Meeting of the International HNE-Club and the University of Graz

Reactive Oxygen Species and Lipid Peroxidation in Human Health and Disease

Thursday, 14 – Friday, 15 September 2017
Graz – Austria
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Dear colleagues,

Welcome to the meeting of the International HNE-Club dedicated to Hermann Esterbauer, who passed away 20 years ago, but whose ideas are still inspiring for all who study pathophysiology of oxidative stress!

Local Organizing Committee
Valery Bochkov (Chairman), Rudolf Joerg Schaur, Brigitte Winklhofer-Roob, Willibald Wonisch, Gholam Ali Khoschsorur, Rudolf Zechner

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- High sensitivity, specificity and accuracy for live cell studies
- Compatible with major components of tissue culture media (phenol red, FBS and BSA)
- Complete set of reagents, including ROS inducers and scavengers
- Suitable for flow cytometry, fluorescence microscopy, and microplate reader applications

Profiling of ROS formation by flow cytometry in HeLa cells. Data represents % positive following treatment with Pyocyanin (ROS/SD inducer), TBHP (ROS inducer), and AMA (superoxide inducer).
Enzo Life Sciences is a pioneer in labeling and detection technologies with expertise in making novel fluorescent probes to visualize cellular responses. Enzo’s ROS-ID® Total ROS/Superoxide detection kit is comprised of two fluorescent probes that enable the simultaneous discrimination of total ROS and specifically superoxide in live cells.

- Distinguishes between different reactive species, such as hydrogen peroxide, peroxynitrite and hydroxyl radicals
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- Compatible with major components of tissue culture media (phenol red, FBS and BSA)
- Complete set of reagents, including ROS inducers and scavengers
- Suitable for flow cytometry, fluorescence microscopy, and microplate reader applications

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Programme

Wednesday, September 13

16:00  **Graz City Tour**
Meeting point: Hotel Mercure, Lendplatz 36-37, 8020 Graz
End point: University of Graz, Schubertstrasse 1, 8010 Graz

18:00  Registration & Get together
University of Graz, Schubertstrasse 1, 8010 Graz

Thursday, September 14
Schubertstrasse 1, 8010 Graz

9:00  Hermann Esterbauer Memorial Session

Chairs

**Neven Žarković** (Zagreb), **Valery Bochkov** (Graz)

Introduction

**Valery Bochkov** (Graz)

Welcome

**Christa Neuper** (Rector, University of Graz)

**Sepp Kohlwein**
(Graz)

**Hermann Esterbauer**
and the University of Graz

**Giuseppe Poli**
(Turin)

**Hermann Esterbauer – a pioneer of lipid peroxidation research**

**Koji Uchida**
(Tokyo)

**The Hermann Esterbauer Decennial Lecture:**
What we know about 4-hydroxynonenal

10:30  **Coffee break**
CLARIOstar®
The most sensitive monochromator-based microplate reader
11:00 Scientific session 1

Intracellular signaling mechanisms activated by lipid peroxidation products

Chairs
Peter Eckl (Salzburg), Randy Jirtle (Raleigh)

Henry Jay Forman
(Los Angeles)
4-Hydroxynonenal-mediated cell signaling

Nikolaus Bresgen
(Salzburg)
Oxidative stress and cell death: The role of iron

Huveyda Basaga
(Istanbul)
Cross-talk between Bcl-2 and IKK in 4-hydroxynonenal-induced apoptosis

Short break

Randy Jirtle
(Raleigh)
Radiation-induced oxidative stress alters disease susceptibility by modifying the epigenome

Fulvio Ursini
(Padova)
GPx4 is the controller of a specific form of programmed cell death executed by lipid peroxidation products

Shlomo Sasson
(Jerusalem)
4-Hydroxyalkenal-activated PPARδ complexes mediate glucohormetic and lipohormetic interactions in diabetes
Tools to Better Understand Oxidized Lipids

- LPO, TBARS, and 8-isoprostane assays available in flexible kit formats
- 8-Isoprostane affinity columns and immunosorbents
- Click chemistry probes, inhibitors, and standards with stable isotope labeled pairs
- Assays to detect ROS and evaluate antioxidant activity
- Contract bioanalysis services are available

Lipid Hydroperoxide (LPO) Assay Kit

Measure LPOs in tissues, cultured cells, plant materials, foods, and biological fluids
13:45  Group photo and lunch break

15:00  Scientific session 2
Analytical approaches for structural and functional analysis of lipid peroxidation products

Chairs
Giuseppe Poli (Turin), Giulia Coliva (Leipzig)

Corinne Spickett
(Birmingham)
Analysis of phospholipid peroxidation and protein lipoxidation products by LC-MS

Maria Fedorova
(Leipzig)
Analytical strategies to uncover the diversity of lipid peroxidation products and their biological effects

Willibald Wonisch
(Graz)
High-throughput screening of oxidative stress biomarkers: Significance, precision and cost-effectiveness

16:15  Coffee break

16:45  Poster session

19:30  Reception by the Lord Mayor of the City of Graz and Conference Dinner
Hotel Wiesler, Salon Frühling, Grieskai 4-8, 8010 Graz
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Friday, September 15

9:00   Scientific session 3

Lipid oxidation products in disease pathogenesis (1)

Chairs
Giovanni Mann (London)
Anne Negre-Salvayre (Toulouse)

Anne Negre-Salvayre (Toulouse)
Pro-atherogenic effects of 4-hydroxynonenal

Gabriella Marisa Leonarduzzi (Turin)
Oxidized lipids in age-related diseases

Norbert Leitinger (Charlottesville)
Oxidized phospholipids and phenotypic polarization of macrophages

Giovanni E. Mann (London)
Enhanced sensitivity to 4-hydroxynonenal and impaired redox signaling in human endothelial cells in gestational diabetes

10:40   Coffee break
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  - 4-hydroxy Nonenal Glutathione-d3 (trifluoroacetate salt) Cat No: 9000876
  - 4-hydroxy Nonenal Alkyne Cat No: 32100
  - 4-hydroxy Nonenal Mercapturic Acid-d3 Cat No: 9000348
  - 4-hydroxy Nonenal-d3 Cat No: 332101
  - 4-hydroxy Nonenal Mercapturic Acid Cat No: 32110
  - 4-hydroperoxy 2-Nonenal3 Cat No: 10004413

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- TBARS (TCA Method) Assay Kit
- TBA Malondialdehyde Standard
- 8-Isoprostane EIA Kit
- 8-Isoprostane Express EIA Kit
- STAT-8-Isoprostane EIA Kit
- Catalase Assay Kit (without Hydrogen Peroxide)
- Antioxidant Assay Kit

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Cat No: 700870
Cat No: 10009202
Cat No: 516351
Cat No: 516360
Cat No: 500431
Cat No: 700910
Cat No: 709001

Offer validity: 13th October 2017
11:10  Scientific session 4
Lipid oxidation products in disease pathogenesis (2)

Chairs
Etsuo Niki (Kyoto), Françoise Guéraud (Toulouse)

Françoise Guéraud (Toulouse)
Dietary 4-hydroxynonenal and other lipid oxidation products in the development of colorectal carcinogenesis

Ana Čipak Gašparović (Zagreb)
Role of 4-hydroxynonenal in communication between cancer stem cells and microenvironment

Huiyong Yin (Shanghai)
The role of lipid peroxidation during the progression of human hepatocellular carcinoma

Tilman Grune (Nuthetal)
Redox regulation in aging: Role of protein aggregates

12:50  Lunch break
14:20 Scientific session 5

Oxidative stress, antioxidants and pharmacological interventions

Chairs
Tilman Grune (Nuthetal), Franz Tatzber (Graz)

Etsuo Niki
(Kyoto)
Oxidative stress and antioxidants: Distress or eustress?

Elżbieta Skrzydlewska
(Bialystok)
Antioxidants and 4-hydroxynonenal in regulation of redox homeostasis

Giancarlo Aldini
(Milan)
Novel molecular approaches for improving enzymatic and nonenzymatic detoxification of 4-hydroxynonenal: Toward the discovery of a novel class of bioactive compounds

Short break
Luigi Iuliano  
(Rome)  
Translational implications of cholesterol autoxidation

Werner Siems  
(Bad Harzburg)  
Lipid peroxidation and pharmaceutical drugs

Giuseppe Poli  
(FRBM Associate Editor)  
Presentation of a special issue of Free Radical Biology & Medicine entitled „4-Hydroxynonenal and Related Lipid Peroxidation Products“

16:50  Coffee break
17:15  Public panel discussion

The Janus face of oxidative stress

Moderation:

Sonja Saurugger (Kleine Zeitung)
Anthony Newman (Elsevier)

Etsuo Niki
(Kyoto)
Oxidative stress: what does it mean?

Randy Jirtle
(Raleigh)
Oxidative stress induced by ionizing radiation

Neven Žarković
(Zagreb)
Pro- and anti-proliferative effects of lipid peroxidation products on cancer cells

18:15  Closing remarks

Neven Žarković
(Zagreb)

Saturday, September 16

All participants are invited to attend the 6th International Symposium of the Human Nutrition & Metabolism Research and Training Center Graz. For free participation, the HNE Club participants need to show their badge of the HNE Club meeting. Students and employees of the universities of Graz have to show the student or employee ID card.

Professional Educational Workshop

How to publish in a scientific journal

University of Graz, Schubertstrasse 1, 8010 Graz

07:45  Registration

08:15  Welcome: Brigitte Winklhofer-Roob (Graz)
Chairs:
Josiane Cillard (President of SFRR-Europe, Rennes, France) and Fritz Spener (Past-Executive Editor of Biochimica Biophysica Acta – Molecular and Cell Biology of Lipids, Graz, Austria)

THE PUBLISHER’S VIEW

08:20 Anthony Newman
(Publisher Elsevier, Amsterdam, The Netherlands)
How to write a great research paper, and get it accepted by a good journal

THE EDITOR’S VIEW

09:20 Giovanni E. Mann (Review Editor, Free Radical Biology and Medicine, London, UK), Henry J. Forman (Editor-in-Chief, Archives of Biochemistry and Biophysics, Merced, USA), Michael J. Davies (Editor-in-Chief, Free Radical Research, Copenhagen, Denmark), Tilman Grune (Editor-in-Chief, Redox Biology, Potsdam, Germany), Torsten Bohn (Editor-in-Chief, International Journal of Vitamin and Nutrition Research, Luxembourg, Luxembourg), Jan Frank (Editor-in-Chief, NFS Journal and Co-Editor Europe, Nutrition, Hohenheim, Germany)

09:50 Discussion
10:00 Coffee break
10:20 Scientific sessions
19:10 End of symposium
Social Programme

Wednesday, September 13
16:00  Graz City Tour  
Meeting point: Hotel Mercure, Lendplatz 36-37, 8020 Graz  
18:00  Arrival at the University of Graz, Schubertstrasse 1, 8010 Graz  
18:00  Get together  
University of Graz, Schubertstrasse 1, 8010 Graz

Thursday, September 14
19:30  Reception by the Lord Mayor of the City of Graz and Conference Dinner  
Hotel Wiesler, Salon Frühling, Grieskai 4-8, 8010 Graz

Saturday, September 16
Excursion to the Fortress of Deutschlandsberg and the Western Styrian Wine Road
10:00  Departure from the University Main Building  
12:00  Lunch at the Castle Deutschlandsberg  
14:00  Archeological Museum Deutschlandsberg  
15:00  Ride along the Schilcher Weinstrasse  
16:00  „Brettjause“ at Restaurant „Jagawirt“  
19:00  Arrival to Graz

Contact and Information
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Institute of Pharmaceutical Sciences  
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- Reduced open access fee for ‘Redox Biology’
- Support for young scientists to attend international meetings
- Meeting the experts on free radicals, oxidative stress, and redox signaling

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Lisbon, Portugal
Presentation abstracts
4-Hydroxy-2-nonenal (HNE) is one of the major products generated during the peroxidation of n-6 polyunsaturated fatty acids, such as linoleic acid and arachidonic acid, and is believed to be largely responsible for the cytopathological effects observed during oxidative stress. In the past decade, considerable progress has been made toward understanding the chemistry and biology of HNE. We now know how abundantly HNE can be generated during lipid peroxidation in vitro and in vivo and how reactive HNE is, especially toward proteins. We also know that the protein-bound HNE could serve as a ligand for a scavenger receptor and function as an immunological trigger for cell signalings and for the production of anti-DNA autoantibodies in autoimmune diseases. These findings strongly suggest that HNE can be causally involved in many of the pathophysiological effects associated with oxidative stress in cells and tissues. In this special meeting, I will give a brief overview on the current status of HNE, focusing mainly on its protein adducts.
HNE-mediated cell signaling

Henry Jay Forman

School of Gerontology, University of Southern California, Davis, 3715 McClintock Avenue, Los Angeles, CA 90089-0191
peroxideman@gmail.com

4-Hydroxy-2-nonenal (HNE) is one of the major α,β-unsaturated aldehydes produced in lipid peroxidation. HNE is a potent messenger in signaling pathways. Aging is associated with increased lipid peroxidation and HNE production, although a cause and effect relation remains unclear. Aging is also associated with significant changes in many signaling pathways with some enhanced and others decreased. One of the issues that is often avoided in studies of HNE is the wide range of exogenous HNE concentrations that have been used. These exposures range from what is found in plasma in unstressed mammals to well beyond real life pathology. Accumulating evidence suggests that HNE modification of signaling proteins occurs at cysteine, histidine, and lysine residues located at particular environments. Finally, HNE-signaling also involves the proteolytic degradation of proteins by proteasomes, lysosomes and autophagy.
Oxidative stress and cell death: the role of iron

Nikolaus Bresgen and Peter Eckl

Department of Cell Biology and Physiology, University of Salzburg, Austria

The partnership between iron and oxygen is indispensable to cell survival, but turns into a life-threatening challenge if it becomes imbalanced. To safeguard proper iron handling, cellular iron management is based on the tight coupling of compartment-specific “labile” (i.e. redox-active) iron pools. In this machinery, the endo-/lysosomal pool of redox-active, labile iron plays a particularly critical role since it couples external and internal iron sources to metabolic iron needs, which involves hetero- and autophagic mechanisms such as the recently described ferritinophagy. Under pro-oxidant conditions, the continued requirement for iron is at the risk of an enhanced, Fenton-reaction based production of hydroxyl radicals which will initiate lipid peroxidation and thus promote the generation of reactive metabolites such as 4-hydroxynonenal (HNE). With respect to lysosomal integrity, HNE-protein adduct formation and enhanced aldehyde-driven protein crosslinking are of considerable relevance since both will hamper lysosome function and increase the risk of lysosomal membrane permeability (LMP). In turn, LMP can directly trigger the onset of an apoptotic or necrotic (lytic) mode of cell death. Hence, the lysosomal labile iron pool represents a critical determinant of lysosomal as well as cellular integrity, especially under conditions of oxidative stress. Based on these findings, a model of lysosomal metastability will be presented which links iron-derived lysosomal stress to cellular live/death decisions.

References:
Cross-talk between Bcl-2 and IKK in 4-hydroxynonenal-induced apoptosis

Huveyda Basaga

Molecular Biology Genetics and Bioengineering program, Sabanci University, 34956 Orhanlı-Tuzla, Istanbul

Apoptosis of macrophage foam cells loaded with modified/oxidized lipids is implicated in destabilization of advanced atherosclerotic plaques in humans. Concentration of HNE, main aldehydic product of plasma LDL peroxidation, elevates in atherosclerotic lesions as well as in cultured cells under oxidative stress. Although this reactive aldehyde has been shown to promote apoptosis with the involvement of p38 MAPK and JNK in various mammalian cell lines, roles of B-cell lymphoma 2 (Bcl-2) family proteins remain to be deciphered. We demonstrated that HNE-induced apoptosis was accompanied by concurrent downregulations of antiapoptotic Bcl-x(L) and Mcl-1 as well as upregulation of proapoptotic Bak. Furthermore, phosphorylation of Bcl-2 at Thr56, Ser70, and probably more phosphorylation sites located on N-terminal loop domain associated with HNE-induced apoptosis in both U937 and HeLa cells while ectopic expression of a phospho-defective Bcl-2 mutant significantly attenuated apoptosis. In parallel to this, HNE treatment caused release of proapoptotic Bax from Bcl-2. Pharmacological inhibition of Inhibitory kappa B kinase (IKK) inhibited HNE-induced Bcl-2 phosphorylation. Similarly, silencing IKKα and -β both ended up with abrogation of Bcl-2 phosphorylation along with attenuation of apoptosis. Moreover, both IKKα and -β coimmunoprecipitated with Bcl-2 and in vitro kinase assay proved the ability of IKK to phosphorylate Bcl-2. In view of these findings and considering HNE inhibits DNA-binding activity of nuclear factor-κB (NF-κB) through prevention of IκB phosphorylation/ubiquitination/proteolysis, IKK appears to directly interfere with Bcl-2 activity through phosphorylation in HNE-mediated apoptosis independent of NF-κB signaling.
Epigenetic Responses to Low Dose Ionizing Radiation

Randy L. Jirtle, Ph.D.

Department of Biological Sciences, NC State University, Raleigh, NC 27695 USA; Department of Oncology, University of Wisconsin, Madison, WI 53706 USA

Two epigenetically regulated subsets of genes that potentially link environmental exposures early in development to adult diseases are imprinted genes and those with metastable epialleles. Genes with metastable epialleles have highly variable expressions because of stochastic allelic modifications in the epigenome. Genomic imprinting is an unusual epigenetic form of gene regulation that results in monoallelic expression in a parent-of-origin dependent manner. The viable yellow agouti (Avy) mouse harbors a metastable Agouti gene because of an upstream insertion of a transposable element.

We previously used this animal model to demonstrate that nutritional and chemical toxicant exposures during early development induce persistent epigenetic changes at the Avy locus that result in alterations in coat color and adult disease susceptibility. We also showed that low doses of ionizing radiation (<7.6 cGy) induce a sex and dose dependent positive adaptive phenotype in Avy offspring (i.e., hormesis) that results from significant alterations in DNA methylation at the Avy locus; an effect that is block by maternal antioxidant exposure. Furthermore, low doses of ionizing radiation alter the epigenetic regulation of the imprinted genes (e.g. Peg3). The importance of these studies, with regards to human health and disease, will be discussed.
GPx4 is the controller of a specific form of programmed cell death executed by lipid peroxidation products

Fulvio Ursini

Department of Molecular Medicine, University of Padova, Italy

The recent identification of a routine of non-apoptotic controlled cell death recapitulates in a physiological perspective the long history of oxygen toxicity and lipid peroxidation. Due to the critical role of iron, this form of controlled non-apoptotic cell death had been named ferroptosis\(^1\). Ferroptosis executes cell death in major neurodegenerative diseases and ischemia-reoxygenation and accounts for of cytotoxicity of drug candidates for cancer treatment\(^2\). Moreover, evidence on embryo development indicates that ferroptosis is also involved in tissue homeostasis\(^3\). What we know is that oxygen, phospholipids containing \(\omega\)-6 fatty acids, iron and a lipoxygenase active on membrane lipids are required. We also know that ferroptosis can only be executed by inactivation the selenoperoxidase GPx\(^4\). The reduction to hydroperoxy derivatives of lipid hydroperoxides inserted in membranes is, indeed, the critical antiperoxidant reaction\(^5\). Seemingly, in the emerging scenario, oxygen metabolically activated in mitochondria, slowly but continuously generates species competent for free radical oxidation of a polyunsaturated fatty acid, evolving into a lipid hydroperoxide. This activates a lipoxygenase active on membrane lipids to produce more hydroperoxides. By decomposition of lipid hydroperoxides, iron propagates chain reactions and generates the electrophiles (aldehydes?) alleged executing cell death. All these events can only, take place when the reaction of GPx4 becomes limiting.

The mechanism of GPx4 reaction has been analyzed up to quantum-mechanical level and calculated structures have been corroborated by MS\(^6\). The interaction of GPx4 with membrane phospholipid has been elucidated by Surface Plasmon Resonance (SPR), supported by molecular dynamics (MD) analysis. We know now how GPx4 works on membranes. A strong electrostatic interaction takes place between specific aminoacid residues in the cationic area on the surface of GPx4 and polar head of phospholipids. This binding drives the orientation of the hydroperoxidic group flipping out of the membrane to precisely interact with the redox center of the enzyme\(^7\). Redox catalysis is operated by proton tunneling leading to the formation of a charge-separated species\(^6\). Interaction of the oxidized selenium with two GSH molecules reacting in sequence lessens the interaction with polar head of phospholipids and permits the “surfing” of the enzyme on the membrane surface to catch and reduce lipid hydroperoxides\(^7\).

In conclusion, metabolism of oxygen in mitochondria provides metabolic energy but can also kill the cells when not sufficiently protected by GPx4 and GSH. About the mechanisms of induction of ferroptosis under physiological conditions we know that GSH concentration can be controlled by the efficiency of the import of cystine for the synthesis of GSH. What is still largely unknown, instead, is how expression/activity of GPx4 could be controlled under physiological conditions.
References:
7) Cozza, G.; Rossetto M.; Bosello-Travail V.; Maiorino, M.; Roveri, A.; Toppo, S.; Zaccarin, M.; Zennaro, L.; and Ursini, F. Glutathione peroxidase 4-catalyzed reduction of lipid hydroperoxides in membranes: The polar head of membrane phospholipids binds the enzyme and addresses the fatty acid hydroperoxide group toward the redox center Antiox Red. Sig. 2017 in press.
4-Hydroxyalkenal-activated PPARδ complexes mediate glucohormetic and lipohormetic interactions in diabetes

Shlomo Sasson

Institute for Drug Research, Faculty of Medicine, The Hebrew University, Jerusalem, Israel

Ligand-bound peroxisome proliferator-activated receptor-δ (PPARδ) induces the expression of genes encoding enzymes that metabolize fatty acids and carbohydrate in various tissues. Attempts to identify cellular endogenous activators of PPARδ produced large lists of different fatty acids and their metabolic derivatives; however, there is no consensus on specific and selective binding interactions of natural ligands with PPARδ. Most models on binding interactions within the ligand binding domain (LBD) of PPARδ have been derived from analyses of PPARδ-LBD crystals formed with synthetic low molecular weight ligands. Nonetheless, crystals of the whole receptor with natural ligands or of its heterodimer with its cognate retinoid X receptor (RXR) are not yet available for analysis. We have found that 4-hydroxyalkenals, non-enzymatic peroxidation products of polyunsaturated fatty acids (PUFA), namely, 4-hydroxy-2E,6Z-dodecadienal (4-HDDE) and 4-hydroxy-2E-nonenal (4-HNE), activate PPARδ in vascular endothelial cells and insulin-secreting beta cells, respectively. In both cases the activated PPARδ complexes induced adaptive responses that allowed the cells to adjust to ambient stressful metabolic conditions induced by high levels of glucose and/or fatty acids. The interactions of 4-hydroxyalkenals with PPARδ and the resulting hormentic interactions in cells exposed to nutrient overload conditions will be presented and discussed.

References:
Analysis of phospholipid peroxidation and protein lipoxidation products by LC-MS

Corinne M. Spickett, Bebiana C. Sousa, Andrew R. Pitt

School of Life and Health Sciences, Aston University, Birmingham, B4 7ET, UK Email: c.m.spickett@aston.ac.uk

Oxidized phospholipids (oxPLs) are produced by the action of free radicals and reactive oxidizing compounds on unsaturated phospholipids, forming a wide range of oxidized products including full-length species or chain-shortened species together with non-esterified breakdown products. These can be further divided into non-reactive versus electrophilic reactive molecules containing carbonyl groups. OxPLs have various biological activities that are thought to contribute to inflammatory-based diseases, although anti-inflammatory effects have also been reported. It is therefore important to be able to characterize the profile of oxPLs that occur in biological situations, and advanced liquid chromatography tandem mass spectrometry (LC-MSMS) techniques have become the method of choice for this purpose. High resolution mass spectrometry allows identification based on accurate mass of the oxidized products, but in complex samples there are advantages to using targeted approaches involving the detection of diagnostic fragment ions. Using such techniques, we have identified many different oxPLs in samples such as human plasma, parasitized red blood cells and LDL from control and diabetic patients. Moreover, reactive oxidized phospholipids are able to attack nucleophilic sites in proteins to form lipid-protein adducts in a process called lipoxidation, which is thought to contribute to the bioactivity of such oxPLs. While lipoxidation by small aldehydes such as acrolein and 4-hydroxynonenal is well established, using semi-targeted LC-MSMS approaches we have also shown the formation of adducts of reactive phospholipids with proteins, including ApoB-100. These methods are currently being developed further to help understand the occurrence of lipoxidation in biological samples, and improve understanding of the mechanisms by which oxPLs may exert effects.
Analytical strategies to uncover the diversity of lipid peroxidation products and their biological effects

Zhixu Ni\textsuperscript{1,2}, Georgia Angelidou\textsuperscript{1,2}, Giulia Coliva\textsuperscript{1,2}, Mike Lange\textsuperscript{1,2}, Maria Fedorova\textsuperscript{1,2}

\textsuperscript{1}Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig, \textsuperscript{2}Center for Biotechnology and Biomedicine, Universität Leipzig, Germany

Many human diseases, including obesity, diabetes and atherosclerosis, are accompanied by chronic inflammation and closely connected to oxidative stress (OS). OS can oxidize virtually all biomolecules of which lipids represent one of the most prominent targets. Lipid peroxidation products (LPPs) are chemically diverse group of biomolecules with a variety of functional activities. Many LPPs were shown to play an important role in the onset and development of OS-related diseases and can serve as diagnostic and prognostic biomarkers. To address the variety of LPPs in biological samples we developed LC-MS based oxLipidomics analytical platform which allows us to target up to six different LPP classes (oxidized, nitrated fatty acids, oxysterols, electrophilic aldehydes, head group modified and oxidized PLs). To facilitate high-throughput workflows several software tools were developed for lipid (LipidHunter) and LPP (LPPTiger) identification. Finally, experimental and publicly available information on oxidized lipids is integrated via knowledge based database LPPdb. oxLipidomics platform was cross-validated using cellular models of oxidative stress (e.g. primary cardiomyocytes) and clinical samples (blood plasma and adipose tissue) from patients with obesity and type II diabetes.
High-throughput screening of oxidative stress biomarkers – significance, precision and cost-effectiveness

Wonisch W.1, Cvirn G.1, Resch U.2, Tatzber F.3

1Institute of Physiological Chemistry, Center for Physiological Medicine, Medical University of Graz, Stiftungtalstrasse 6 M1/D/3, 8010 Graz, Austria
2Department of Vascular Biology and Thrombosis Research, Medical University of Vienna, Schwarzspanierstrasse 17/1, 1090 Vienna, Austria
3Center of Molecular Medicine, Institute of Pathophysiology and Immunology, Medical University of Graz, Heinrichstrasse 31a, 8010 Graz, Austria

Since Reactive Oxygen (ROS) and Nitrogen Species (NOS) provoke a wealth of molecular, biochemical and immunological modifications, it is important to develop biomarkers to estimate the oxidative stress (OS) burden. In recent decades, several oxidative stress biomarkers have been developed to determine both antioxidative defense mechanisms as well as indicators for the pro-oxidative radical attack. Due to the interlocked network of antioxidants, free radicals, immunological reactions and lipidperoxidation products, a battery of biomarkers is needed to provide an overall impression with respect to the pro- and anti-oxidative balance in humans. This is of great importance to prevent carelessness administration of highly dosed antioxidant supplements as well as to contribute to diagnostics in health and disease.

These aspects were the basis and challenge in designing distinct biomarkers at predetermined junctions, i.e. the measurement of antioxidants in the first line - with special attention to the kinetics of diverse antioxidants including TAC® (Total Antioxidant Capacity)1, PPm®(Polyphenols microtitre)] and the endogenous antioxidant system, e.g. peroxidase activity (EPA®)2. Total peroxides [TOC®] indicate short-term as well as long-term oxidative stress on lipids and proteins and are diagnostically as conclusive as isoprostanes3. Last but not least, antibodies against oxidized LDL are essential in assessing the overall health of individuals. Accordingly, different ELISA methods are available for IgM [MDA-LDL IgM®] and IgG antibodies [oLab®] for both malondialdehyde (MDA)-modified LDL as well as copper-oxidized LDL4. As a remarkable feature we equipped the IgM MDA-LDL antibody ELISA with standards of a human monoclonal antibody emerging from the fusion of a female B-cell with a mouse myeloma cell line.

These biomarkers were designed as high-throughput methods to determine a multitude of samples within a short space of time with high sensitivity, thereby minimizing the cost of a single determination. We have developed an assortment of assays to determine the interplay of pro- and antioxidative variables at distinct key events in the course of lipid peroxidation. Such tools are suitable for both scientific purposes and routine diagnosis to supply clinicians as well as scientists with additional information on the OS status.

In conclusion, the main advantages of these biomarkers can be listed as follows: high capacity, low cost, suitability for every body fluid, high precision and sensitivity, specificity and short time of analysis. These methods have proved to be reliable and sensitive5 in such varied settings as epidemiologic studies on OS as a function of increasing body-mass index6 or the coincidence of deteriorating performance and increasing OS in top athletes7.
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Proatherogenic effects of 4-hydroxynonenal

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Among the various mechanisms involved in atherogenesis, the oxidative theory of atherosclerosis relies on the oxidation of low density lipoprotein (LDL) in the vascular wall and their implication in the formation of early atherosclerotic lesions. Reactive oxygen species (ROS) and oxidants generated by activated endothelium, initiate LDL oxidation in the intima, which generates a huge variety of lipid peroxidation products (LPPs), exhibiting atherogenic, pro-inflammatory and pro-apoptotic properties. Reactive carbonyl compounds (RCCs), are a family of highly reactive agents generated during polyunsaturated fatty acid (PUFA) peroxidation. RCCs covalently bind to nucleophilic group of proteins, peptides, phospholipids and nucleic acids, thereby generating a “carbonyl stress”. Among RCCs, 4-hydroxy-2-nonenal (HNE) exerts its atherogenic effects through several mechanisms, by targeting lipoproteins or cellular components. HNE generated during LDL oxidation is able to form HNE-apoB adducts, which are recognized by scavenger-receptors of macrophagic cells, thereby leading to foam cell formation. HNE can be released during the degradation of oxLDL, or generated through oxidative stress and PUFA peroxydation in cell membranes. The biological effects of HNE on vascular cells depend on its local concentration and on the expression of detoxifying systems, such as glutathione S-transferase, aldose reductase, and aldehyde dehydrogenase (ALDH), which rapidly neutralize and remove HNE from cells. Physiological concentrations (0.1-1 µmol/L) of HNE induce hormetic and adaptive responses, and transcription factors (Nrf2) that increase cell resistance to oxidative attack and other stresses, while moderate HNE concentrations, (1 to 10 µmol/L), trigger the accumulation of HNE-adducts and a variety of biological responses, such as inflammation and cell proliferation. Higher HNE concentrations, (above 10-20 µmol/L), induce cell dysfunction and apoptosis. However, important variations are observed in atherosclerotic lesions, from the lipid core to the periphery of the plaque, with very different local outcomes. HNE can modify signaling proteins involved in atherosclerotic plaque remodeling, particularly growth factor receptors (PDGFR, EGFR), cell cycle proteins, mitochondrial and endoplasmic reticulum components or extracellular matrix proteins, which progressively alters smooth muscle cell proliferation, angiogenesis and induces apoptosis. HNE-adducts accumulate in the lipidic necrotic core of advanced atherosclerotic lesions, and may locally contribute to macrophage and smooth muscle cell apoptosis, which contributes to plaque destabilization and rupture, thereby increasing the risk of athero-thrombotic events.
Oxidized lipids in atherosclerotic plaque instability

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A large body of evidence suggests a mechanistic link between oxidized lipids and atherosclerosis. Oxysterols and 4-hydroxynonenal (HNE), the major products deriving from LDL oxidation, are consistently present in inflamed and atherosclerotic arteries and they play a key role in the development of atherosclerosis (1,2). Due to the fact that atherosclerosis is a leading cause of mortality in Western countries, it is of increasing importance to understand the various molecular mechanisms induced by oxysterols and HNE, as well as to identify new markers to distinguish between stable and unstable atherosclerotic plaques.

Since chronic inflammation and matrix degradation might play a key role in plaque instability, we investigated the effect of oxysterols and HNE on various inflammatory molecules and MMP-9 expression in promonocytic U937 cells. In U937 cells, both oxysterols and HNE induced the expression of several cytokines and MMP-9 through TLR4 activation. These oxidized lipids also sustained inflammation by upregulating COX-2 and mPGES-1 levels, enzymes that cooperate to catalyze the conversion of arachidonic acid to PGE2. Inhibition of inflammatory molecule formation decrease MMP-9 release by macrophages, underlying the crucial role of inflammatory response in MMP-9 overexpression, a major marker of atherosclerotic plaque instability. Recently, it has been reported that proprotein convertase PCSK6, a new marker of plaque instability, is overexpressed in symptomatic carotid plaques. In connection with this, we are now investigating whether an oxysterol mixture and HNE can modulate the expression of PCSK6. Preliminary results indicate that both oxysterols and HNE upregulate PCSK6 in U937 cells. The downregulation of PCSK6 by siRNA significantly reduced MMP-9 activity induced by oxidized lipids, underlying a possible link between PCSK6 activity and MMP activation.

Our results suggest that oxysterols and HNE contribute to atherosclerotic plaque instability by enhancing the inflammatory response and favouring matrix degradation through MMP-9 upregulation. Moreover, preliminary results indicate that PCSK6, upregulated by oxysterols or HNE, might play a key role in plaque instability by increasing MMP activity. These results might provide strong incentive for the development of new therapeutic strategies to counteract the risk of atherosclerotic plaque rupture and thrombosis, which cause acute coronary syndromes.

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Oxidized phospholipids and phenotypic polarization of macrophages

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Oxidized phospholipids (OxPL) were shown to have pro-as well as anti-inflammatory effects, activating a variety of cell types. In particular, we have previously shown that macrophages respond to OxPL by drastically changing their gene expression pattern and function (1). However it is not known how macrophages adapt their metabolism to changes in tissue oxidation status. We found that macrophages respond to phospholipids containing oxidized fatty acid moieties by reprogramming their metabolism to support either redox homeostasis or inflammatory responses. OxPL-treated macrophages (Mox) adopt a metabolic profile, which is strikingly distinct from M1 and M2 macrophages and characterized by accumulation of antioxidant metabolites involved in glutathione synthesis. The metabolic adaptation in Mox macrophages coincided with Hif1α- and Nrf2-dependent gene expression. On the other hand, OxPL suppressed mitochondrial respiration by a mechanism involving TLR2-dependent ceramide production. Treatment of macrophages with truncated OxPL suppressed mitochondrial respiration and promoted expression of genes controlling redox homeostasis, while non-fragmented oxygenated OxPL induced pro-inflammatory gene expression, without inhibiting bioenergetics.

Using a targeted lipidomics approach, we demonstrate that both truncated and oxygenated OxPL species were abundant in healthy, lean adipose tissue. Accordingly, we identify a prominent population of previously unrecognized CX3CR1$^+$F4/80$^{lo}$CD11b$^+$ cells in lean adipose tissue, which are also positive for HO1$^+$ and Txnrd1$^+$, resembling the Mox phenotype. These cells are characterized by a quiescent bioenergetic profile, which can be mimicked by macrophages polarized with truncated OxPL. Surprisingly, we found that high-fat diet feeding led to a disproportional increase of oxygenated unfragmented OxPL species. In obese adipose tissue, the predominant macrophage population was CX3CR1$^+$F4/80$^{hi}$CD11b$^+$ positive, expressing both CD11c$^+$ and CD206$^+$ and characterized by a highly energetic metabolism.

Our findings demonstrate that macrophages respond to OxPL and adapt their metabolism to control redox homeostasis. The data suggest that by sensing OxPL, macrophages translate tissue oxidation status into antioxidant and inflammatory responses via modulation of metabolism and bioenergetics.

References:
Enhanced sensitivity to 4-hydroxynonenal and impaired redox signaling in human endothelial cells in gestational diabetes

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Fetal exposure to gestational diabetes (GDM) in utero is strongly associated with a higher risk of cardiovascular disease and insulin resistance in later life, and accumulating evidence suggests this may be a consequence of fetal programming potentially involving epigenetic influences.1 Notably, offspring of mothers with GDM exhibit elevated blood pressure and reduced endothelium-dependent reactivity.

We previously reported abnormal nitric oxide production, insulin resistance and reduced cell proliferation in fetal umbilical vein endothelial cells (HUVEC) from GDM pregnancies,2,3 and in parallel studies with endothelial cells from pre-eclamptic pregnancies, associated with in utero oxidative stress, identified abnormalities in NO production and regulation of [Ca²⁺].4,5 These phenotypic changes are maintained during culture, highlighting the involvement of fetal programming and epigenetics. As placental oxidative stress is propagated to the maternal and fetal vasculature via circulating lipid peroxides and H₂O₂ in GDM, we hypothesised that sustained oxidative stress in the fetal vasculature in utero may impair endogenous antioxidant defences.

We established that activation of the redox sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2)6 and its downstream target antioxidant enzymes is markedly inhibited in HUVEC from GDM pregnancies. Fetal endothelial cells exhibited marked deficits in glutathione synthesis, increased basal mitochondrial superoxide production, reduced nuclear translocation of Nrf2, and diminished adaptive increases in the expression of the Nrf2 target genes heme oxygenase-1 (HO-1) and/or NAD(P)H quinone oxidoreductase 1 (NQO1) in response to the lipid peroxidation product 4-hydroxynonenal (4-HNE). A proteomic analysis of normal and GDM HUVEC confirmed the altered GDM phenotype, characterised by markers of increased oxidative stress, reduced antioxidant protection and reduced cell proliferation.7 More recently, we have confirmed increased 4-HNE induced damage and attenuated Nrf2-regulated gene expression in umbilical artery smooth muscle cells from GDM mothers. The altered phenotype of fetal venous endothelial and arterial smooth muscle cells derived from GDM pregnancies may underlie an increased risk of developing type 2 diabetes and cardiovascular disease in childhood and early adulthood.8-12

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Dietary HNE and other lipid oxidation products in the development of colorectal carcinogenesis

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Based on epidemiological studies and meta-analyses, the World Cancer Research Fund (WCRF) panel stated that red meat is a convincing cause of colorectal cancer. Recently, the IARC/WHO classified red meat consumption as “probably carcinogenic to humans” (Group 2A). Red meat contains high concentrations of heme iron that induces lipid peroxidation in the colon lumen and the subsequent formation of secondary lipid oxidation products such as 4-hydroxynonenal (HNE). HNE was found in foodstuffs in relatively high concentration, particularly in meat and processed meat. Moreover, HNE formation possibly occurs in the digestive tract when rats receive heme iron and a PUFA-rich oil at the same time.

In vivo studies in rats show that the development of preneoplastic lesions is well correlated with heme-iron and HNE content of the diet, and also with the urinary excretion of the major metabolite of HNE, the mercapturic acid of 1,4-dihydroxynonenone (DHN-MA). Secondary oxidation products of PUFA, and especially HNE, could be one of the missing links between heme iron, enhanced luminal lipid peroxidation and colon cancer development.

In vitro studies in mouse epithelial colon immortalized cells show that the cells mutated on the Apc (Adenomatous polyposis coli) gene, an early and frequent mutation during the development of human colorectal cancer, were more resistant to HNE than non-mutated cells. This resistance is due to a better metabolization capacity of those cells towards HNE. The enzymes involved are under the transcriptional control of Nrf2/antioxidant responsive element (ARE) pathway, a pathway that is upregulated in Apc mutated cells.

Nrf2 natural inducers, as curcumin, have a protective effect both in vitro and in vivo.
Role of 4-hydroxynonenal in communication between cancer stem cells and microenvironment

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Cancer stem cells are a subpopulation of cancer cells with great plasticity, slow cell cycle and are capable of dormating for long time. Today, these cells are recognized as a major factor of malignancy causing metastasis, relapse, failure of cancer treatment and therapy resistance. The mechanisms which keep in control or activate these cells are still not fully revealed, but microenvironment certainly has a specific role. Proteins of the extracellular matrix can modify processes which cells are undergoing and changes of these proteins consequently affect cell. In addition to the environmental signals, oxidative stress may modulate and change signals cells are recieving thereby creating different outcome. Among the molecules produced during oxidative stress, 4-hydroxynonenal (HNE) was shown to modulate many signaling pathways thereby inducing differentiation, proliferation or apoptosis. Here, we provide evidence that HNE can change cancer stem cell responce to chronic oxidative stress through interactions with collagen, representative of extracellular matrix protein. Hence, HNE may be the factor that modulates cellular responce to stress or therapy via interactions with extracellular proteins and thereby changing the outcome of the disease.
The role of lipid peroxidation during the progression of human hepatocellular carcinoma (HCC)

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Hepatocellular Carcinoma (HCC) is ranked 5th among the most diagnosed cancer and the 3rd most common cause of cancer-related death worldwide. Emerging evidence suggests that HCC metastasis through portal vein tumor thrombus (PVTT) is the leading cause for poor prognosis in HCC patients. However, the mechanisms underlying initiation and progression of HCC remain to be defined. Altered redox status in cancer cells has been linked to lipid peroxidation induced by reactive oxygen species (ROS) and subsequent formation of reactive lipid electrophiles, especially 4-hydroxy-nonenal (4-HNE). Emerging evidence suggests that cancer cells manipulate redox status to acquire anti-apoptotic phenotype. Cardiolipin (CL), a mitochondria-specific inner membrane phospholipid, is critical for maintaining mitochondrial function. Paradoxically, liver tissues contain tetralinoleoyl cardiolipin (TLCL) as the major CL in mitochondria yet emerging evidence suggests that ROS generated in mitochondria may lead to CL peroxidation and activation of intrinsic apoptosis. It remains unclear how CL oxidation leads to apoptosis and its relevance to the pathogenesis of hepatocellular carcinoma (HCC). We employed a mass spectrometry-based lipidomic approach to profile lipids in human tissues of HCC and found that CL was gradually decreased in tumor comparing to peripheral non-cancerous tissues, accompanied by a concomitant decrease of oxidized CL and its oxidation product, 4-HNE. Incubation of liver cancer cells with TLCL significantly restored apoptotic sensitivity accompanied by an increase of CL and its oxidation products when treated with staurosporine (STS) or Sorafenib (the standard treatment for late stage HCC patients). Our studies uncovered a novel mechanism by which cancer cells adopt to evade apoptosis, highlighting the importance of mitochondrial control of apoptosis through modulation of CL oxidation and subsequent 4-HNE formation in HCC. Thus manipulation of mitochondrial CL oxidation and lipid electrophile formation may have potential therapeutic value for diseases linked to oxidative stress and mitochondrial dysfunctions.
Redox regulation in Aging: role of protein aggregates

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Reactive oxygen species (ROS) are formed continuously in the organism even under physiological conditions. Proteins are prominent targets of oxidation reactions. Such oxidized proteins are either degraded or form protein aggregates. Such protein aggregates are accumulating during cellular aging and influence the metabolism in an aged cell.

One of the potential adverse reactions of protein aggregates in aged cells is the aggregate-dependent formation of reactive oxygen species. Protein aggregates are able to incorporate metals, especially iron, which is redox active and can only be partially accessed by chelators. We were able to show that such protein aggregates are contributing substantially to the changes in the pro-oxidative status of aged cells.

A special role in this formation of protein aggregate-dependent, metal-induced oxidative stress is played by iron containing proteins, which may be included into the protein aggregates. We could show a contribution of both, cytosolic/ferritin and mitochondrial iron sources.

While research is often focusing on the pro-oxidant role of mitochondria in aged cells, other sources – as protein aggregates – of oxidants have to be taken into account.
Oxidative stress and antioxidants: Distress or eustress?

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Oxidative stress, a signal which affects redox balance and induces oxidative modification of biological molecules, may have multiple faces. Reactive oxygen and related species (ROS) protect us from invading xenobiotics and act as physiological redox signaling messenger. The production of ROS is tightly regulated and the reactions are selective. On the other hand, ROS produced by unregulated manner induce random oxidative modification of lipids, proteins, and nucleic acids, which gives rise to deleterious effects and has been implicated in the pathogenesis of many diseases. Thus, ROS may become good stress (eustress) or bad stress (distress).

Unsaturated lipids such as linoleic acid (LA), arachidonic acid (AA), and cholesterol, both free and ester forms, are vulnerable to oxidation by multiple oxidants to give diverse products. Lipid hydroperoxides, the major primary product, are toxic per se and may exert deleterious effects by producing reactive secondary products including HNE. We aerobic organisms are protected from lipid oxidation products by inhibiting their production and by inducing adaptive response. It has been shown that multiple antioxidant compounds and enzymes are induced by lipid oxidation products to prepare for subsequent deleterious oxidative stress.

The lipid oxidation products produced by random manner may not be physiological signaling messenger, but rather xenobiotics. It has been observed that levels of these products are associated with the onset and progress of diseases such as atherosclerosis.

We are protected from oxidative stress by an array of defense system in which multiple antioxidants with diverse functions play their respective roles including reduction of hydrogen peroxide and hydroperoxides, sequestration of metal ions, scavenging of reactive oxidants, repair of damage, and excretion of toxic products. Scavenging of ROS is one of the important functions of antioxidants. It may be noted that the efficacy of scavenging oxidants depends on the nature of oxidants. Further, it is unlikely that antioxidants scavenge such oxidants that act as physiologically important signaling messenger.

Interestingly, higher levels of lipid oxidation products produced by peroxyl radicals, peroxynitrite, hypochlorite, 15-lipoxygenase, and singlet oxygen have been found in atherosclerotic lesions than in normal arteries and in plasma of patients than healthy subjects, suggesting that multiple antioxidants are required to inhibit deleterious lipid oxidation in vivo.
Antioxidants and HNE in the regulation of redox homeostasis

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Under physiological conditions, cells are in a stable state known as redox homeostasis, which is maintained by the balance between continuous ROS/RNS generation and several mechanisms involved in antioxidant activity. ROS overproduction results in alterations in the redox homeostasis that promote oxidative damages to major components of the cell, including the biomembrane phospholipids. Lipid peroxidation subsequently generates a diverse set of products, including α,β-unsaturated aldehydes. Among these compounds, 4-hydroxy-2-nonenal (HNE) is the frequently studied aldehyde on the basis of its involvement in cellular physiology and pathology. Depending on its level, HNE exerts harmful or protective effects associated with the induction of antioxidant defense mechanisms. These effects make HNE a key player in maintaining redox homeostasis, as well as producing imbalances in this system that participate in aging and the development of pathological conditions. Increased level of lipid peroxidation products including HNE has been observed in typical inflammatory diseases such as Rheumatoid arthritis (RA) and Lyme arthritis (LA). It has been revealed the comparison of the HNE level in plasma can be helpful for RA monitoring and in differential diagnostic between RA and LA. Additionally HNE because of its high chemical reactivity is able to generate HNE-protein adducts, thus HNE participates in multi-step regulation of cellular metabolic pathways that include signaling and transcription of antioxidant enzymes, pro-inflammatory factors, and anti-apoptotic proteins. Accumulation of the 4-HNE-His protein adducts is observed in plasma of the RA patients indicating on the importance of lipid peroxidation in the disease progression. The most widely described roles for HNE in the signaling pathways are associated with its activation of kinases, as well as transcription factors that are responsible for redox homeostasis. Increased level of HNE in UV irradiated cells suggests that this reactive molecule through direct interactions with NFκB and/or Nrf2 inhibitors activate these factors, what is also confirmed by the expression of proteins that transcription depends on NFκB or Nrf2 activity. However, skin cells treatment with simple exogenous antioxidant such as ascorbic acid or polyphenols leads to decrease in HNE level, and thus inhibits signalling based on HNE-transcription factor interactions. Observed effects make HNE a key player in maintaining redox homeostasis, as well as inducing its imbalance resulting in the development of pathological conditions.
Novel molecular approaches for improving enzymatic and nonenzymatic detoxification of 4-hydroxynonenal: toward the discovery of a novel class of bioactive compounds

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Reactive carbonyl species (RCS) such as α,β-unsaturated aldehydes are endogenous or exogenous byproducts involved in the pathogenic mechanisms of different oxidative-based disorders. Detoxification of RCS by carbonyl quenchers is a promising therapeutic strategy. Among the most studied quenchers are aminoguanidine, hydralazine, pyridoxamine, and carnosine; their quenching activity towards four RCS (4-hydroxy-trans-2-nonenal, methylglyoxal, glyoxal, and malondialdehyde) was analyzed and compared by using an innovative method based on high-resolution mass spectrometry (HRMS). The reactivity of the compounds was RCS dependent: carnosine efficiently quenched 4-hydroxy-trans-2-nonenal, pyridoxamine was particularly active towards malondialdehyde, aminoguanidine was active towards methylglyoxal and glyoxal, and hydralazine efficiently quenched all RCS. Hence carnosine was found to be a selective detoxifying agent of α,β-unsaturated aldehydes and could therefore be used as a pharmacological tool to assess the role of HNE overproduction in different animal models. Since carnosine can also act as a pro-histaminic compound following the carnosinase hydrolytic cleavage to histidine, a derivative resistant to the hydrolysis catalyzed by carnosinase was then designed: FL926, (2S)-2-(3-amino propanoylamino)-3-(1H-imidazol-5-yl)propanol, which is a novel derivative of carnosine with high oral bioavailability and resistant to carnosinases. FL926 showed a suitable ADMET profile and was determined to have the greatest potency and selectivity toward α,β-unsaturated aldehydes (e.g. 4-hydroxynonenal, HNE) of all others so far reported. In rodent models of diet-induced obesity and metabolic syndrome, FL926 dose-dependently attenuated HNE-adduct formation in liver and skeletal muscle while simultaneously mitigating inflammation, dyslipidemia, insulin resistance, and liver steatosis. These improvements in metabolic parameters with FL926 were not due to changes in energy expenditure, physical activity, adiposity or body weight. Collectively, our findings illustrate a pathogenic role for RCS and in particular of α,β-unsaturated aldehydes in obesity-related metabolic disorders, and provide validation for a promising new class of carbonyl-scavenging therapeutic compounds rationally derived from carnosine.
**Lipid peroxidation and pharmaceutical drugs**

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There exists a multitude of pharmaceutical drugs which effects include the formation of oxidants and, therefore increased lipid peroxidation or which at least deteriorate the redox balance within cells, tissues or organs. In many of those cases, the generated oxidants are an important part of the drug efficiency. Well known examples of those drugs and xenobiotics are anticancerogenic drugs (chemotherapeutics), antihelminthics, dermatics, tuberculostatics and various antibiotics. In anticholinergics and spasmyotics an increased generation of oxidants and lipid peroxides seems to be rather a side effect than a specific pharmacological effect. That seems true also for all xenobiotics stimulating the catecholamine formation.

The increased free radical generation and lipid peroxidation by chemotherapeutic drugs and cytostatics, by many antibiotics, tuberculostatics, and dermatics contributes to therapy of different types of cancer, microbially induced infectious diseases – by bacteria, viruses, or fungi – autoagression, or psoriasis.

Doxorubicin / daunorubicin is an example for anthracyclin-antibiotics used in the therapy of malignant tumors such as breast and lung tumors, gynecological sarcomas, lymphomas, and hepatocellular carcinoma (HCC). Within its fine mechanisms of this substance one finds intercalant transcription inhibiting effectivity, inhibition of topoisomerase II activity, and promoting an overwhelming formation of oxidants and lipid peroxidation products. Last mechanism leads also to strong side effects of doxorubicin such as depression of the bone marrow and cardiotoxicity.

In contrast other pharmaceutical drugs reduce the concentration of oxidants or even directly deliver antioxidants, such as antiallergics, nootropics (antidementives), geriatrics, antiphlogistics, prostaglandins and prostacyclins such as iloprost, drugs influencing gout or hyperuricemia such as inhibitors of purine degradation, roborantia and vitamine preparatives, also anticoagulative acting drugs, and hypnotics. The use of GSH can be useful, since the dysfunction of the GSH redox system appears to cause a variety of diseases including neurodegenerative disorders. However, the effectiveness of GSH as therapeutic agent is limited because of its low bioavailability.

Another aspect of the formation of oxidants and increased lipid peroxidation during and after application of pharmaceutical drugs is the detoxification of drugs preferably by the liver. The metabolic reactions involved in the detoxification of drugs and further xenobiotics present one of the main sources for the generation of oxidants in humans and animals treated with pharmaceutical drugs. The cytochrome P450 systems plays an important role within total oxygen radical and oxidant generation in human body.
Poster abstracts
Systems biology view on lipid oxidation - reconstructing lipid peroxidation products (LPPs) metabolic networks

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Many human diseases, including obesity, diabetes and atherosclerosis, are accompanied by chronic inflammation and closely connected to oxidative stress (OS). OS can oxidize virtually all biomolecules of which lipids represent one of the most prominent targets. Lipid peroxidation products (LPPs) are chemically diverse group of biomolecules with a variety of functional activities. Many LPPs were shown to play an important role in the onset and development of OS-related diseases and can serve as diagnostic and prognostic biomarkers. However, to include LPPs in a systems medicine view on obesity, the information on their structures, activities and functions as well as associations with various pathological conditions need to be collected and summarized.

Based on a comprehensive meta-study including over 170 publications focusing on the enzymatic and non-enzymatic LPPs production, networks of enzymatic and free-radical-driven oxidative reactions were reconstructed for the ten most abundant PUFAs (18:2, 18:3 n-3, 18:3 n-6, 20:3 n-6, 20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-3, 22:5 n-6, and 22:6 n-3). Reconstructed networks allowed to illustrate differences and similarities in PUFAs oxidation mechanisms and were further used to design in silico oxidation algorithms. PUFAs based LPP networks will be further enriched with available information on LPPs involvement in different cellular pathways and integrated into more complex metabolic networks of phospholipids oxidation.
Induction of oxidative stress in barley by drought and chilling

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Environmental stresses such as draught, chilling, salinity, high light induce oxidative stress in plants (1). The measurements of reactive oxygen species (ROS) and lipid peroxidation provide information about oxidative state of the exposed plants (2). Peroxidases are important antioxidant enzymes with role in utilizing H2O2 (3). In this work we investigated the effect of draught and chilling on barley seedlings. Plants were grown in growth chamber with a light intensity of 100 µmol m-2 sec-1 at 22 °C and 80% humidity for seven days on a 16/8-hour day/night cycle. Draught was induced by omitting water for 10 days while maintaining normal humidity, temperature, and 16/8 day/night light cycle. For induction of chilling stress plants were exposed to 0°C for 4 h prior to measurements. We measured level of thiobarbituric acid reactive substances (TBARS) as a marker of lipid peroxidation, concentration of hydrogen peroxide and activity of total peroxidases using guiacol as a substrate.

Results showed that both stresses induced oxidative damage of membranes by increasing lipid peroxidation levels. Higher levels were observed in plant exposed to chilling stress in comparison with drought. Concentration of H2O2 and activity of guiaicol peroxidase were higher in plants exposed to draught. This observed differences could be due to the time of exposure to the particular stress as well as barley genotype.

References:
 Detecting lipoxidation: diagnostic tools for the translation and commercial application

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Oxidative stress is strongly associated with the pathophysiology of inflammation diseases such as sepsis, diabetes and cardiovascular diseases. The oxidants formed in inflammation can cause oxidative damage to lipids, producing short chain electrophilic oxidized lipid products that can react with nucleophilic amino acid residues of proteins, a process known as lipoxidation [1]. 4-hydroxy-2-nonenal (HNE) is one of the quantitatively most important products of lipid peroxidation, and can react by Michael addition or form Schiff’s base adducts with proteins, leading to cellular dysfunction. As lipoxidation products have potential as biomarkers of oxidative stress and disease, development of rapid assays for them is required. The aim of this work was to produce antibodies that recognize HNE-modified human serum albumin for future use in diagnostic assays of plasma.

Human serum albumin (HSA), the most abundant protein of the human sera, was reacted with HNE at a 1:10 ratio. Analysis of intact HSA-HNE by direct infusion ESI-MS suggested the presence of (on average) 4.7 molecules of HNE/HSA and MALDI-TOF analysis showed an increase in mass corresponding to the addition of 3 molecules of HNE/HSA. Proteomic analysis of tryptic digests gave with confident identification only of HNE Michael addition, on the histidine of peptide VH²⁴²TECCHGDLLECADDR. To complement the results, an ELISA assay was performed with a commercial anti-HNE antibody and specific binding to HSA-HNE was observed, in contrast to low binding of un-modified HSA. The HSA-HNE adducts were subsequently immunized into sheep for generation of polyclonal antibodies and ELISA assays performed with enriched antibodies showed specific binding to HNE-modified proteins. In parallel, a potential reactive peptide sequence (LQQC₃₄PFE; m/z = 862.3) from HSA was synthesised and reacted with HNE. Using ESI-MS, HNE Michael adducts (m/z = 1018.4) on the cysteine residue were confirmed and Schiff’s base adducts (m/z = 1000.4), probably on the free N-terminal group, were also observed. These adducts were purified by reverse phase HPLC and immunized in rabbit and sheep for generation of antibodies anti-HNE-modified LQQCPFE.

In conclusion, HNE adducts on HSA were confirmed and used for the generation of polyclonal antibodies. Enriched antibodies showed binding to HNE-modified HSA, but some cross-reactivity to HSA. Future work includes the improvement of the antibody purification process and testing anti-sera from rabbit and sheep immunized with LQQC(HNE)PFE.

References:
Lipid droplets carbonylation - a new pathway to eliminate lipid peroxidation products?

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Lipid peroxidation products (LPPs) generated by oxidative stress (OS) have been recognized as biomarkers of numerous human disorders including atherosclerosis, neurodegenerative diseases, type 2 diabetes, chronic inflammation, aging, and cardiovascular diseases. To evaluate the effect of OS and related LPPs on cardiac system, rat primary cardiomyocytes treated with peroxynitrite donor SIN-1 were used as a cellular model of mild nitroxidative stress. Carbonylated molecules were visualized by fluorescent microscopy using cell permeable dye 7-(diethylamino)coumarin-3-carbohydrazide (CHH). Interestingly, increase in lipid droplets (LDs; visualized using Nile Red staining) as well as their enrichment in carbonylated LPPs was observed upon SIN-1 treatment of cardiomyocytes. Furthermore, using Nile Red and CHH co-staining heterogeneity in LDs carbonylation was demonstrated. Role of autophagy-lysosomal degradation pathway in removal of carbonylated LPPs from LDs was further evaluated using selective inhibitors including 3-methyladenine (inhibitor of an autophagosome formation), chloroquine (inhibitor of an autophagosome and lysosome fusion), orlistat (lipase inhibitor), E64d and Pepstatine A (proteases inhibitors). Significant role of autophagy-lysosomal degradation pathway in elimination of oxidized lipids incorporated in OS induced LDs was demonstrated.
LC-MS\textsuperscript{3} method for the analysis of Lipid Peroxidation Products (LPPs) positional isomers

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Lipid peroxidation is an enzymatic or non-enzymatic transformation of polyunsaturated fatty acids (PUFAs) leading to the formation of various lipid peroxidation products (LPPs) including low molecular weight aldehydes (i.e. 4-hydroxy-trans-2- nonenal, acrolein), truncated lipids (i.e. alkanals, alkenals), and hydroxy-alka(e)nals), isoprostanes, hydroperoxy-, hydroxy-, keto- and epoxy-derivatives. Numerous studies shown the involvement of lipid peroxidation in the onset and progression of inflammatory based diseases such as diabetes, Alzheimer, Parkinson diseases and cardiovascular diseases[1]. LPPs are known to modulate different cellular signaling pathways by inducing changes in the biological membranes[2], protein lipoxidation, and interaction with cell surface (e.g. scavenger receptors and TLRs)[3] and intracellular (e.g. PPARs) receptors [4].

Various biological activities of LPPs are mainly determined by their chemical diversity. In order to understand LPPs structure-functional relationships, specific and sensitive analytical tools allowing separation and identification of structural isomers are required. Liquid chromatography coupled on-line to mass spectrometry (LC-MS) allows high-throughput characterization of LPPs in biological samples. However, majority of the methods are not capable to distinguish LPP structural isomers.

Here a novel LC-MS\textsuperscript{3} method for analysis of isomeric LPPs was developed. MS and tandem MS analysis was performed in negative ion mode. Anions of oxidized PUFAs, produced by collision induced dissociation of LPP precursors, were further used for data-driven MS3 analysis yielding structure specific fragment ions necessary to assign type and position of oxidation within PUFA alkyl chain. Method was validated using in vitro oxidized standard PLs and further translated for analysis of complex biological samples. Thus, using novel LC-MS\textsuperscript{3} based approach it was possible to reveal structural diversity of LPPs in a high-throughput manner.

References:
Exogenous NOS inhibitor 7-NI influence ADMA production and improve redox status in plasma of spontaneously hypertensive rats

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Introduction: NO inhibition can influence blood pressure regulation and increase hypertension. Asymmetric dimethylarginine ADMA, is an analogue of L-arginine and its elevation inhibits NO synthesis, impairs endothelial function and is risk marker of cardiovascular diseases. In hypertension also reactive oxygen metabolites influence changes in signalisation and cell damages. In our study we focus on changes in ADMA level and oxidative stress in NO synthase inhibitors (L-NAME and 7-nitroindazole) treatment.

Materials and Methods: Spontaneously hypertensive rat (SHR) were chronically treated (up to 6 weeks) by L-NAME (50mg/kg) or 7NI (10mg/kg) and were comparing with control group. HPLC method with fluorescence detection has been used to simultaneously analyse the levels of L-arginine and methylated stereoisomers: ADMA and SDMA. Plasma samples underwent solid-phase extraction followed by derivatization. Data acquisitions were achieved either using a classical standard method from calibration curve of an internal standard N-monomethyl-L-arginine- MMA (method 1) or using the method of several standard addition from linear regression extrapolation (method 2). Redox status has been determined by comparison of TTL vs. ROM level in plasma. Reactive oxygen metabolites – dROMs assay (Diacron, Grosseto, Italy) is a spectrophotometric method for determination of the hydroperoxides concentration reacting with a chromogenic substrate to develop a colored derivative. Total thiols level -TTL assay (RelAssay, Gaziantep, Turkey) is based on the ability of free thiol groups to develop a colored complex when reacted with 5,5-dithiobis-2-nitrobenzoic acid (DTNB). The color intensity is directly related to the thiol groups which are not affected by oxidation. ROM and TTL plasma biomarkers were measured on a clinical autoanalyzer Unicel DxC 800 (Beckman-Coulter, Woerden, the Netherlands).

Results and discussion. Effects of the two potential exogenous NO-inhibitors: L-NAME and 7-NI, were compared. ADMA level, ROS and TTL have not been changed in the L-NAME group comparing to control SHR. However, the administration of 7-NI markedly decreased the ADMA over-production in plasma (7NI 0,0630±0,012 µM vs Control 0,208± 0,033µM) and also increased and improve TTL/ROM redox level comparing to L-NAME group.

It has been observed that 7-NI exogenous NO-inhibitor can correlated levels of ADMA, ROM and TTL in plasma of spontaneously hypertensive rats.

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Differences in rutin effect on membrane phospholipids in skin fibroblasts irradiated with UVA and UVB

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Chronic exposure of the skin to solar UV radiation induces a number of biological alterations, including a redox imbalance; therefore, there is a constant need for protective compounds, particularly natural antioxidants. The aim of this study was to determine the effect of rutin on interactions between membrane phospholipid and antioxidant proteins expression in UVA or UVB irradiated fibroblasts. Following exposure to UVA and UVB irradiation cells were incubated with rutin 12h before and/or up to 24h after irradiation, and the structural and metabolic changes were examined at selected time intervals.

Rutin penetration through the fibroblast phospholipid bilayer was aided by UVA-induced bilitranslocase activity, while lipidomic analysis revealed that following UVB-irradiation rutin transport into cell is enhanced by changes in membrane permeability resulting from UVB-induced lipid peroxidation. Moreover, rutin partially prevented UVA/B-induced increase in phosphatidylethanolamine and phosphatidylcholine and decrease in sphingomyelin, as well as their membrane localization. However, the inhibition of phospholipase A2 activity and increase in ROS level resulted in protection against the reduction of phospholipids/free arachidonic and linoleic acids and the increase in the level of the lipid peroxidation product 4-HNE, which demonstrates the antioxidant proteins regulatory properties. The antioxidant activity of rutin, effectively prevented the enhanced ROS generation as well as antioxidant system destruction. Proteome analysis show that rutin treatment more strongly protects against UVA-induced rather than UVB-induced increases in the total expression of proteins involved in antioxidant response (such as SOD, TrxR, and Prxs 1/2). However, in the case of UVB-irradiated cells, rutin additionally enhances the levels of disulfide-isomerase – an enzyme that is responsible for the formation and breakage of disulfide bonds, what in the case of Nrf2/ARE pathway has significant meaning in the Nrf2 transcriptional activity level and leads to the synthesis of cytoprotective proteins.

In conclusion, UVA and UVB radiation in partially different ways affect rutin interactions with the fibroblast biomembrane lipids as well as antioxidant proteins. Rutin membrane penetration is promoted by UVA-induced bilitranslocase activity, while UVB irradiation enhanced membrane permeability to facilitate the interaction of rutin with phospholipids. Moreover, rutin particularly influences UVA-induced antioxidant proteins expression and UVB-induced transcription signaling.
Catechins and other flavonoids as protectors against undesired modifications of biomolecules

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Undesired modifications of biomolecules (proteins, lipids and nucleic acids), mainly oxidation by reactive oxygen species, underly many diseases and apparently aging. Compounds counteracting these modifications can therefore be candidates for potential drugs and aging-modifying agents. Naturally occurring compounds are especially interesting in this respect since knowledge of their properties may be a basis for dietary recommendations and functional food production. The aim of our studies was to compare the antioxidant properties and efficiency of a range of flavonoids and other natural antioxidants in protection against protein modifications and lipid peroxidation. We compared the ABTS*-scavenging activity capacity of a range of flavonoids and selected other antioxidants, their iron binding capacity estimated by the ferrozine competition test and strong iron binding evaluated by a modified deoxyribose degradation test, their potency to prevent peroxynitrite reactions in three different test systems, based on fluorescein bleaching, tyrosine nitration and serum albumin thiol oxidation as well as oxidation of dihydrorhodamine and fluorescein, and protein tyrosine chlorination by hypochlorous acid. The sequence of protective capacity of the antioxidants was different in various systems. Correlation analysis revealed that the hydroxyl group at the R04 position of the flavonoids contributes significantly to prevention of fluorescein bleaching, R3-OH is important for prevention of thiol oxidation while R5-OH and R03-OH are significant in prevention of tyrosine nitration. The total number of hydroxyl groups correlated with the ability of flavonoids to prevent oxidation reactions and the presence of vicinal hydroxyl groups correlated with flavonoid reactivity in all systems used [1]. Flavonoids were also active in preventing nitration of intracellular proteins. Flavonoids and other antioxidants were also compared as protective agents against glycation of serum albumin induced by various sugars (glucose, fructose and ribose) [2] and reactive aldehydes (glyoxal and methylglyoxal) [3] and blood plasma lipoproteins against lipid peroxidation induced by various agents (AAPH, peroxynitrite and hypochlorous acids). Catechins showed the highest ABTS*-scavenging capacity, the highest stoichiometry of Fe³⁺ reduction in the FRAP assay and belonged to the most efficient compounds in protection SIN-1 induced oxidation of dihydrorhodamine 123, AAPH-induced fluorescein bleaching and hypochlorite-induced fluorescein bleaching. (+)-Catechin and (-)-epicatechin were the most effective compounds in protection against AAPH-induced erythrocyte hemolysis while (-)-epicatechin gallate, (-)-epigallocatechin gallate and (-)-epigallocatechin protected at lowest concentrations against hypochlorite-induced hemolysis. The results demonstrate high potency of flavonoids in protection against undesired modifications of biomolecules, even at micromolar concentrations.
The study was supported by grants 2011/01/M/NZ3/02065 and 2014/14/A/ST4/00640 from the Polish National Science Center.

References:
Mild therapeutic hypothermia decreases oxidative damage and increases glutathione levels in post-cardiac arrest patients

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Background: The post-cardiac resuscitation syndrome is a pathophysiologic state after the successful cardiopulmonary resuscitation in cardiac arrest patients. Ischemia-reperfusion-induced oxidative stress is one of the main mechanisms of tissue injury after cardiac arrest [1]. A decrease in antioxidant defenses may contribute to ischemia-reperfusion injury [2]. The mild hypothermia treatment may improve the tissue damage in post-cardiac arrest patients [3]. Mild therapeutic hypothermia is known to decrease the levels of oxidative damage biomarkers, as previously shown by our research group [4]. The mechanisms involving cardiac arrest pathophysiology and hypothermia treatment are not well elucidated.

Methods: Intensive care unit patients admitted in 2011 and 2012, victims of in-hospital or out-of-hospital cardiac arrest were screened for the study. The sample consisted of 31 patients under controlled normothermia (36°C) and 11 patients treated with mild therapeutic hypothermia (33°C). Mild hypothermia was induced 4-5h after successful cardiopulmonary resuscitation for 24 h. Clinical data and venous blood samples were collected 6, 12, 36 and 72 h post-cardiac arrest. We investigated lipid (malondialdehyde levels) and protein (carbonyl levels) damage biomarkers, and also investigated the levels of the antioxidants glutathione, vitamin C and vitamin E, and nitric oxide levels at 6, 12, 36, and 72 h after cardiac arrest. Data were compared by multivariable logistic-regression models with generalized estimating equations and pairwise comparisons of estimated means by the post-hoc Bonferroni method.

Results: Serum malondialdehyde and plasma carbonyl levels were decreased in hypothermic group at 6, 12, 36, and 72 h after cardiac arrest in hypothermic patients [4]. Also, erythrocyte glutathione levels were elevated by mild therapeutic hypothermia at all time-points, while serum Vitamin C levels decreased significantly at 6 and 12 h after cardiac arrest in hypothermic patients, coinciding with the period of therapeutic hypothermia. Serum vitamin E and erythrocyte nitric oxide levels were not altered by hypothermic treatment.

Discussion: The present results evidence that hypothermia can reduce oxidative damage in post-cardiac arrest patients. Moreover, our findings reported, for the first time, increased glutathione levels after cardiac arrest in hypothermic patients as compared to all time points in normothermic patients. There is no previous evidence or possible mechanism corroborating a decrease in vitamin C levels as a consequence of hypothermia.

Conclusions: These findings suggest that mild therapeutic hypothermia may reduce oxidative damage to lipids and proteins, and concomitantly elevate glutathione levels. Therefore, mild therapeutic hypothermia may contribute to the tissues protection from ischemia-reperfusion injury after cardiac arrest by decreasing oxidative stress.

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References:
New N-9- sulfonlpurine derivatives induce changes in mitochondrial function and ROS accumulation in carcinoma and leukemia cells in vitro

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Our recent studies showed significant antiproliferative capacity of newly synthesized N-9-sulfonlpurine derivatives on human carcinoma, lymphoma, and leukemia cells. These derivatives behave as antimetabolites exploiting cellular metabolism to induce cytotoxicity in treated tumors’ cells (1 - 3). Based on previously obtained results on N-9-sulfonlpuridine derivatives, a new series of N-9-sulfonlpurine derivatives were synthesized and tested on biological potential.

The aim of this study was to determine if measured antitumor activity of N-9-sulfonlpurine derivatives is linked with ROS accumulation and mitochondrial membrane destabilization in human cervix adenocarcinoma (HeLa), human chronic myelogenous leukemia (K562) and human Burkitt’s lymphoma (Raji) cells. Changes in the mitochondrial membrane potential (ΔΨm) of tested compounds were determined by flow cytometry, measuring fluorescence of JC-1 dye in tumor cells after 24 hours of treatment. Intracellular accumulation of ROS was determined after 1 hour in cells exposed to N-9-sulfonlpurine derivatives by flow cytometry as well. Tested derivatives induced increased accumulation of ROS and mitochondrial disruption in more than 80% of HeLa and Raji cells. The effect of tested derivatives on K562 cells is reflected by the change of mitochondrial potential in more than 70% with slightly reduced accumulation of ROS. Based on obtained results we can conclude that newly synthesized N-9-sulfonlpurine derivatives are good candidates for further antitumor studies.

References:
Mechanisms of triacylglycerides oxidation in artificial lipid droplets

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Lipid droplets (LDs) are inducible cellular organelles that have a pivotal role in physiological processes. Depending on their size and host cell type they serve functions ranging from lipid storage (e.g. in adipocytes), cytoprotection (in non-adipose tissue) and inflammation (in macrophages and leukocytes).[1] LD are micelles consisting of a neutral lipid core (triglycerides - TAG, cholesteryl esters - CE and fatty acids - FA) surrounded by a monolayer of phospholipids (PL). Under conditions of oxidative stress (e.g. cancer) lipid droplets have been found to contain increased levels of oxidized TAG (oxTAG). [2]

Oxidized lipid species have been shown to be involved in the disturbance of cellular processes by various mechanisms but are also known to serve regulatory functions [3]. Non-enzymatic lipid oxidation is based on an oxygen dependent radical-propagation mechanism. Due to the highly unpolar milieu in the LDs core, polar oxygen-centered radicals (e.g. hydroxyl radical) cannot penetrate through the outer LDs phospholipid monolayer indicating that other mechanisms of radical reactions might govern generation of oxidized lipids in LDs, either involving longer lived, hydrophobic organic radicals or radical propagation from PL monolayer into the LD core.

LDs of specific composition (molar ratio: PC/PE 3:1; PL/TAG 1:20) and defined size (140 nm) were prepared by differential ultracentrifugation steps. Size and composition of generated micellar structures was elucidated by nanotracking light scattering microscopy and fluorescence spectrophotometry. Generated micelles were oxidized by either Fenton chemistry (hydroxyl radicals) or by AAPH (organic radicals) and further analyzed by LC-MS using RPC-ESI-QTOF or ESI-LTQ-Orbitrap.

References:
Cysteinylated and glycated human serum albumin levels are increased in human heart failure

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Cysteinylated human serum albumin (Cys-HSA) is a post-translational modification of human serum albumin (HSA) where the sulphhydryl group (SH) of cysteine in position 34 (Cys34) becomes oxidized by a cysteine [1] and this oxidation easily occurs when exposed to oxidative stress [2]. The degree of oxidized Cys34 in HSA is correlated with oxidative stress related pathological conditions [3]. Indeed, increased Cys-HSA levels have been detected in patients undergoing end stage renal disease [4], coronary artery disease [2], chronic liver disease or diabetes mellitus [3] as well as in pregnant women with intrauterine growth restriction [5]. Functional loss of HSA due to post-translational modification could influence homeostasis, which may contribute to the progression of chronic diseases. For example, Oettl et al. reported that, in advanced liver disease, oxidative damage impairs the binding properties of HSA resulting in increased tissue distribution of toxic endogenous compounds, and thus enhancing the risk of tissue damage related to complications [6]. Similarly, the loss of significant oxidant buffering capacity of HSA due to Cys-HSA may lead pregnancy complications such as intrauterine growth restriction [5].

In this study we examined for the first time the extent of Cys-HSA in plasma of patients with heart failure [NYHA class III and IV (n class III =10, n class IV = 10)] compared with healthy subjects (n=11) by using a direct infusion ESI-MS.

Blood samples were collected in citrate tubes and centrifuged immediately after collection. Plasma fraction was stored at -80 °C. Before the analysis, plasma samples were diluted 1:200 in water containing 50% acetonitrile and 0.1% formic acid and infused into a triple quadrupole mass spectrometer (Xevo TQ-S from Waters). The relative content of the HSA isoforms (Cys-HSA and glycated-HSA) were determined by measuring their relative intensities.

Results showed a significant increase of Cys-HSA in heart failure (HF) patients (13.11%±5 and 17.31%±3.6 for NYHA III and IV, respectively) with respect to age-matched healthy subjects (9.87%±3). Furthermore, the levels of glycated-HSA were higher in HF patients (7.72%±1.49 and 8.04%±0.85, for NYHA III and IV, respectively) in comparison with age-matched healthy subjects (6.535%+0.54). Finally, HF patients showed a significant decrease in the total level of HSA as expected [7].

In conclusion, this study revealed an increased level of cysteinylation and glycation of HSA in patients with HF expanding the current knowledge that different residues on HSA can undergo certain post-translational modifications in specific environments in oxidative stress-related diseases.

Future studies will establish the utility of monitoring the redox status of Cys34 in HSA as a marker for oxidative stress in HF and its potential role in the onset and progression of the disease. Finally, since HSA has an established role as a blood stream carrier, it will be interesting and exciting to test how these modifications can affect drug delivery of various pharmacological treatments in HF.
References:
[3] DOI: 10.1371/journal.pone.0085216
[7] DOI: 10.1016/j.ahj.2010.05.022
Tiron is Protective Against ROS-Induced Damage in Human Bronchial Epithelial Cells

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Background: Reactive oxygen species (ROS)-induced damage and resultant oxidative stress have been documented within the lung and have been implicated in the development of pulmonary diseases such as chronic obstructive pulmonary disorder and asthma (1). Antioxidant supplementation has been used to inhibit accumulation of oxidative damage such as DNA strand breaks and lipid peroxidation in inflammation (1,2). Tiron, a superoxide scavenger, has previously been shown to reduce murine airway remodelling and associated inflammation as well as offer protection against UV and H2O2-induced mitochondrial DNA (mtDNA) damage in human dermal fibroblasts (2,3).

Aim: This study aimed to investigate the use of the Tiron to prevent ROS-induced damage in human bronchial epithelial cells (BEAS-2B).

Methods: BEAS-2B cells were pre-treated with 3mM Tiron in BEGM (Lonza, UK) for 24 hours prior to induction of ROS damage (1 hour H2O2 (0.25mM) or exposure to 120 hour hypoxic (1% O2) conditions). Simultaneous Tiron treatment and ROS-induction was also assessed. The level of ROS production was assessed via flow cytometric analysis using the DCFDA assay (Abcam, UK). MtDNA strand breaks were assessed using a qPCR 1kb amplification following 83bp alignment (4). Concentration of the lipid peroxidation biomarker malondialdehyde (MDA) was quantified using a colourimetric assay (Sigma, UK). All data was normalised to the cell controls where appropriate and summarised as mean ±SEM, analysed by one-way ANOVA and Dunnett’s posthoc test (p≤0.05).

Results: Both pre-treatment and the addition of Tiron at the time of ROS-induction demonstrated 100% protection (±0.18 vs. H2O2; ±0.06 vs. hypoxia pre-treatment and ±0.30 co-treatment) against both H2O2 and hypoxia induced mtDNA strand breaks. This was supported by a significant (p≤0.001) reduction in the production of ROS and diminished formation of MDA with both H2O2 and hypoxic conditions compared to untreated controls.

Conclusion: This study demonstrates a promising use for Tiron as a supplement against both H2O2 and hypoxia-induced ROS in BEAS-2B cells. However, the precise mechanism of Tiron’s action requires further elucidation. The findings of this study indicate a potential use for Tiron in the treatment and prevention of pulmonary diseases associated with oxidative stress.

References:
Antioxidative response and photosynthetic performance of common fig (Ficus carica L.) leaves after short-term chilling stress

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Common fig (Ficus carica L.) is widely cultivated Mediterranean species. Such warm-climate species are adapted to elevated temperatures and are susceptible to chilling stress (0-12°C)[1]. However, occasional short chilling periods are common during growing season in temperature areas what can affect functionality of the plant[2]. The aim of this work was to investigate influence of short-term low temperature (chilling) on PSII photochemistry and antioxidative response in young, still developing leaves of common fig. Leaves were detached from the tree, acclimated at room temperature in dark for 12h and then exposed to low temperature (10°C) and low irradiation (50 µmolm⁻²s⁻¹) for 4h. Dark adapted leaves were considered as the control. Photosynthetic performance was analyzed by measuring in vivo chlorophyll fluorescence increase (JIP test). The production of H₂O₂, lipid peroxidation (TBARS) and activity of antioxidative enzymes: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and guaiacol peroxidase (GPOD) were measured as well.

Maximum quantum yield of PSII (Fv/Fm) and overall photosynthetic performance (Ptotal) decreased in leaves exposed to the chilling stress. Exposure to low temperature decreased absorption of light energy (ABS), trapping (TR0) of absorbed light energy and electron transport (ET0) further than primary acceptor (QA-) while energy dissipation (DI0) remained the same compared to the control. Moreover, reduction of the end electron acceptor (RE0) at photosystem I (PSI) also decreased after exposure to low temperature. The obstruction of photosynthetic electron transport flow is well documented reason for increased H₂O₂ production in leaves exposed to short-term chilling stress[1, 3]. In spite of increased H₂O₂ accumulation, the increase in TBARS level was not observed in the investigated leaves. Low temperatures inhibited activities of SOD and GPOD, while the CAT, APX and GR activities increased compared to the control. Our results suggests that antioxidative system was enough efficient to prevent the oxidative damage in biomembranes of fig leaves exposed to the chilling stress at 10°C.

References:
Analytical strategies for the identification and characterization of protein adducts with HNE and related compounds

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Advanced Lipoxidation Endproduct (ALEs) are modified proteins that can act as pathogenic factors in several chronic diseases, like diabetes and cardiovascular diseases [1]. These covalent adducts belong to a heterogeneous class of compounds derived from the protein adduction by reactive carbonyl species (RCS), which are generated upon lipid peroxidation. A similar class of compounds, Advanced Glycation Endproducts (AGEs), exhibit the same damaging effects as ALEs, partly due to binding to the Receptor for Advanced Glycation End products (RAGE).

Using this receptor as a stationary phase for affinity chromatography, modified proteins could be potentially entrapped and enriched from any sample, to identify and characterize the origin of the modification. Applying this innovative approach, it has been shown already that AGEs can be captured, enabling the full characterization of the adducted moieties and site of modification using a bottom-up approach [2]. In order to validate this strategy for ALEs, and with the aim to understand whether ALEs are also binder of RAGE, fully characterized ALEs were produced in-vitro, by incubating human serum albumin (HSA) with glyoxal (GO), methylglyoxal (MG), 4-hydroxynonenal (HNE), acrolein (ACR) and malondialdehyde (MDA).

The formation of ALEs was confirmed using a top-down MS approach by direct infusion on a triple-quadrupole mass spectrometer and the modifications and sites of adduction fully characterized by a bottom-up approach. The in-vitro produced ALEs were then subjected to VC1 Pull-Down relying on magnetic beads bound to VC1, the domain of RAGE necessary for binding, which can easily be separated. ALEs will be retained by VC1 and unbound protein can be easily removed, enriching ALEs in the sample. After binding, ALEs are eluted from the magnetic beads and subjected to GeLC-MS/MS to identify and localize the modification. Data obtained using this method were analysed using a targeted approach based on setting known modifications. Results obtained using VC1 Pull-Down were compared to the results of the in-solution digested ALEs and showed that ALEs containing a cyclic moiety induced by the modifications, are better retained by VC1, including pyrimidine, pyridine, pyraline and imidazolone adducts. Another observation is the binding of ALEs containing a carboxy-derivative, since these adducts exhibit a negative charge and increases the binding specificity to VC1, which has a positive charge. Semi-quantitative analysis also showed an enrichment of ALEs from VC1 Pull-Down, compared to unenriched sample. Results showed that VC1 can be used as a stationary phase to selectively enrich ALEs, depending on the structure and nature of the modification. Different applications of this technique are underway to identify and characterize ALEs and AGEs from samples of patients affected by diseases involving oxidative stress. In conclusion, we have found that besides AGEs, also ALEs are RAGE binders. ALEs involvement in the RAGE dependent proinflammatory cascade is currently under investigation.
References:
A new role for Carbonyl Reductase 1 on 4-hydroxynonenal detoxification

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4-Hydroxy-2-nonenal (HNE) is one of the main products of lipid autoxidation of unsaturated fatty acid (1). It is an highly reactive molecule and has received a particular attention for its biological activity and its role in different diseases (2).

HNE metabolism is reported to mainly occur through its conjugation with glutathione and the subsequent formation of 3-glutathionyl-4-hydroxynonenal (GSHNE) (3). This molecule is susceptible to both reductive and oxidative transformations, which occur through the action of either the NADPH-dependent action of aldose reductase or the NADP+-dependent activity of aldehyde dehydrogenase, respectively. In this context it has been identified a role of carbonyl reductase 1 (CBR1) in the detoxification of GSHNE through its oxidation to the corresponding 3-glutathionyl-nonanoic-δ-lactone (4). More recently it has been also reported the capability of the enzyme to reduce GSHNE, together with a number of glutathionylated aldehydes, to the corresponding GS-dihydroxynonane (GSDHN) (5).

Thus GSHNE, through its equilibrium between the open aldehyde form and its cyclic hemiacetal acts, with respect to CBR1, as a dual substrate. Being the two red/ox processes linked to the same red/ox cofactor (NADP+/NADPH), it turns out that a CBR1-catalyzed disproportion of GSHNE occurs.

The high catalytic efficiency of CBR1 in the GSHNE processing, would suggest this molecule as one of the main physiological substrate of the enzyme. In this contest besides contributing to detoxification processes, CBR1 may be involved in the production of a signaling molecule, GSDHN, which is reported to activate inflammatory processes mediated by NFκB (6). These results could add new relevance on the inhibition of CBR1 activity by specific molecules that could play an anti-inflammatory role.

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Modulation of the cell growth by Aloe Vera extract and hydrogen peroxide

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Reactive oxygen species (ROS), reactive nitrogen species, and their counterpart antioxidant agents are essential for physiological signaling, while imbalances between oxidants and antioxidants may provoke pathological reactions causing a range of diseases including cancer. In addition, ROS can stimulate signal transduction pathways and lead to activation of key transcription factors such as Nrf2 and NF-κB modulating cell growth and redox balance.[1]

The well-known medical plant Aloe vera contains an abundance of antioxidants, which are capable of neutralising ROS [2]. This work evaluates the effects of the genuine Aloe vera extract (AV) on human cells in vitro in respect to the toxic effects of hydrogen peroxide.

After propagating Aloe vera for 1 year in 0.5 kg plastic pots, the first fully developed leaf, fourth from the top, was harvested and subjected to biochemical tests for determination of vitamin C, carotenoids and total soluble phenolic content, while total antioxidant capacity of the plant extract was analysed using Brand-Williams method [3].

Afterwards, four different human cell lines, notably HeLa (human cervical cancer), HMEC (human microvascular endothelial cells), HaCat (human keratinocytes) and HOS (human osteosarcoma) were treated with 2 concentrations of AV for 1 hour after one hour pre-treatment with ranging concentrations of H₂O₂, thus inducing oxidative stress. The viability of the cells was determined by an MTT-based vitality assay, EZ4U (Biomedica, Vienna, Austria).

The plant extract expressed strong antioxidant capacities (1.1 mmol of Trolox eq./g FW), mostly likely due to the combined effects of its antioxidants ingredients. However, while H₂O₂ inhibited in a concentration dependent manner the growth of all cell lines except HOS, the extract of AV did not show any particular effects except a slight stimulation of the HeLa and HMEC cells. We may assume that antioxidants of the plant extract are not involved in the observed growth modifying effects on the different cell lines used. More likely, some components of the plant extract could interfere with the cellular antioxidants and redox signalling and its effects together with hydrogen peroxide. The above actions of AV confirmed that it can potentially act as relatively safe natural source of medical remedies since there were no toxic effects observed, while further studies on the mechanisms of its actions are needed.

References
Growth on HNE modified collagen induce Nrf2 in breast cancer stem cells

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Oxidative stress is an important factor in carcinogenesis. In addition, radiotherapy, chemotherapy and inflammation increase oxidative stress in tumors. Lipid peroxidation end product, a reactive aldehyde 4-hydroxy-2-nonenal (HNE), is considered to be second messenger of oxidative stress. HNE modifies the metabolism of cancer cells by interacting with proteins, lipids and DNA. Along with oxidative stress, cancer stem cells have been recognized as the crucial factor in cancer malignancy and are considered responsible for metastasis occurrence, therapy resistance and, finally, recurrence of the disease. For these reasons we examined the effect of HNE induced oxidative stress and HNE modulation of the extracellular matrix on cancer stem cells metabolism, proliferation and their antioxidative mechanisms. Collagen was used as a representative protein of extracellular matrix and was modified with HNE. Cells were plated on native and HNE-modified collagen as well as polystyrene surface, and were additionally treated with HNE every second day. Afterwards, we determined viability and proliferation with MTT and 3HT test. Antioxidant cell capacity was assessed by glutathione and catalase assay. Dot-blot analysis was performed in order to quantify Nrf2 and HNE histidine conjugates. We confirmed specificity of dot-blot findings by performing western blot analysis of Nrf2. The results suggest that the aggressive cancer stem cell phenotype is enhanced during chronic oxidative stress. Extracellular matrix alterations resulting from oxidative stress may cause an adaptation of cancer stem cells, enabling them to survive increased oxidative stress.

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New Small Molecular Weight Antioxidants and Pro-oxidants Control Melanoma Cell Proliferation and Spreading in vitro

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Melanoma, the most dangerous skin cancer, originates from the melanocytes and has a high tendency to invade neighboring tissues, and metastasize. Both antioxidant and pro-oxidant appear to be involved in modulating melanocyte transformation, melanoma progression and invasion. Consequently, potent antioxidants and pro-oxidant may prevent cell transformation and tumor progression. Skin melanoma, the most common malignancy in United States, there are 2 million cases diagnosed in US annually. Among the novel therapies there is the use of bioactive compounds, which have proven to show increase response (IRR) and overall survival (OS) rates. In fact Dacarbazine is the only FDA approved chemotherapeutic bioactive compound for melanoma treatment. We have identified eight bioactive compounds with antioxidant and pro-oxidative activities as anti-melanoma/anti-invasion agents. They were previously found to possess in vitro antioxidant or pro-oxidant activity, Compounds 1, 2, 3, 4, 5, 6, 7 and 8 were found to be most potent anti-melanoma agent. These compounds are now tested for intracellular free radical quenching and ROS producing role in skin melanoma cells in vitro and their ability to reduce proliferation and spreading.

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Antibody against low density lipoproteins (oLAb), procalcitonin and neopterin as prognostic marker during sepsis and systemic inflammatory response syndrome (SIRS)

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INTRODUCTION: Aim of this study was to evaluate the impact of lipid peroxidation during the course of Sepsis and SIRS in ICU patients. As a biomarker for lipid peroxidation we used antibodies against oxidized LDL (oLAb). Furthermore, we applied Procalcitonin (PCT) and C-reactive protein (CRP) as markers for inflammation as well as Neopterin (NPT) as a marker for macrophage activation. oLAb play an important role in diseases associated with lipid peroxidation, e.g. liver disease, autoimmunological diseases, adipositas, heart and circulatory failure as well as infectious diseases like sepsis or SIRS. The hypothesis concerning the prognostic value of oLAb in sepsis indicates that increasing titers seem to be wholesome in contrast to decreasing values, which seem to show overwhelming lipidperoxidation.

PATIENTS AND METHODS: 25 patients with verified sepsis (n=13; 7 survivor, 6 non-suvivor) and SIRS (n=12; 7 survivor, 5 non-survivor). Patients stayed 48 hours at the ICU under the criteria according to Roger C. BONE and Jukka TAKALA. Biomarkers were determined every day, as clinic score we used APACHE II Score during the first 24 hours. In addition, we determined Cytokines (i.e. IL-1, IL6 and IL-8).

RESULTS: Surviving patients showed significant increasing oLAb titres (p<0,001), as well as significant decreasing levels of PCT, CRP, Neopterin and Interleukins. In non surviving patients we observed a decreasing oLAb titre (p<0,05) and a constant increase of inflammatory parameters (CRP, PCT, NPT).

CONCLUSION: Even though the number of patients was rather small we conclude that oLAb seems to be an indicative parameter for the follow up of sepsis and SIRS besides general inflammatory markers like CRP, PCT and NPT. Beyond that, the use of antioxidant drugs like Pentoxifylin, Ascorbic acid, Selenium, Alpha-Tocopherol as well as Coenzyme Q10, Allopurinol and N-Acetyl-cystein might be useful to reduce lipid peroxidation and to improve the chance to survive. Patients receiving parenteral or enteral nutrition or via infusion of those substances would show a better outcome.
Anti-inflammatory action of polyphenols from grape seed extracts in primary human endothelial cells

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Background: Endothelial cells lining the inner surface of blood vessel walls play a key role in tissue homeostasis. These cells embody an active organ which selectively regulates permeability barrier and vasomotoric tone to maintain tissue homeostasis. Endothelial dysfunction in response to pathogens, physical, chemical and nutritional (hyperglycemia, hyperlipidemia) cues results in the production of pro-inflammatory and pro-thrombotic products, which are critically involved in the initiation and progression of atherosclerosis, cardiovascular disease and numerous other age-related diseases. The consumption of fruits and vegetables plays a role in preventing disease and the beneficial effects are allied to bioactive dietary polyphenols (PP) present in plant-derived foods which have antioxidant properties. Here we investigated the effect of PPs extracted from grape seeds on pro-inflammatory gene expression in primary human endothelial cells (HUVEC) in vitro.

Method: TNF-induced immediate-early gene expression of adhesion molecule E-selectin (SELE), endothelial nitric oxide synthase (eNOS, NOS3) and prostaglandin G/H Synthase 2 (COX2) upon simultaneous or subsequent to a 1h preincubation with grape seed extracts from various sources were measured using real-time PCR, gallic acid and ferulic acid served as respective controls. SELE protein-expression and IkBα-degradation were measured by in-cell-Western. PP-content of grape-seed extracts were measured by a commercially available colorimetric assay.

Results: We show that grape seed extracts differentially modulate TNF-induced pro-inflammatory SELE, COX2 and NOS3 gene expression, dependent of on experimental conditions and PP-content.

Conclusion: Grape seed extracts exert anti-inflammatory properties on primary human endothelial cells in vitro.
β-Carotene and its biological oxidation products: are they good modulators of pro-oxidant reactive species in human blood and neutrophils assay systems?

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β-carotene is the most abundant carotenoid found in the diet and human tissues [1]. High ingestion of carotenoids has been associated with low incidence of degenerative diseases and it has been mainly attributed to their ability to scavenge physiologically relevant reactive species. In humans, β-carotene is metabolized by oxidative cleavage into trans-β-apo-8’-carotenal and β-ionone [2]. Therefore, it is crucial to disclose the potential antioxidant effects of β-carotene and its oxidation products.

In this sense, the main aim of this work was to elucidate the potential antioxidant effects of β-carotene, trans-β-apo-8’-carotenal and β-ionone, at relevant physiologic concentrations. The two selected biological systems were human blood, a complex matrix that most approximately mimic the in vivo biologic environment; and human neutrophils, the first line of defense cells utterly implicated in the immune and inflammatory responses. Two fluorescent probes were used in both matrices: 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) that unspecifically detects hydrogen peroxide (H2O2), hydroxyl radical, nitric oxide and peroxynitrite anion, and amplex red (AR) that specifically detects H2O2.

In both matrices, none of the tested carotenoids showed any ability to stimulate reactive species production. As so, they do not seem to be pro-oxidants. For the study of the antioxidant potential, phorbol 12-myristate 13-acetate was used to stimulate reactive species production by human blood cells. Interestingly, in what concerns the human blood matrix, none of the tested carotenoids (0.3 - 49 µM) showed antioxidant activity. In what respects human neutrophils, also none of the tested compounds (0.3 - 100 µM) was able to inhibit DCFH-DA oxidation; trans-β-apo-8’-carotenal was the only one that showed a slight ability to inhibit AR oxidation, suggesting some modulation of H2O2 production.

The obtained results show that β-carotene and its oxidation products, at biological relevant concentrations, did not provide acute pro- or antioxidant activity, in the studied experimental conditions. These results indicate that carotenoids and/or its oxidation products probably require different types of exposure on biological systems to enable a better incorporation in the lipidic system opening a window to new studies with this kind of compounds.

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References:
A mass spectrometry approach for the identification and localization of lysozyme modifications by acrolein

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Lipids containing polyunsaturated fatty acids are primary targets of oxidation, leading to the formation of a variety of reactive products (1). The new oxidised products are generated with different number of oxygen molecules bound to the acyl chain, increasing the lipid molecular weight. However, the alkoxy radicals can generate short-chain oxidation products including short-chain aldehydes, which can covalently modify proteins in a process called lipoxidation (2). Acrolein, with only three carbons, is the shortest alkenal identified as a short-chain oxidation product. It is also by far the strongest electrophile and therefore the most reactive, especially with thiol groups on proteins (3). These oxidative post-translational modifications influence cell behaviour and can be involved in inflammatory diseases (4). The exact nature of many of these adducts, and their relationship with cellular effects are still unclear. There is also a need to develop sensitive mass spectrometry (MS) methods that are well-adapted for the identification of these adducts in complex biological systems. The aim of this work was to develop a mass spectrometry approach for the analysis of protein-aldehyde adducts. Lysozyme from chicken egg white was used to investigate the formation of short-chain aldehyde-containing lipoxidation products. The protein was first reduced with dithiothreitol to break disulphide bounds between cysteine residues, generating free thiols available for modification. Acrolein was then added to modify the reduced protein. The protein-aldehyde adducts formed were stabilized by reduction with sodium borohydride and identified using mass spectrometry to analyse the intact protein and tryptic digests. Analysis of intact acrolein-modified lysozyme showed that multiple sites (up to 8) could be modified. All modifications were found to be Michael adducts, increasing the protein mass by 58 Da per adduct. Analysis of tryptic digests allowed the localization of the adducts to specific amino acid residues. Six cysteine and two lysine residues were shown to be modified, which corroborates observation of up to 8 acrolein adducts per protein in the intact protein analysis. By the same proteomic approach it was possible to identify amino acid-specific fragmentations that may be helpful in the identification of specific acrolein adducts. In summary, this study can be seen as a model testing mass spectrometry for the analysis of protein-aldehyde adducts. The combined use of direct infusion and LC-MS/MS methods helped to identify the type, the number and the location of acrolein modifications in lysozyme. This shows that MS methods provide a powerful tool for the evaluation of these modifications and further aldehydes are now being investigated. Potentially, this MS approach can be applied to discover biomarkers of adductions in cells and tissues under pathophysiological environments.
Altered redox homeostasis and signaling in Cerebral Cavernous Malformation disease: towards a complex but unifying pathogenic mechanism and therapeutic implications

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Cerebral Cavernous Malformation (CCM) is a major cerebrovascular disease of genetic origin affecting 0.3-0.5% of the population and still awaiting therapies other than neurosurgery. It is characterized by abnormally enlarged and leaky capillaries, which predispose to seizures, neurological deficits and intracerebral hemorrhage (ICH), and may occur sporadically or is dominantly inherited with incomplete penetrance and variable expressivity. Three disease genes have been identified, \textit{KRIT1} (\textit{CCM1}), \textit{CCM2} and \textit{PDCD10} (\textit{CCM3}), whose loss-of-function mutations are major pathogenic determinants, accounting for the main phenotypic hallmarks of CCM disease, including destabilization of endothelial cell-cell junctions and increased vascular permeability [1]. However, accumulating evidence in animal models clearly demonstrate that homozygous loss of CCM genes is not fully sufficient to cause CCM lesion formation and disease progression, suggesting the necessary contribution of additional determinants, including microenvironmental stress events [1]. Indeed, the clinical behavior in individual patients, including development of numerous and large lesions, and risk of ICH, remains highly unpredictable, while novel pharmacological strategies are particularly needed to limit disease progression and severity in susceptible individuals [1].

Useful insights into innovative approaches for CCM disease prevention and treatment are emerging from a growing understanding of the biological functions of the three known CCM proteins. Previously, we found that CCM proteins, including KRIT1, play an important role in maintaining intracellular redox homeostasis through the modulation of master regulators of ROS production/detoxification and cell responses to oxidative stress, thereby limiting altered redox signaling and oxidative damage, and preserving cellular resistance to oxidative stress [1-3]. Consistently, recently we demonstrated that KRIT1 loss-of-function causes upregulation of NADPH oxidase-mediated redox signaling, leading to enhanced endothelial cell sensitivity to oxidative stress and inflammation, and decreased microvessel barrier function, further suggesting that altered redox signaling and oxidative stress contribute to CCM pathogenesis [4]. Moreover, preliminary results indicate that these events involve also carbonyl compounds generated through the lipid peroxidation process, and a sustained upregulation of Nrf2, suggesting a complex but unifying pathogenic mechanism that reconciles both the pleiotropic functions of CCM proteins and the distinct therapeutic approaches proposed so far. In addition, we identified genetic modifiers influencing disease severity, including polymorphisms in genes related to inter-individual variability in susceptibility to oxidative stress [5].
Taken together, our findings point to a major role for altered redox signaling in CCM pathogenesis, and indicate that inter-individual variability in cell responses to oxidative stress may impact disease onset, progression and severity, suggesting novel preventive and therapeutic approaches.

References:
The formation of 4-HHE and 4-HNE during cooking and in vitro gastroduodenal digestion of meat and fish

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Meat and fish have varying contents of haem-Fe and polyunsaturated fatty acids (PUFAs) and are hence sensitive to oxidation during its preparation and gastrointestinal digestion. However, studies comparing the sensitivity of meat and fish to oxidation during digestion are scarce. In this experiment, we measured 4-HHE and 4-HNE following the preparation and in vitro digestion of a red meat product (pork), white meat (chicken), a fatty fish (salmon) and a lean fish (cod). Secondly, we investigated if the formation of these compounds in pork is inhibited by nitrite-curing (nitrite salt added at 20 g/kg), or by seasoning with Provençal herbs (added at 10 g/kg). All meats and fish were purchased as fresh as possible and cooked for 70 min in a warm water bath at 70°C. Fatty acids (FA) were analyzed by GC according to Raes et al. (2001). In vitro gastrointestinal digestion was performed with 5 replicates per sample according to Van Hecke et al. (2014). Following their reaction with cyclohexanedione, 4-HHE and 4-HNE in cooked meats and digests were measured by HPLC. The salmon had the highest contents of PUFA (4.43 g/100g), with approximately equal contributions of n-3 and n-6 PUFA. In contrast, chicken and pork had 9.5- to 13-fold lower levels of n-3 PUFA (0.15 g/100g) than n-6 PUFA (1.43 and 1.95 g/100g respectively), whereas n-3 PUFA were the most abundant FA in cod (0.27 g/100g), amounting 6-fold higher than the n-6 PUFA (0.04 g/100g). Of all meats and fish prior to digestion, salmon had the highest 4-HHE levels (8.34 ± 0.97 AUC×10^3/g) followed by pork, chicken and cod (2.39 ± 0.14, 1.89 ± 0.15 and 1.09 ± 0.41 AUC×10^3/g respectively). In contrast, 4-HNE was highest in pork (4.72 ± 0.52 AUC×10^3/g) followed by chicken, and the lowest values were detected in salmon and cod (1.86 ± 0.05, 0.51 ± 0.13 and 0.37 ± 0.27 AUC×10^3/g respectively). Following in vitro gastrointestinal digestion, salmon digests contained the highest levels of 4-HHE (23.5 ± 3.1 AUC×10^3/mL) with 4-HNE concentrations among the lowest (1.93 ± 0.29 AUC×10^3/mL). In contrast, pork digests contained the highest levels of 4-HNE (11.1± 1.6 AUC×10^3/mL) with 4-HHE concentrations among the lowest (3.17 ± 0.13 AUC×10^3/mL). Compared to pork, chicken digests had 30% less 4-HNE and approximately equal 4-HHE concentrations (2.80 ± 0.29 AUC×10^3/mL). Compared to pork and chicken, cod digests had higher 4-HHE levels (5.77 ± 0.56 AUC×10^3/mL) with negligible 4-HNE concentrations (0.69 ± 0.02 AUC×10^3/mL). Both seasoning of the pork with Provençal herbs or nitrite-curing decreased 4-HHE (5-8-fold respectively) and 4-HNE (9-14-fold respectively) levels in the meat prior to digestion. Also after digestion, 4-HNE levels remained low (0.59 ± 0.28 and 0.42 ± 0.08 AUC×10^3/mL respectively) and 4-HHE was below the detection limit. This study showed 4-HHE to be present in higher concentrations in fish digests, whereas meat digests contained more 4-HNE, which formation can be inhibited by antioxidants.
References:
Amaranth oil in prevalent pulmonary arterial hypertension: changes in fatty acid panel and products of lipid peroxidation

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Pulmonary arterial hypertension (PAH) is a severe illness associated with the dismal prognosis. Oxidative stress (OS) plays an important role in the pathobiology of PAH and its signs are not eliminated with the long-term treatment by the PAH specific medication -sildenafil (SLD) [1,2]. Use of the compounds that improve regulation of the redox process [3] may offer benefit for the management of PAH.

We studied profile of polyunsaturated fatty acids derived from phospholipids and main products of lipid peroxidation in the serum of patients with PAH, who received long-term treatment with sildenafil. Patients were divided into two groups: group 1 continued treatment with SLD; group 2 in addition to SLD received Amaranth oil (AmO) (1ml per 60 kg of body weight) for one months. Group 3 consisted of healthy volunteers who also received AmO in the above-mentioned dosing.

Prevalent PAH patients were characterized by some decrease of linoleic acid (LA), arachidonic acid (AA), docosahexaenoic acid (DHA) and significant lowering of eicosapentaenoic acid (EPA) level. Interestingly that the level of gamma-linolenic acid (GLA, omega-6) increased 5.5-fold comparing to control. With continuation of SLD therapy deficit of mentioned fatty acids, especially EPA, was further progressing, however, in subjects treated with SLD only this decrease was less prominent. High levels of GLA were lowering in both groups but remained to be significantly elevated comparing to control.

Supplementation with AmO reduced initially elevated levels of hydroxynonenal (HNE) by 13 %, while in patients on SLD therapy only 40% increase of HNE was observed. The level of oxononenal (ONE), which initially was 2-fold elevated, was normalized with treatment in both groups, while the levels of hydroxyhexanal and malonic dialdehyde lowered insignificantly only in group 1. Worsening of OS signs with SLD treatment in PAH was further confirmed by GSH/GSSG ratio, which was 4.95 before treatment and 10.3, 3.0 after intervention in group 1 and 2 respectively. Mild prooxidant action of AmO was confirmed in group 3 (healthy volunteers) which showed increase in phospholipid derived fatty acids (LA, AA, GLA, EPA by 20%, 54%, 20% and 103% respectively). This effects may promote development of the optimal profile of lipid-derived regulatory molecules involved in management of inflammation, immune response, and in general improved OS resistance. In addition to changes in the lipid profile, increases in HNE (by 38%), ONE (by 65%) and 2-fold lowering of GSH/GSSG were observed, which may be suggestive about the involvement of these molecules in the redox signalling.
Changes in the profile of the fatty acids and products of lipid peroxidation in PAH patients and healthy volunteers are suggestive that maintaining mild prooxidant activity (hormetic reaction) may offer some benefit in the long-term management of severe chronic diseases such as PAH. The mechanisms involved in the development and maintenance of this processes need further clarification.

References:
The workload of heavy workers is associated with increased low-grade inflammation, emotional and oxidative stress

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Background: There is limited knowledge with regard to oxidative stress in different working categories e.g. between heavy workers and desktop workers. Therefore, the aim of this study was to evaluate the antioxidative as well as oxidative stress profile exactly in these occupational groups.

Methods: We enrolled 79 male subjects i.e. 27 employees as desktop workers (age 38.8 ± 9.1) and 52 heavy workers in a slaughterhouse (age 40.8 ± 8.2). Blood was drawn in the morning after an 8-hour work shift from an antecubital vein. Total antioxidant capacity (TAC), uric acid, total polyphenols (PPm) and endogenous peroxidase-activity (EPA) were determined to evaluate the antioxidative potential while total peroxides (TOC), malondialdehyde (MDA), myeloperoxidase (MPO), were used as oxidative stress biomarkers. In addition, we analysed adrenocorticotropic hormone (ACTH), hsCRP and interleukin-6 (IL-6) and the brain-derived neurotrophic factor (BDNF) as biomarkers for inflammation and emotional stress.

Results: These two working groups show significant differences between BMI (p=0.0026). Furthermore, we observed significant increased concentrations for TOC (p<0.001), TAC (p=0.026), ACTH (p<0.001) and hsCRP (p=0.002) in the heavy workers group. In contrast, EPA, BDNF (p<0.001) and polyphenol concentrations were significantly higher in desktop workers. No differences were found for uric acid, MDA, MPO and IL-6.

Conclusion: These results indicate a distinct association between occupational category and prevalence for oxidative stress. Desktop workers were at an advantage over heavy workers for each biomarker indicating a stress situation except for the total antioxidant status. It is a fact that uric acid is an excellent antioxidant and becomes increased during oxidative stress. Thus, we determined uric acid and observed a high correlation to TAC, which is an explanation for this paradox – in spite of the fact, that there was no significant difference for uric acid concentrations between both groups. Although classical biomarkers as MDA, MPO or IL-6 were insensitive to indicate differences in these two working groups, we still achieved significant differences with sensitive and high-throughput methods to the disadvantage for heavy workers, disclosing the linkage between low-grade inflammation emotional and oxidative stress. Further studies are needed to verify these preliminary results and to complement different working groups.

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