinoside) fortification in rye-buckwheat ginger cakes on Maillard reaction progress and antioxidative properties.

**Strategy and Methods:** Ginger cakes were formulated on rye flour and light buckwheat flour or flour from roasted buckwheat groats. The recipe of rye-buckwheat ginger cakes was enriched by adding low and high amounts of rutin to the mixture of flours. Before baking ginger cakes dough was incubated in the fermentable chamber of the oven during 72 hours at 21ºC. Further, analysis of total phenolics and rutin contents, and antioxidative capacity determined against 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation and superoxide anion radicals were performed. For the characterization of the Maillard reaction progress, markers of early (furosine), advanced (carboxymethyllysine- CML and fluorescence of intermediary compounds) and final (melanoidins) stages were determined.

**Main Results:** The rye-buckwheat ginger cakes formulated on light buckwheat flour or flour from roasted buckwheat groats and both enriched with high dose of rutin showed 83.9 and 78.7 mg of rutin per 100 g dry matter, respectively. Enrichment of ginger cakes with rutin improved their antioxidant properties. Moreover, an inhibition of furosine formation was in the range from 38 to 78%. Furthermore, high rutin dose decreased 33% of the total amount of fluorescent compounds. The loss of nutritional quality of cakes enriched with rutin was observed. This effect was related to the formation of CML at the advanced stage of Maillard reaction. Rye-buckwheat ginger cakes formulated on light buckwheat flour and enriched with rutin reduced by 35% formation of CML whereas no effect was found in those cakes formulated on flour from roasted buckwheat groats. Moreover, rutin supplementation stimulated the Maillard reaction progress to the melanoidin formation. The found melanoidins were positively correlated with antioxidant capacity measured against superoxide anion radical ($r=0.76$), total phenolics ($r=0.64$) and rutin contents ($r=0.64$).

**Conclusions:** It can be concluded that consumption of 250 g of ginger cakes fortified with high dose of rutin may provide to the organism enough amount of rutin to observe the health effect. Moreover, enrichment of ginger cakes with rutin influenced the antioxidant potential. This study also indicates that rutin beneficially increases the amount of favorable Maillard reaction products (melanoidins) whereas decreases unfavorable ones (furosine, CML).
PREPARATION OF LIPOSOMES USING GLYCATED LIPIDS FOR THE RAPEUTIC PURPOSES

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Main Objectives: Liposomes are closed vesicles comprising phospholipids, which are under extensive investigation for improving the delivery of therapeutic agents. Liposomes are generally prepared with phosphatidylcholine (PC), whereas when both PC and phosphatidylethanolamine (PE) are used, such surface-modified liposomes can show unique behavior in vivo. On the other hand, with regard to PE molecule, we have found that PE can react with sugar, yielding Amadori-glycated PE (Fig. 1A)¹. Hence, it is hypothesized that using both PC and glycated PE, liposomes having new properties (e.g., enhancement of physical stability and intestinal absorption) can be formed. In this study, this possibility was evaluated. A therapeutic agent, curcumin (CUR), was entrapped in liposomes.

Methods: (1) By using different amounts of PC, (native) PE and CUR, liposomes (single unilamellar vesicles (SUVs)) were prepared. (2) Glycated PE was prepared by reacting PE with sugar (glucose or lactose). (3) We tried to prepare SUVs by using suitable amounts of PC, glycated PE (or control non-glycated PE) and CUR. Once SUVs were obtained, their particle size and zeta potential were evaluated. Investigation about incorporation of liposomal CUR into cultured cells was performed.

Main Results: (1) When the ratio of PE against PC was less than about 35 wt.%, multilamellar vesicles (MLVs) could be prepared. SUVs were subsequently made from MLVs by using Mini-Extruder. The resultant SUVs contained sufficient amount of CUR (10 wt.%). (2) We prepared glycated PE by reacting PE with sugar (glucose or lactose). HPLC-MS/MS analysis revealed that more than half of PE was glycated in the present experimental conditions. (3) It was found that SUVs (particle size, about 200 nm; CUR entrapment, 10 wt.%) could be prepared, regardless of glycated or non-glycated PE used. Interestingly, zeta potential of SUVs prepared from PC and glycated PE showed significantly lower value (Fig. 1B). This might be related to the fact that when the SUVs were subjected to cell culture assay, a high cellular incorporation of CUR was found.

Conclusions: By using PC and glycated PE, we could prepare SUVs. These SUVs showed different properties (zeta potential and cellular incorporation of CUR) compared to control SUVs prepared from PC and non-glycated PE. We are now performing animal experiments to evaluate SUVs-CUR incorporation into animal body.

DETECTION OF METHYLGLYOXAL DERIVED HYDROIMIDAZOLONE OF CREATINE (MG-HCR) IN MEAT

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Main Objectives: Creatine is linked to energy metabolism especially in muscle via the creatine/phosphocreatine system. For that reason creatine can be found in mammalian muscle (meat), poultry as well as in fish with amounts of 4 to 5 g/kg (30 to 40 mmol/kg). Recently we could identify the methylglyoxal derived hydroimidazolone of creatine (MG-HCr) as a specific AGE formed from creatine (Cr) and methylglyoxal (MG) in model incubations. Furthermore, we detected MG-HCr in urine of vegetarians indicating its formation in vivo. MG is physiologically formed mainly from the degradation of triosephosphates as a by-product of the glycolysis and contributes to the so called “carbonyl stress”. Trapping of methylglyoxal by creatine might be a strategy to reduce carbonyl stress and its consequences in vivo. Since meat is the major dietary source of creatine the question arises if MG-HCr is formed in meat and if MG-HCr is ingested by a diet containing meat.

Strategy and Methods: In samples of beef, pork, chicken and salmon MG-HCr was determined. Therefore the samples were analyzed raw and fried (well-done). Additionally, meat products like burger and sausages like salami, lyoner, dry-cured and cooked ham were analyzed. All samples were purchased from local shops and fast food restaurants. After homogenization, samples were defatted with dichlormethane, extracted with 0.05 N HCl and deproteinized with acetonitrile. Quantitation of MG-HCr in meat samples was carried out via standard addition and hydrophilic interaction chromatography coupled to tandem mass spectrometry, as published recently for the quantitation of MG-HCr in urine.

Main Results: The analytical method for MG-HCr in urine was successfully adopted for meat and meat products. The reaction product of MG and creatine MG-HCr could be detected in low amounts in raw meat samples of mammalians (beef, pork), poultry (chicken) and fish (salmon) in concentrations of 0.2–0.7 µmol/kg. Defined frying of these meat samples resulted in a 100-fold increase. The amount of MG-HCr ranged from 16 to 100 µmol/kg, accounting for less than 1% of the present creatine. The quantitation of MG-HCr in different meat products showed a broad range between 0.5 µmol/kg for salami and 60 µmol/kg for cooked ham. This might be referred to varying manufacturing processes. Milk is also known to contain creatine. The analysis of UHT milk revealed a MG-HCr concentration 0.1 µmol/L.

Conclusions: Meat is known to be the major source of creatine in food. The occurrence of MG-HCr already in raw meat, points to its formation in vivo. The high creatine content in meat enables the distinct increase of MG-HCr by cooking processes. Therefore meat should be the major dietary source of MG-HCr. Other food from animal origin might also contribute to the overall MG-HCr intake. MG-HCr could be detected in UHT milk in minor amounts. In further studies we want to investigate whether creatine can scavenge dicarbonyl compounds during food processing and protect amino acids and proteins from glycation. Another focus will be on the question if dietary MG-HCr contributes to its load in humans.
ANTI-HYPERTENSIVE EFFECT OF SOY SAUCE IN SALT-SENSITIVE HYPERSENSITIVE RATS

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Main Objectives: Excessive intake of salt promotes reactive oxygen species generation. High sodium food ingestion has been assumed to induce hypertension, glomerular sclerosis, kidney dysfunction and stomach cancer, possibly via induction of oxidative stress. Hence typical Japanese high-salt seasonings such as Soy source and Miso, are often main targets of salt reduction campaign. Nevertheless, Soy sauce contains various bioactive components such as peptides, minerals, polyphenol and Maillard reaction products. Soy Sauce is considered to show anti-oxidative, anti-carcinogenic, cholesterol-lowering and anti-hypertensive effects. In this context, we hypothesize that Soy Sauce ingestion would have different biological influence from singly salt ingestion. In this study, we intended to elucidate the anti-hypertensive effect of Soy Sauce using salt-sensitive hypersensitive rats.

Strategy and Methods: Salt-sensitive hypersensitive rats were divided two groups (Soy Sauce or salt) based on their body weight and blood pressure (Tail-cuff). After acclimatized, they were given free access to water and commercial rodent powder food (MF, ORIENTAL YEAST CO., LTD, Tokyo, Japan) containing the same salinity of Soy Sauce or a salt solution throughout the experimental period. Urine and feces samples were collected using metabolic cage at the last experimental week. We measured diets and water intake, blood pressure, oxidative stress (PCOOH, TBARS), genetic analysis (Mn-sod, Cu-sod, Zn-sod, CAT and Gpx1-3) and enzyme activity (Gpx, SOD) in kidney, and biochemical parameters in blood and excretion. Moreover, kidney, heart and inferior vena cava stained by PAS and HE staining for morphological observation.

Main Results: The Soy Sauce ingestion group significantly suppressed the increase of blood pressure, whereas salt ingestion raised blood pressure with experimental period. Food and water intakes and body weight did not show significantly differences between groups throughout experimental period. Calculated sodium ingestion value was also not different between groups. Minerals in blood, mineral excretion, and morphological observation did not show significantly different. A noteworthy facts were that Soy Sauce ingestion group show significantly high expression of anti-oxydative enzymes mRNA and suppressed PCOOH concentration.

Conclusions: In conclusion, Soy Sauce ingestion significantly suppressed the increase of blood pressure with the relation of anti-oxidative effects of Soy Sauce.
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由来と特徴
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糖尿病性脳症
SDT fatty ラットでは、5週齢からの血糖上昇と共に尿糖、脂質も早期から認められます。病理組織学的検査では、尿細管上皮の腫脹形成や尿細管の拡張が8週齢より認められ、慢性性的の先細胞障害を呈します。メサンギウムの増生も認められます。40週齢以降より結節様病変および線維性硬化が出現し、尿細管間質の炎症および線維化が認められます。

糖尿病性神経障害
SDT fatty ラットの潮謝障害に伴い、糖尿病末梢神経障害が認められます。尿細管神経伝導速度の低下は24週齢時には既に認められており、その低下は加齢と共に明るかとなります。また、尾部血流量の低下および神経組織内ソルビトールの個々でセリトール含有の増加が確認されています。

糖尿病性骨粗鬆症
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