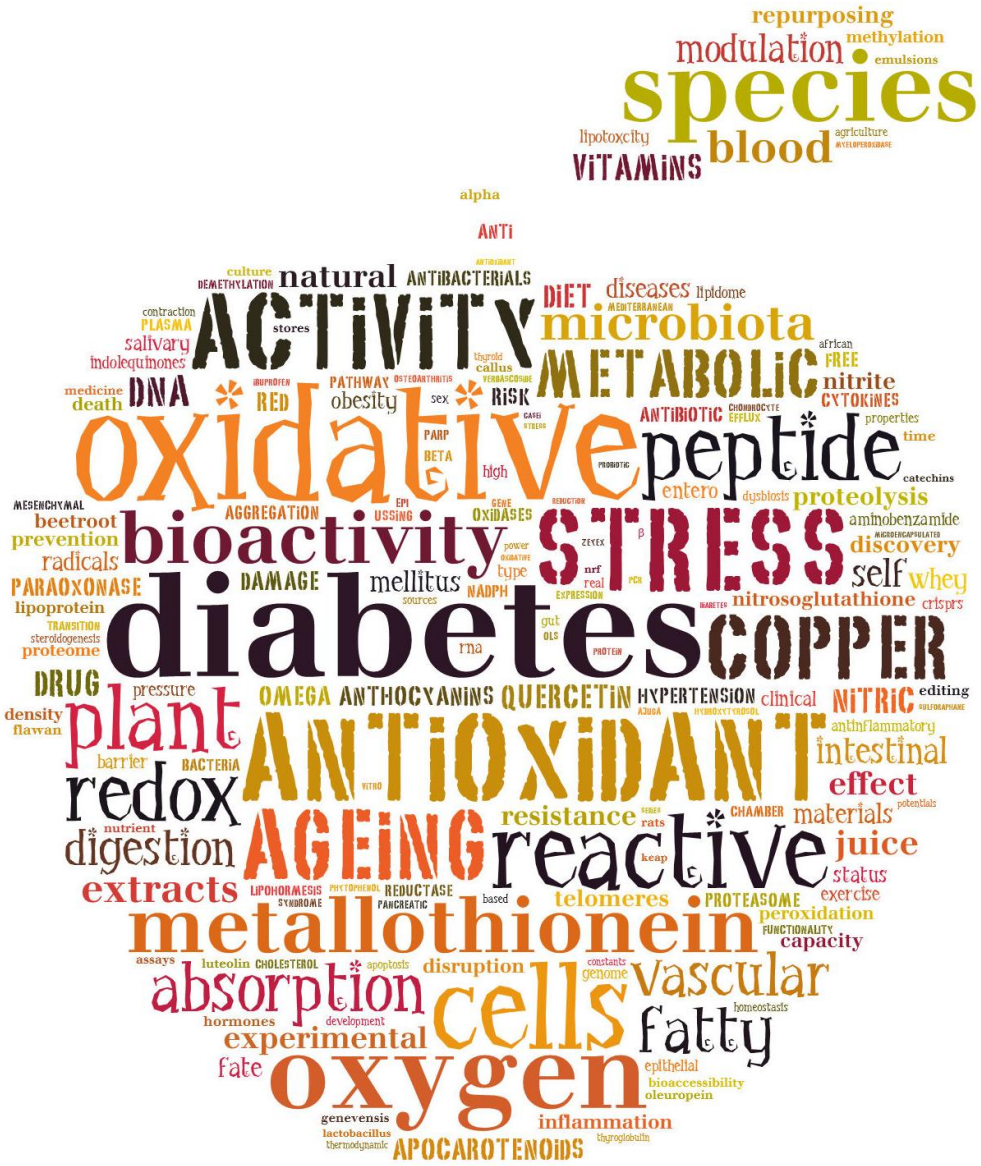
The NutRedOx COST Action CA16112 & the 6th NutriOx Atelier 2017

Preventing Age-Related Diseases with Redox-Active Compounds: a taste of controversy?

Strasbourg, ECPM, University of Strasbourg
27 – 29 September 2017



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Local Organizing Committee

Dr Mourad Elhabiri, University of Strasbourg, Strasbourg, France
Dr Caroline Gaucher, University of Lorraine, Nancy, France
Professor Claus Jacob, University of Saarland, Saarbrücken, Germany
Professor Mustapha Cherkaoui Malki, University of Burgundy, Dijon, France
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Vrushali Khobragade, University of Strasbourg, Strasbourg, France
Didier Boettger, University of Strasbourg, Strasbourg, France
Nathalie Bancod, University of Burgundy, Dijon, France

Secretariat

Mourad Elhabiri, UMR 7509 CNRS-University of Strasbourg, European School of Chemistry, Polymers and Materials, 25, rue Becquerel 67200 Strasbourg, France
Email: elhabiri@unistra.fr
Phone: (+33) 3 68 85 26 85
Fax: (+ 33) 3 68 85 27 42

and

Nathalie, Bancod, BioPeroxIL, EA7270, Laboratoire de Biochimie du Peroxysome, Inflammation & Métabolisme Lipidique, University of Burgundy, 6, Boulevard Gabriel, 21000 Dijon, France
Email: nathalie.bancod@u-bourgogne.fr
Phone: (+33) 3 80 39 62 37
Fax: (+33) 3 80 39 62 50

Congress website: http://blog.u-bourgogne.fr/cost-nutredox/events/event/cost-nutredoxnutriox-joint-meeting-strasbourg/
Contact: Dr Pierre Andreoletti
Email: pierre.andreoletti@u-bourgogne.fr

Foreword

On behalf of the International Scientific Committee and of the Local Organization Committee, it is our great pleasure to cordially welcome you at the University of Strasbourg – Alsace/Région Grand Est in Strasbourg, France to attend the joint scientific meeting "NutRedOx COST Action CA16112 & Postgraduate Training Atelier "NutriOx" 2017". This book gathers the abstracts of the 2 keynote lectures, 31 oral communications, and 26 poster communications that will be presented by about 120 participants (coming from 36 countries) during the two-day meeting held at the European School of Chemistry, Polymers and Materials (ECPM) of the University of Strasbourg. Besides the University of Strasbourg (Mourad Elhabiri), this meeting will be organized in cooperation with the University of Lorraine (Caroline Gaucher), the University of Saarland (Claus Jacob) and the COST Action CA16112 (Mustapha Cherkaoui-Malki and Agnieszka Bartoszek).

The NutriOx Network brings together emerging junior scientists from the region and experts from various disciplines, ranging from analytical and organic chemistry to medicinal, pharmaceutical, nutritional or agricultural sciences to cite a few. This long-term network aims to address questions of nutrition and health. Every year, the NutriOx network runs a workshop for early stage and experienced scientists in the form of an open and informal "Atelier" that is supported by the French-German University. In the tradition of the previous NutriOx workshops (University of Saarbrucken in September 2012, University of Burgundy in September 2013, University of Metz in October 2014, Hospital Kirchberg in Luxembourg in November 2015 and University of Kaiserslautern in September 2016), this Postgraduate Training Atelier 2017 will undoubtedly provide an international forum for early stage scientists, graduate and PhD students as well as Post-Docs.

Exceptionally, this 6th NutriOx Atelier 2017 will be organized jointly with the NutRedOx COST Action annual meeting (CA16112), so that the participants can benefit from the advantages of these two intimately related networks. The NutRedOx COST Action (CA16112) entitled "*Personalized nutrition in aging society: redox control of major age-related diseases*" started in March 2017 and is led by Prof. Mustapha Cherkaoui Malki (University of Burgundy, France) and co-led by Prof. Agnieszka Bartoszek (Gdansk University of Technology, Poland). Its scientific items are closely linked to the NutriOx network. The NutRedOx COST Action is gathering experts from more than 35 European and Mediterranean countries, and from different disciplines that are involved in the study of biological redox active food components that are relevant to the ageing organism, its health, function and vulnerability to diseases.

This joint meeting entitled *"Preventing Age-Related Diseases with Redox-Active Compounds: a taste of controversy?* will focus on up-to-date topics concerning nutrichemicals and health with a particular focus on age-related diseases. The title of this meeting is deliberately challenging and will focus on redox active compounds, with special themes such as their beneficial (or deleterious) use in nutrition for the prevention and eventually treatment of age-related diseases. Nutrition and Ageing is undoubtedly a matter of substance in our society with the raised perennial question: how to stay healthy and prevent disease in a near-natural manner? Maybe "simply" in our daily nutrition? But with which compounds? From which sources? Furthermore, are redox-active natural compounds or nutrients (*i.e.* there are many in our daily diets) beneficial or deleterious for the human health particular for the elderly? The answers are not straightforward and the understanding of diets and dietary components effects on ageing and age-related diseases requires deeper investigations

through complementary pluri- and interdisciplinary approaches emanating from analytical, chemical, biochemical and pharmacological, neuroscience, cardiovascular medicine, and nutritional sciences. Many hot-topics have to be studied such as the effects and the mechanisms of actions on cell function of natural or plant-derived constituents (*i.e.* vitamins, minerals and other micronutrients, as well as non-nutrients) and the potential of these nutrients to modulate environmental and genetic factors linked to age-related diseases.

We are wishing to all of you the best profit from your participation at this joint NutRedOx COST Action CA16112 meeting & NutriOx Atelier 2017 and a pleasant stay in the historical city of Strasbourg and in the Alsace region.

Sincerely yours,

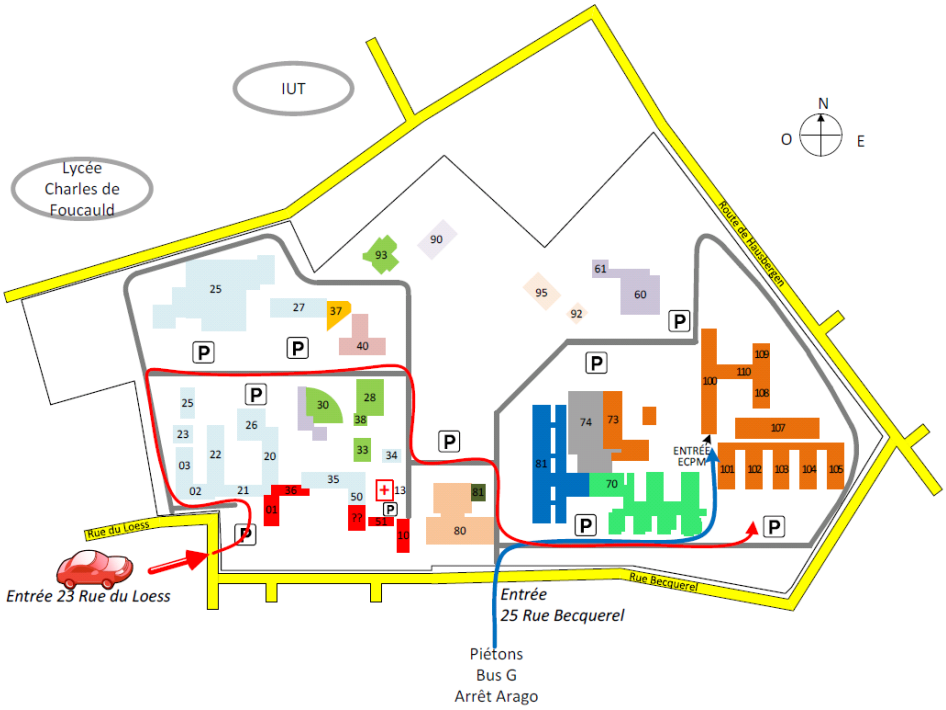
The Organisers:

Dr. Mourad Elhabiri (Laboratoire de Chimie Moléculaire, UMR 7509 CNRS-University of Strasbourg, Strasbourg, France)
Dr. Caroline Gaucher (CITHEFOR, EA 3452, University of Lorraine, Nancy, France)
Pr. Claus Jacob (University of Saarland, Saarbrucken, Germany)
Pr. Mustapha Cherkaoui-Malki (Laboratoire Bio-PeroxIL, EA 7270, University of Burgundy, Dijon, France)

NutRedOx COST Action CA16112 & Postgraduate Training Network "NutriOx" 2017
Strasbourg, 27 – 29 September 2017

Venue: Amphi Lambla & Wurtz room, European School of Chemistry, Polymers and Materials (ECPM) Strasbourg, 25 rue Becquerel 67200 Strasbourg http://ecpm.unistra.fr/en/contact/access-map/
Date: 27 & 28 September 2017

MC member meeting: Amphi Lambla & Wurtz room, European School of Chemistry, Polymers and Materials (ECPM) Strasbourg, 25 rue Becquerel 67200 Strasbourg
http://ecpm.unistra.fr/en/contact/access-map/
Date: 29 September 2017



From downtown & Gare de Strasbourg railway station

Cars and pedestrians access: 23, rue du Loess

Pedestrian access (keycard access only from 9:30 am to 05:00 pm): 25, rue Becquerel

From Gare de Strasbourg railway station (15 min) - Take the bus line G "Espace Européen de l'Entreprise" and leave at "Arago" stop.

From 7:30 to 9:30 am and from 5.00 to 7:00 pm, you can access the ECPM from 25 rue Bequerel.

Between 9:30 am and 5:00 pm, you will need to access campus from the left, 23 rue du Loess, and to walk through the campus following the road signs.

From Strasbourg – Entzheim Airport (45 min) - Take the shuttle train to Strasbourg railway station (http://www.sncf.com/ - every 10 minutes)

Take the bus line G "Espace Européen de l'Entreprise" and leave at "Arago" stop.

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Grateful acknowledgments to:

NutRedOx COST Action CA16112 & Postgraduate Training Network "NutriOx" 2017
Strasbourg, 27 – 29 September 2017

CONFERENCE PROGRAM

Wednesday 27. September 2017

8:00-8:40 Arrival and registration, coffee

8:40-9:00 Welcome and Opening remarks,

Dr. Mourad Elhabiri (Unistra) & Dr Caroline Gaucher (U. Lorraine) & Prof. Claus Jacob (U. Saarland) – NutriOx

Prof. Mustapha Cherkaoui Malki (U. Burgundy) & Prof. Agnieszka Bartoszek (U. Gdansk) – NutRedOx COST Action CA16112

Session 1: Isolation, (Bio)characterization, Analysis and Effects of Antioxidants

Chairman Prof. Claus Jacob

9:00 -9:40 *1st keynote lecture*: Prof. Peter Faller (U. Strasbourg, France) **The production of reactive oxygen species by Cu-amyloid-β: mechanism, inhibitors and relevance to Alzheimer's**

9:40 -10:00 *Communication 1*: Dr. Svetlana Dinić (Department of Molecular Biology, Institute for Biological Research, University of Belgrade, Belgrade, Serbia) **Natural products with antioxidant activities in diabetes treatment**

10:00 - 10:20 *Communication 2*: Prof. Torsten Bohn (Luxembourg Institute of Health, Luxembourg) **Anti-inflammatory properties of carotenoids – an overview**

10:20 - 10:40 *Communication 3*: Muhammad A. Farooq (U. Strasbourg, France). **The omega-3 EPA:DHA 6:1 formulation improves ageing-related blunted endothelium-dependent relaxations and increased contractile responses in the mesenteric artery: role of oxidative stress and cyclooxygenases**

10:40-11:10 Poster session and networking + Coffee Break

Chairman Prof. Josep Antoni Tur Mari

11:10 - 11:30 *Communication 4*: Prof. Elke Richling (U. Kaiserslautern, Germany) **Antioxidative effects of a fruit juice rich in anthocyanins in humans**

11:30 – 11:50 *Communication 5*: Prof. Bojana Vidovic (Department of Bromatology, Faculty of Pharmacy, University of Belgrade, Serbia University of Belgrade, Serbia) **Effect of different nutraceuticals on paraoxonase 1 enzyme activity and oxidative stress status in middle-aged subjects**

11:50 – 12:10 *Communication 6*: Prof. Ileana Antohe (University of Medicine and Pharmacy "Grigore T Popa"" Iasi, Roumania) **Natural polyphenols' antioxidant intervention in a murine experimental model of *Diabetes mellitus***

12:10 – 12:30 *Communication 7*: Alberto Corrochano (Teagasc Food Research Centre, Moorepark, Fermoy, Co.Cork, Ireland) **Antioxidant activity of whey protein: survival during gut transit and bioavailable to target cells?**

12:30 - 12:50 *Communication 8*: Andreia Gomes (Instituto de Biologia Experimental e Tecnológica, Estação Agronómica Nacional, Oeiras - Portugal) **Daily consumption of a (poly)phenol enriched diet prevents death of hypertensive rats: exploring the role of gut microbiota**

12:50–14:20 Poster session and networking + Lunch

Session 2: Nutrition & Health

Chairman Prof. Agnieszka Bartoszek

14:20-15:00 *2nd keynote lecture*: Prof. Eric Marchioni (U. Strasbourg, France) **Analysis of bioactive compounds in local and traditional foods. Application to diabetes and their complications**

15:00-15:20 *Communication 9*: Prof. Wim Vanden Berghe (University of Antwerp, Belgium). **Characterisation of chemosensitizing effects of the electrophilic steroidal kinase inhibitor withaferin A in therapy resistant multiple myeloma**

15:20-15:40 *Communication 10*: Prof. Norbert Latruffe (University of Burgundy & Head of the NMS association) **Presentation of the Mediterranean diet and health association. A link with NutriOx/NutRedOx purposes**

15:40-16:00 *Communication 11*: Prof. Armen Trchounian (Yerevan State University, Armenia) **Armenian plants and their callus cultures in biotechnology and medicine: novel results and future study**

16:00-16:30 Poster session and networking + Coffee Break

Chairman Prof. Elke Richling

16:30-16:50 *Communication 12*: Prof. Claus Jacob (University of Saarland, Germany) **Redox active natural products: a fountain of inspiration and inexhaustible well of well-being**

16:50-17:10 *Communication 13*: Prof. Niki Chondrogianni (Institute of Biology, Medicinal Chemistry & Biotechnology, Athens, Greece) **Proteasome activators in our diet: a promising strategy against aging and aggregation**

17:10-17:30 *Communication 14*: Cristina Bouzas (University of Balearic Islands, Spain) **Dietary antioxidant intake and physical activity level among metabolic syndrome elderly patients: preliminary evidence in the Balearic Islands subgroup of the PREDIMED-PLUS trial**

17:30-17:50 *Communication 15:* Manuela Abbate (University of Balearic Islands, Spain) **An energy restricted Mediterranean diet does not compromise dietary antioxidant intake: preliminary evidence in the Balearic Islands subgroup of the predimed-plus trial**

17:50-18:10 *Communication 16*: Prof. Hafida Merzouk (University of Tlemcen, Algeria) **Effects of vitamins on *in vitro* lymphocyte proliferation, cytokine release and oxidant/antioxidant status in obese aged subjects: impact on telomere length**

18:10-18:30 *Communication 17*: Dr. Caroline Gaucher (University of Lorraine, France) **Thiol based nitric oxide donors for cardiovascular diseases treatment**

20:15 Conference Dinner

Thursday 28. September 2017

Session 3: Biological Activity, Intracellular effects and nutrition

Chairman Dr. Linda Giblin

9:00-9:20 *Communication 18*: Prof Gerald Thiel (U. Saarland, Germany) **Identification of the c-Fos and IL8-encoding genes as delayed response genes following resveratrol stimulation**

9:20-9:40 *Communication 19*: Prof. Paul G. Winyard (University of Exeter Medical School, Main Medical School Building, Exeter, U.K.) **Functional effects of dietary inorganic nitrate in humans**

9:40-10:00 *Communication 20*: Justine Bonetti (University of Lorraine, France) **Intestinal absorption of nitric oxide donors**

10:00-10:20 *Communication 21*: Romain Schmitt (University of Lorraine, France) **S-nitrosoglutathione and intestine barrier integrity on an *ex vivo* rat model of LPS-induced inflammation**

10:20-11:00 Poster session, networking and coffee break

Chairman Dr. Elisabeth Davioud-Charvet

11:00-11:20 *Communication 22*: Prof. Shlomo Sasson (Faculty of Medicine, The Institute for Drug Research, The Hebrew University of Jerusalem, Israel) **Lipid peroxidation, lipohormesis and lipotoxicity in insulin secreting beta cells**

11:20 – 11:40 *Communication 23*: Prof. Henrik E. Poulsen (Bispebjerg Frederiksberg Hospital, University of Copenhagen, Copenhagen, Danmark) **Evidence for accelerated ageing in type 2-diabetes: RNA oxidation predicts survival in newly diagnosed as well as in manifest type 2-diabetes**

11:40 – 12:00 *Communication 24*: Prof. Carsten Carlberg (School of Medicine, Institute of Biomedicine University of Eastern Finland, Kuopio, Finland) **Nutrigenomics of vitamin D: the personalized vitamin D response index**

12:00- 12:20 *Communication 25*: Esma Yagdi (LBMCC - Hopital Kirchberg, Luxembourg) **Autophagy modulates survival during mitotic arrest by involving p62 protein in colon cancer**

12:20-14:00 Lunch, poster session & coffee

Chairman Prof. Mustapha Cherkaoui Malki

14:00-14:20 *Communication 26*: Prof Mohamed Zaibi (School of Science and Postgraduate Medicine, The University of Buckingham, Buckingham, United Kingdom) ***Leptadenia hastata* leaf extracts reduce bodyweight gain and improve insulin sensitivity In two animal models of obesity and insulin resistance**

14:20-14:40 *Communication 27*: Dr Barbora Orlikova (LBMCC - Hopital Kirchberg, Luxembourg) **Atypical degradation of PARP-1 and necrotic cell death mediated by methylated indolequinone in chronic myeloid leukemia cells is prevented by new off target function of 3-aminobenzamide**

14:40-15:00 *Communication 28*: Prof. Kyriakos E. Kypreos (University of Patras School of Health Sciences, Department of Medicine, Pharmacology Laboratory, Rio Achaias, Greece) **HDL protein composition as a modulator of lipoprotein functionality: evidence from clinical cases and animals studies**

15:00-15:30 Poster session, networking and coffee break

Chairman Prof. Nina Hermans

15:30-15:50 *Communication 29* Melita Vidakovic (Institute for Biological Research "Siniša Stanković", University of Belgrad, Serbia) **Cellular reprogramming *via* Epi-CRISPRs-induced targeted DNA methylation**

15:50-16:10 *Communication 30*: Prof. Mustafa Atalay (Institute of Biomedicine, Physiology, University of Eastern Finland, Kuopio, Finland) **The protective role of metabolic antioxidant alpha lipoic acid and exercise training in diabetes**

16:10-16:30 *Communication 31*: Dr. Isabel Moreno-Indias (Biomedical Research Institute of Malaga , University Hospital of Malaga, Malaga, Spain) **Gut microbiota as a central mediator of the metabolic status of the host**

16:30-17:00 Discussion "NutriOx", feedbacks and future actions - Closing: Dr. Mourad Elhabiri (Unistra), Dr. Caroline Gaucher (U. Lorraine) & Prof. Claus Jacob (U. Saarland)

17h10 -18h10 Core Group Meeting **CA16112**

Friday 29. September 2017

The program will be sent by Prof. Mustapha Cherkaoui Malki **CA16112 (MC Members only)**

POSTER COMMUNICATIONS

P1) Virginie Aires, Jean-Jacques Michaille, Dominique Delmas, Fatima Djouadi, Jean Bastin, Mustapha Cherkaoui Malki & Norbert Latruffe.
Mirna Modulation by Resveratrol in Human Fibroblasts with Fatty Acid Oxidation-Deficiency as Consequence of Carnitine-Palmitoyl Transferase 2 (CPT2) Mutation

P2) Monika Baranowska, Klaudia Suliborska, Wojciech Chrzanowski, Jacek Namieśnik, Agnieszka Bartoszek
The Impact of Flawan-3-Ols on Expression of Genes Related to Oxidative Stress and Antioxidant Defence

P3) M.M. Bibiloni, J. Karam, A. Julibert, E. Argelich, J.M. Gámez, I. Llompart, A. Sureda, A. Pons, J.A. Tur
Estimation of Dietary Fatty Acid Intake in Mediterranean Old Adults

P4) Kateřina Valentová, David Biedermann, Jiří Vrba, Jitka Ulrichová, Vladimír Křen
2,3-Dehydroflavonolignans as the Active Principle of Silymarin

P5) Corte-Real J., Guignard C., Gatenbein M., Bernard W., Burgard K., Hoffmann L., Richling E., Bohn T.
Interactions of Divalent Minerals and Carotenoids during Digestion

P6) Karym El-Mostafa, 2, Bouchab Habiba, Badreddine Asma, Thomas Nury, Abdelkhalid Essamadi, Khadija Moustaid, Pierre Andreoletti, Mustapha Cherkaoui-Malki, Gérard Lizard, Boubker Nasser
The protective Effect of Opuntia *Ficus indica* Seeds against the Cytotoxicity Induced by Iron Overload: Chemical Analyses and *in vitro* Studies in the Protozoan Tetrahymena pyriformis

P7) Mario Caruana, Neville Vassallo, Ruben J.Cauchi, Michelle Briffa, Stephanie Ghio, Johanna Neuner, Alison J.Gauci, Rebecca Cacciottolo, Christelle Marchal, Christopher Cullin
Mediterranean Plant Extracts Ameliorate Cellular and Animal Models of Neurodegenerative Diseases

P8) Azubuike P. Ebokaiwe
Chemopreventive Role of Quercetin and Vitamin E on Bonny Light Crude Oil-Induced Redox Alterations in Testicular Function of Wistar Rats

P9) E.C.C. Ejike Chukwunonso
African Plants with Redox Modulatory Properties Relevant in Medicine and Agriculture

P10) Bruno Fink, Coy Brunßen, Henning Morawietz, Alexander Rabovsky
Multifunctional Supplement is that the Key to Modulate Redox Signaling in Metabolic Syndrome Volunteers: Cellular Metabolic Index

P11) Marko Gerić, Goran Gajski, Vera Garaj-Vrhovac
Is the Future of Human Diet Green: A Genome Damage Study

P12) M. Iddir, Y. Devaux, C. Guignard, B. Appenzeller, P. Borel, Y. Larondelle, T. Bohn
Influence of Proteins on Carotenoid Digestion and Aspects of Bioavailability

P13) Zübeyir Elmazoğlu, Sait Yarayıcı, Cem Nuri Aytekin, Berivan Bitik, Berna Göker, Çimen Karasu
Targeting Redox Signaling by Phytophenols in Chondrocytes Isolated from Human Osteoarthritic Articular Cartilage *in vitro*

P14) M. Khvedelidze, T. Mdzinarashvili, E. Shekiladze
Incorporation of Vitamins in DPPC Nanoparticles as Novel Approach for Drug Delivery to Tumorous MDCK Cells

P15) Maija Dambrova, Andrejs Skesters, Janis Liepins
NutriOX related "Whos and Wheres" from Latvia

P16) T. Mdzinarashvili, M. Khvedelidze, N.Turkadze, I. Papukashvili, E. Lomadze
Physical-Chemical Factors Influence on Bacteria Behavior

P17) Karol Parchem, Agnieszka Bartoszek
Impact of Dietary Oxidized Phospholipids on Human Health and Methods of their Profiling and Assessment in Foods

P18) C. Perrin-Sarrado, V. Salgues, P. Giummelly, P. Leroy, I. Lartaud
Ability of Different S-Nitrosothiols to Induce Vascular Storage of Nitric Oxide and to Decrease Vasoconstrictive Capacities of Isolated Rat Aortae

P19) N. Sahakyan, M. Petrosyan, A. Trchounian
The Radical Scavenging Activity of *Ajuga genevensis L. In vitro* Culture

P20) Alice Santoro, Nina Ewa Wezynfeld, Wojciech Bal, Peter Faller
Cysteine Triggers the Copper Transfer from Aβ4-16 Peptide to Metallothionein-3

P21) Katarina Smilkov, Tanja Petreska Ivanovska, Tatjana Ruskovska, Kristina Mladenovska
Influence of Microencapsulated Probiotic Intake on Myeloperoxidase Activity in TNBS-Induced Colitis in Rats

P22) Klaudia Suliborska, Monika Baranowska, Agnieszka Bartoszek, Claus Jacob, Jacek Namieśnik, Wojciech Chrzanowski
Potentiometric Titration as a Reliable Method of Determination of Standard Reduction Potentials of Antioxidant Compounds on the Way to the of Antioxidant Power Series

P23) Antoni Sureda, Carla Busquets-Cortés, Xavier Capó, Silvia Tejada, Alejandra Arenas, David Mateos, Antoni Pons, Josep A Tur
A Mediterranean Diet Nutritional Intervention Lowers Blood Pressure and the Production of Reactive Oxygen Species by Immune Cells

P24) Panos G. Ziros, Eleni Ntalampyra, Gerasimos P. Sykiotis
Testing the Effects of Redox-Active Compounds on Thyroid Cells: a Call for Collaborations

P25) Zélie Triaux, Léa Briard, Odile Petit, Eric Marchioni, Diane Julien-David
Antioxidant Capacity of Plant Extracts

P26) E. M. Karym, A. Badreddine, T. Nury, M. Cherkaoui-Malki M., B. Nasser, G. Lizard, A. Vejux
Contribution of Iron Overload and Cholesterol Oxidation in the Pathogenesis of Alzheimer's Disease

ORAL COMMUNICATIONS

The Production of Reactive Oxygen Species by Cu-amyloid-β: Mechanism, Inhibitors and Relevance to Alzheimer's

Peter Faller

Biometals and Biology Chemistry, Institut de Chimie (CNRS UMR 7177), University of Strasbourg, 4 rue B. Pascal, 67081 Strasbourg Cedex (France).

Oxidative stress has been reported to be implicated in a number of diseases, including Alzheimer's disease (AD). Copper ions can play an important role in oxidative stress and they are one the one hand involved in the defense (e.g. Cu in super-oxide dismutase) and on the other hand are very efficient in catalyzing the production of reactive oxygen species (ROS) (often loosely bound Cu).

In AD Cu ions are bound to amyloid-β peptides (Aβ) in amyloid plaques, a hallmark of AD, ii) in vitro Cu ions are able to modulate Aβ aggregation and to form oligomeric forms, supposed to be more toxic and iii) Cu-Aβ complexes are able to catalyze the production of hydrogen peroxide and hydroxyl. [1]

During the recent years we were interested in the mechanism of the ROS production by Cu-Aβ complexes. The data obtained of Cu-Aβ and Aβ several derivatives (mutations, substitutions etc.) point to an original mechanism of the ROS production, involving a low populated "in-between" state that is responsible for the ROS production. [see e.g. 2] Very recently we were able to make a detailed proposition of the structure of this "in-between" state,[3] and we could show that ROS can oxidize Aβ itself, which changes the Cu-coordination. This different Cu-site is able to produce faster ROS.[4]

Another line of research concerns the investigation of inhibitors, molecules that are able to inhibit the production of ROS by Cu-Aβ complexes. On possibility is to withdraw Cu from Cu-Aβ and sequester Cu in a redox-inert environment. A lot of research groups investigate Cu-chelators in this respect [5]. We were also interested in natively present proteins, like metallothioneins or serum albumin, that could suppress Cu-Aβ catalyzed ROS production.[6] Based on data from us and the literature, guidelines and concepts for the design of Cu-sequestering chelators in the context of Cu-Aβ linked to AD can be proposed.

References:

1. Nasica-Labouze J, al. *Chem. Rev.,* **115**, 3518-63 (2015)
2. Reybier, K. et al. *Angew. Chem. Int. Ed.* **55**, 1085-1089 (2016).
3. Cheignon, C. et al. *Chemical Science*, **8**, 5107-5118 (2017)
4. Cheignon, C. et al. *Metallomics,***8**, 1081-1089 (2016)
5. Telpoukhovskaia MA, & Orvig C. *Chem Soc Rev.*;**42**, 1836-46 (2013)
6. Meloni et al. Nat Chem Biol., 4, 366-72 (2008)

Keywords: *copper, amyloid, reactive oxygen species, metallothionein, self-assembly, oxidative stress, peptide oxidation*

Natural Products with Antioxidant Activities in Diabetes Treatment

Svetlana Dinić

Department of Molecular Biology, Institute for Biological Research, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia

Diabetes is a metabolic disorder accompanied by a chronic hyperglycemia that occurs as a result of the lack or reduction of insulin action. Oxidative stress is one of the basic mechanisms that underlies diabetes development and progression through insulin producing pancreatic beta-cells destruction and disfunction. In addition, chronic hyperglycemia promotes the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which further contribute to oxidative stress condition in diabetes and promote the development of diabetes associated complications. One of the main goals of our research is the studying of the mechanisms of action of antioxidant compounds that prevent or slow down the process of beta-cell loss and dysfunction, as well as the development of diabetes complications. Herbal preparations, as a complex combination of chemical compounds, especially polyphenols, could alleviate oxidative stress through synergistic, additive and antagonistic interactions of redox active substances at different levels. Using in vitro (beta-cell lines treated with redox active compounds) and in vivo (streptozotocin (STZ)-induced diabetes in rats) models of diabetes, our group has elucidated several mechanisms that underlie the antioxidant effect of alpha-lipoic acid, a powerful antioxidant and a compound which stimulates cellular glucose uptake, and plant extracts isolated from sweet chestnut (*Castanea sativa*), edible mushrooms (*Lactarius deterrimus*), european centaury (*Centaurium erythraea* Rafn) and natural products containing beta-glucan, in the treatment of diabetes. These findings revealed that examined compounds possess the ability to significantly lower the risk of oxidative stress mediated beta-cell death and disfunction, as well as the ability to increase organism's resistance to the onset of oxidative stress related diabetes complications.

Keywords: *diabetes, oxidative stress, plant extracts, antioxidant activity*

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Strasbourg, 27 – 29 September 2017

Anti-Inflammatory Properties of Carotenoids – an Overview

Bohn, Torsten

Luxembourg Institute of Healt

E-mail: Torsten.bohn@gmx.ch

Dietary intake and tissue and blood levels of carotenoids have been associated with reduced risk of developing several number of chronic diseases. While earlier direct antioxidant properties were proposed to constitute the responsible mechanisms, it has become apparent that influences on gene expression, such as via transcription factors and nuclear receptors, are more likely to be responsible for the proposed health benefits. However, it is often unclear whether native carotenoid or their metabolites are more bioactive, and whether there exists a clear dose-response relation.

In this presentation, we highlight the role of carotenoids and their metabolites in relation to mechanisms associated with their proposed health benefits. A review together with own findings is provided, covering aspects of carotenoid absorption, prominent and potential metabolites, and their relation to health effects, especially focusing on molecular pathways related to inflammatory aspects and oxidative stress.

A special focus is dedicated to anti-inflammatory and anti-oxidant aspects of carotenoids, which appear to be linked, at least in part, to reduced NF-κB translocation to the nucleus and increased translocation of Nrf2 [(1)]. Proteomic and transcriptomic studies with vegetables and fruits rich in carotenoids support their bioactivity on a cellular level [(2)]. In addition, several studies have suggested that rather the more polar apocarotenoid metabolites (following BCO1/2 cleavage) or polar derivatives of native carotenoids have a stronger affinity to interact with cellular signal transduction cascades [(3)]. Finally, also several metabolites of lycopene (apo-lycopenoids), have shown to interact with RXR/RAR receptors, suggesting vitamin A like activity [(4)]. Very recently, also the differentiation of adipocytes has been related to carotenoids and their metabolites, emphasizing the relation of carotenoids to lipid metabolism, obesity and related complications [(5)].

In conclusion, carotenoids and their metabolites do influence cellular signaling pathways and may be involved in regulating transcription factors important for the bodies' own anti-inflammatory and anti-oxidant defenses, respectively. It also appears that several metabolites, due to their higher polarity, being more electrophilic, and better solubility in the cytosol, may be better activators of related pathways. A U-shaped curve of their bioactivity is proposed, and gaps of our knowledge are emphasized.

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Keywords: *apocarotenoids, transcription factors, bioactivity, intestinal solubility, absorption*

The Omega-3 EPA:DHA 6:1 Formulation Improves Ageing-Related Blunted Endothelium-Dependent Relaxations and Increased Contractile Responses in the Mesenteric Artery: Role of Oxidative Stress and Cyclooxygenases

Muhammad A. Farooq, Lamia Amoura, Sébastien Gaertner, Zahid R. Niazi, Shinhee Park, Abdul W. Qureshi, Min-Ho Oak, Florence Toti, Valérie B. Schini-Kerth, Cyril Auger

UMR CNRS 7213 Laboratoire de Biophotonique et Pharmacologie, Faculté de Pharmacie, Université de Strasbourg, Illkirch, France

Introduction: Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to cause endothelium-dependent nitric oxide (NO)-mediated relaxations of isolated blood vessels, with EPA:DHA 6:1 being a superior formulation. The aim of the present study was to determine whether EPA:DHA 6:1 improves ageing-related endothelial dysfunction, and, if so, to determine the underlying mechanism.

Methods: Male Wistar rats (20 months-old) received daily 500 mg/kg of either EPA:DHA 6:1 (omega 3), corn oil (control), or water for 2 weeks. Young male Wistar rats (12 weeks-old) were used as control.

Results: In the main mesenteric artery, ageing was associated with an endothelial dysfunction characterized by a blunted NO-mediated component, an abolished endothelium-dependent hyperpolarization (EDH)-mediated component, and also by the induction of endothelium-dependent contractile responses (EDCF, in the presence of NO and EDH inhibitors) sensitive to indomethacin (a cyclooxygenase inhibitor) in response to acetylcholine. Endothelial dysfunction was associated with an increased level of vascular oxidative stress and expression of COX-2, but not of COX-1, in the mesenteric artery. The EPA:DHA 6:1 treatment improved both the NO- and EDH-mediated relaxations, and reduced EDCF, vascular oxidative stress and expression of COX-2 in the old mesenteric artery.

Conclusion: The present findings indicate that intake of EPA:DHA 6:1 prevented the development of the ageing-related endothelial dysfunction in rats. The beneficial effect involves an improvement of both the NO- and the EDH-mediated relaxations as well as a reduction of endothelium-dependent contractile response most likely by preventing vascular oxidative stress.

Keywords: *Omega 3 fatty acids, endothelium, vascular oxidative stress, cycloxygenases*

Antioxidative Effects of a Fruit Juice Rich in Anthocyanins in Humans

Bakuradze, Tamara[1]; Tausend, Angelina[1]; Groh, Isabel[2]; Marko, Doris[2]; Richling, Elke[1]

[1] *Department of Chemistry, Division of Food Chemistry and Toxicology, University of Kaiserslautern, Kaiserslautern, Germany;*
[2] *Department of Food Chemistry and Toxicology, University of Vienna, Vienna, Austria*

Anthocyanin-rich fruit consumption is attributed to antioxidant, anti-inflammatory and chemopreventive properties and are associated with health beneficial effects [1, 2]. We have performed an eight week randomised placebo controlled intervention study with 57 healthy volunteers to investigate DNA protective and antioxidative effects of anthocyanin rich fruit juice. After seven days of washout the volunteers consumed a bolus of 750 ml anthocyanin rich red fruit juice (group A, n=30) or an ascorbic acid enriched placebo beverage (group B, n=27), respectively. In the following four weeks, the volunteers consumed daily 750 ml of the respective beverage in three equal portions (à 250 ml). During the study, blood and urine samples were collected. In the blood samples the modulation of biomarkers of DNA damage and antioxidant defence enzymes were analysed. Additionally, body weight/composition as well as energy and nutrient intake of volunteers were investigated. The results showed a significant reduction of spontaneous and total DNA strand breaks already after 24 h ($p < 0.001$) as well as 1, 4, and 8 weeks ($p < 0.001$) of intervention. Plasma SOD activity was increased after 56 days intervention with red fruit juice (group A, $p < 0.05$), whereas after placebo beverage ingestion (group B) no increase could be observed. Interestingly, consumption of red fruit juice was associated with a decrease in body fat ($p < 0.05$) and an increase in fat free mass ($p < 0.001$). Overall, the consumption of anthocyanin-rich red fruit juice revealed DNA-protective and antioxidative effects in healthy volunteers.

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Keywords: *anthocyanins, human study, red juice, DNA damage*

Effect of Different Nutraceuticals on Paraoxonase 1 Enzyme Activity and Oxidative Stress Status in Middle-Aged Subjects

Bojana Vidović[a], Ivana Djuričić[a], Danica Michalickova[a], Vanja Todorović[a], Jelena Kotur-Stevuljević[b], Brižita Đorđević[a], Slađana Šobajić[a]

[a] *Department of Bromatology, Faculty of Pharmacy, University of Belgrade, Serbia*
[b] *Department of Medicinal Biochemistry, Faculty of Pharmacy, University of Belgrade, Serbia*

Email: bojana@pharmacy.bg.ac.rs

Paraoxonase 1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme that possesses anti-atherogenic and anti-inflammatory properties. Low PON1 activity has been found in a variety of oxidative stress conditions such as obesity, diabetes, cardiovascular and neurodegenerative diseases. Therefore, pharmacological and nutritional modulation of expression and PON1 activity could constitute a useful approach for the prevention of some age-related chronic diseases.

We investigated the effects of omega-3 fatty acids, alpha-lipoic acid and tomato ethanol extract in 3 separate intervention studies on PON1 activity and oxidative stress markers in apparently healthy middle-aged subjects, with one or more cardiovascular risk factors.

In a randomized controlled crossover trial carried out in 35 middle-aged dyslipidemic subjects receiving 2 g fish oil /day (in capsules) or 150 g of smoked salmon fish 2 times per week, during 8 weeks, both sources of omega-3 fatty acid significantly increased PON1 activity. These effects were possibly a consequence of an enzymatic response to the initially observed pro-oxidant effect of omega-3 fatty acids. Alpha-lipoic acid (500 mg/day) resulted in a significant increase in plasma total antioxidant capacity with a decrease in the plasma lipid malondialdehyde conncentrations and PON1 activity for 12 weeks. Supplementation with tomato juice fortified with 1000 mg tomato ethanol extract for 8 weeks increased the plasma total antioxidant capacity in middle-aged hypertensive subjects, but had no effect on PON1 activity.

Our results indicate that different nutraceuticals may modulate paraoxonase activity independent of oxidative stress status. Nutrigenetic aspect response to nutraceuticals will need to be considered in large clinical studies.

Keywords: *nutraceuticals, oxidative stress, paraoxonase, cardiovascular risk factors*

Natural Polyphenols' Antioxidant Intervention in a Murine Experimental Model of Diabetes Mellitus

Ileana Antohe, Carmen Boldea Colcear Pleşoianu, Codruţa Bădescu, Mihaela Elena Carausu, Manuela Ciocoiu

University of Medicine and Pharmacy "Grigore T Popa" Iaşi

Natural polyphenols, major vegetal constituents, attract medical attention due to their potential benefic effects in human pathology. Among these effects the reduction of oxidative stress, of inflammatory markers and immune modulation are in the front line.

The present experimental model of diabetes mellitus was induced in 48 white Wistar rats, with a mean weight of 250-280 g. The animal population was divided in 4 groups (12 rats each): a witness group (W), a diabetic group (D), a diabetic group treated with *Sambucus nigra* (D+S) and a diabetic group treated with *Aronia melanocarpa* (D+A). Administration of natural polyphenols induced significant decrease of glicated hemoglobin (HbA1c) in diabetic rats. A significant decrease of total antioxidant status (TAS) in diabetic group versus non-diabetic rats was observed. In the two groups treated with natural polyphenols extracts (D+S and D+A) TAS was significantly increased compared with the diabetic group. TAS improved in diabetic rats receiving polyphenols, the greater effect being noticed in those receiving *Sambucus nigra* extract. Administration of polyphenols in diabetic rats increased significantly plasma antioxidant enzymes such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD).

Polyphenols administration also reduced inflammatory changes encountered in diabetic rats, as shown through white cells and fibrinogen reduction. It also improved latency time in nociception tests on diabetic rats.

Due to these multiple benefic effects we consider that natural polyphenols could be used as additional protective therapy in diabetic cardiomyopathy and ischemic involvement in diabetes mellitus patients.

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Keywords*: natural polyphenols, antioxidant effect, experimental diabetes mellitus*

Antioxidant Activity of Whey Protein: Survival during Gut Transit and Bioavailable to Target Cells?

A. R. Corrochano[1, 2], E. Arranz[1], A. Ferrareto[3], M. Bottani[3], M. Stuknyte[4], V. Buckin[2], P. Kelly[1], I. De Noni[4] and L. Giblin[1]

[1]Teagasc Food Research Centre, Moorepark, Fermoy, Co Cork, Ireland
[2]School of Chemistry & Chemical Biology, University College Dublin, Ireland
[3]Department of Medical Chemistry, Biochemistry and Biotechnology, L.I.T.A., Segrate, Milan, Italy, [4]Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy

Cellular metabolic processes inherently produce free radicals. In healthy cells, antioxidant mechanisms function to neutralise these free radicals (Fusco et al., 2007). Dietary antioxidants can help to boost cellular anti-oxidant processes thereby avoiding cell damage by oxidative stress Sustained damage by oxidative stress is considered a causative agent of, or aggravates, several chronic disorders. Recently the dairy protein, whey, has received considerable attention for its antioxidant bioactivity (Power et al., 2013). The objectives of this research were to investigate the efficacy of whey as a cellular antioxidant by following (1) its bioactivity through gut transit, (2) its bioavailability across the intestinal barrier and (3) its antioxidant effects on downstream target cells. The COST INFOGEST static method (Minekus et al., 2014) was used to perform an *in vitro* gastrointestinal digestion on commercial whey ingredients. Antioxidant activity of test samples, pre and post gastric digestion, were determined using ORAC, ABTS and FRAP assays. The intestinal barrier co-culture model, Caco2-HT-29, were then exposed to test samples for two hours. Apical and basolateral solutions were analysed for peptide content by Ultra-Performance Liquid Chromatography/Electrospray Ionisation-High Resolution Tandem Mass Spectrometry (UPLC/ESI-HR-MS/MS). Peptides previously described as bioactive and peptides with unknown bioactivity were discovered in the basolateral side. Several peptides were synthesised and tested for reduction of free radicals on target organs using cells from muscle and liver. We provide evidence that gut transit functionalises whey proteins producing bioactive peptides. Some of these peptides can pass through the intestinal barrier and modulate the redox state of target cells.

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Keywords: *Antioxidant, whey, digestion, peptides, bioactive*

Daily Consumption of a (Poly)phenol Enriched Diet Prevents Death of Hypertensive Rats: Exploring the Role of Gut Microbiota

A. Gomes[1,2*], C. Oudot[3], A. Màcia[4], M.C López de Las Hazas[4], A. Foito[5], D. Stewart[5,6], M.J. Motilva[4], C. Brenner[3], C. N. Santos[1,2]

[1]iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901 Oeiras, Portugal. e-mail: andreiagomes@itqb.unl.pt; [2]Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal; [3]INSERM UMR-S 769-LabEx LERMIT, Université Paris-Sud, 5 rue Jean-Baptiste Clément 92296 Châtenay Malabry, France; [4]Food Technology Department, Agrotecnio Center, Escuela Técnica Superior de Ingeniería Agraria, University of Lleida, Lleida, Spain; [5]Enhancing Crop Productivity and Utilisation, James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK [6]School of Life Sciences, Heriot-Watt University, Edinburgh EH14 4AS, Scotland, United Kingdom*

Through the years, many alterations in human dietary and lifestyle patterns has been affecting human health, particularly cardiovascular health. Epidemiological studies have shown a consistent beneficial relationship between the consumption of fruits and vegetables, rich sources of (poly)phenols, and a reduced risk of cardiovascular diseases (CVD) susceptibility. Abundant dietary (poly)phenols in our diet are not necessarily able to result in the highest tissue concentrations, owing to considerable differences in bioavailability. Thus, a detailed and profound knowledge on the specific bioactive (poly)phenol metabolites and their molecular targets is a key point to ensure the safe use of these active phytochemicals as a nutraceutical/therapeutic agent in a nearest future.

Our goal was to identify the (poly)phenol metabolites derived from a berries diet present in urine, faeces and kidney in an animal model of hypertension (Dahl-salt sensitive rats) where was observed an improvement in cardiovascular function. Moreover, we also intent to evaluate the influence of diet in the rat's gut microbiota. A berries mixture composed of blueberries, blackberries and raspberries, as well as, wild Portuguese crowberry and strawberry tree fruit was supplemented to the rat's diet. Rats divided into 4 groups have been fed with different diets for 9 weeks: Low Salt (LS), Low Salt and Berries (LSB), High Salt (HS) or High Salt and Berries (HSB) diet. Urine, faeces and kidney were processed for phenolic extraction and analysed by UPLC-MS/MS. Faeces were also processed for Genomic DNA Isolation, 16S rRNA Gene Sequencing and identification of the microbial composition.

Hypertension-induced by HS diet was associated with cardiac hypertrophy and a decrease in the cardiac function. Five rats in HS group died from stroke before the end of trial while all LS, LSB and HSB survived. Interestingly at 9 weeks, HSB diet prevented the cardiac damage independently of changes in systolic pressure. Moreover, an increase in kidney weight index in HS rats was detected that was attenuated in HSB rats; additionally, in this organ we could identify polyphenols metabolites exclusively present in the HSB group. Differential urinary and faeces phenolic profile is also observed for HBS rats that agree with kidney results. Regarding rat's microbiome the presence of high amounts of salt and the berries in the diets affected the abundance of the different phyla.

In conclusion, we accomplished to identify the metabolites putatively responsible for the cardioprotective effect observed, as well as the influence of diet in microbiota diversity. Further investigations will be important to fully elucidate the mechanisms of biological activity of these metabolites in CVD cell and subcellular models.

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Keywords: *Cardiovascular diseases, hypertension, (poly)phenols, metabolic fate, microbiota*

Analysis of Bioactive Compounds in Local and Traditional Foods. Application to Diabetes and their Complications

Eric Marchioni

[1]*Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France.*

The search for new bioactive molecules of natural origin unquestionably requires the development of high-resolution bioguided screening methods. Our aim is to provide the Research Teams with a tool powerful enough to screen a wide range of molecules present in natural resources of all origins. Although bioguided screening techniques are widely used today, they suffer from many disadvantages such as their very long implementation time and the very important risk of discovering a molecule already discovered and widely described in the scientific literature. Moreover, the increasingly fine separation of compounds often present in trace amounts in a very complex matrix leads to the separation of molecular species which, when active together, they are separated. The work to be presented concerns the implementation of an analytical method that allows on-line detection of the presence of compounds with radical scavenging activities, to isolate them on the molecular scale and to identify them thanks to hyphenation with a detection by high-resolution mass spectrometry. Separation methods and different methods of detection will be presented, the most original of which being the hyphenation of a liquid chromatography with a detection by electron paramagnetic resonance spectrometry which makes it possible to unambiguously detect the radical scavenging activities of compounds present in a complex mixture.

Characterisation of Chemosensitizing Effects of the Electrophilic Steroidal Kinase Inhibitor Withaferin A in Therapy Resistant Multiple Myeloma

Chandra Sekhar Chirumamilla, Claudina Pérez-Novo, Xaveer Van Ostade, Wim Vanden Berghe

Laboratory of Protein Science, Proteomics and Epigenetic Signaling (PPES), Department of Biomedical Sciences, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, Wilrijk, Belgium.

Email: wim.vandenberghe@uantwerpen.be

Redox networks in the cell integrate signaling pathways that control metabolism, energetics, cell survival, and death. The physiological second messengers that modulate these pathways include nitric oxide, hydrogen peroxide, and electrophiles. Electrophiles are produced in the cell via both enzymatic and nonenzymatic lipid peroxidation and are also relatively abundant constituents of the diet. The signaling pathways through which electrophiles function have unique characteristics that could be exploited for novel therapeutic interventions; however, development of such therapeutic strategies has been challenging due to a lack of basic understanding of the mechanisms controlling this form of redox signaling.

The dietary medicinal phytochemical withaferin A (WA), isolated from Withaferin somnifera (popular Indian name 'Ashwagandha') holds promise as a novel electrophilic steroidal anti-cancer agent which modulates hormone receptor and Nrf2 oxidative proteotoxic stress pathways. By means of peptide array based tyrosine phosphopeptide fingerprinting and kinome activity profiling, we further demonstrate that WA inhibits various tyrosine kinases which are hyperactivated in therapy resistant multiple myeloma cells, including TEC, BTK and HCK kinases. Altogether, we propose a novel mechanism of WA-dependent kinase inhibition via electrophilic covalent targeting of cysteine residues in conserved kinase activation domains (kinase cysteinome), which could underlie its pleiotropic therapeutic effects to overcome cancer therapy resistance in multiple myeloma.

Presentation of the Mediterranean Diet and Health Association (NMS). A Link with Nutriox/Nutredox Purposes

Norbert Latruffe

University of Burgundy, Laboratory BioPeroxIL, 6, Bd Gabriel, F-21000 Dijon, France

The cultural and the nutritional aspects of the multisecular Mediterranean civilization include wine as a central element of health and wellbeing if it is used with moderation, especially if associated with the Mediterranean diet. Indeed, mediterranean meals provide food microcomponents including polyphenols, vitamins, fibers, poly-unsaturated fatty acids and oligo-elements brought by fruits, vegetables, olive oil, fish, infusions or/and wine. In addition, red wine provides additionnal unique polyphenols with anti-oxidant properties. For instance, resveratrol, procyanidines, and monophenols including hydroxytyrosol and tyrosol.

The purpose of the NMS association is to provide comprehensive data on Mediterranean diet, wine and health and their positive impact on human physiology (cardiovascular, aged-linked disorders and s.o.) and especially the effects of polyphenols as anti-oxidants; and from a humanity point of view, the tasting properties, society perception and the societal image.

The aims are to promote and sustain the developpment of scientific and medical recherches on the beneficial effects of mediterranean diet on health and to allow the largest diffusion of knowledge on this topic to a large public by organizing in a optimal manner the information transfert on this subject.

We must point out that mediterranean diet is obviously associated to mediterranean climat which is tempered and characterized par by hot and dry summers and mild and humids winters. Mediterranean climat and diet is not restricted to mediterranean sea area but also in similar regions of the world located between 30 and 40° parallel of latitude. For instance the west face of continents like California, center of Chili, Cap region in south Africa and sud and west of Australia. In all of these countries food and beverage are quite similar and provide good health to the populations. Think to the sarde and cretan islands centenary living poeple.

Armenian plants and their callus cultures in biotechnology and medicine: novel results and future study

A. Trchounian

Department of Biochemistry, Microbiology and Biotechnology, Biology Faculty, Yerevan State University, 1 A. Manoogian Str., 0025 Yerevan, Armenia

Email: Trchounian@ysu.am

Oxidative stress is a risk factor in the pathogenesis of numerous chronic and age diseases. Free radicals and other reactive oxygen species (ROS) are recognized to be involved in the pathogenesis of different diseases as well as to be responsible for the human aging. In addition, nowadays, antibiotic resistance has become one of the most urgent challenges of humanity. Investigation of plant materials as a source of biologically active compounds can play an important role for the solution of these problems. Armenian flora is very rich of plants which can be used in medicine, food and cosmetics. But a great number of plants are considered to be endangered, scarce or included in Red books of Armenia; therefore biotechnological methods of obtaining of valuable metabolites are also of interest. Thus, during last years the Department of Biochemistry, Microbiology and Biotechnology and Research Laboratories of Biochemistry and Microbiology, Bioenergetics and Biotechnology, YSU, adopted ways which suggest comprehensive solution of these problems. Studies were divided into several areas: screening of flora for revealing plants with antioxidant, antibacterial, anti-diabetic, hypoglycemic and hypolipidemic activities (1); investigation of their chemical composition (2); obtaining of selected plants isolated callus cultures and investigation the biological activity of intact plants and their *in vitro* culture extracts (3,4), essential oils activity (2) as well as plants with anti-diabetic activity which are grown on hydroponics (5). The novel results are summarized; significance and further research respective to NutriOx are discussed.

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Keywords: *Plant materials; reactive oxygen species, free radicals, antibiotic resistance, antioxidants, antibacterials, anti-diabetics*

Redox Active Natural Products: a Fountain of Inspiration and Inexhaustible Well of Well-Being

Claus Jacob

Bioorganic Chemistry, Department of Pharmacy, Saarland University, Building B 2.1, Campus, D-66123 Saarbruecken, Germany

E-mail: c.jacob@mx.uni-saarland.de

Plants endow us with a truly colourful treasure-chest of redox-active products, from simple, low molecular weight sulfur compounds in garlic to complex, macromolecular polyphenols found in fruits and berries. Amazingly, many of these substances are biologically rather active, yet at the same time have formed part of our normal daily nutrition for centuries. It is therefore feasible to use nutrition as a simple, yet effective supplier of active materials which may greatly benefit human health, especially in an older population, where a demand for supplementation cannot simply be met by excessive medication.

Here, our own focus resides on redox active sulfur-containing secondary metabolites. Whilst those substances are traditionally considered as antioxidants, quite a few of them also classify as “Reactive Sulfur Species” (RSS) [1]. These more oxidizing sulfur compounds post-translationally modify selected cysteine residues of proteins and enzymes of a cellular redox control and feedback network we have recently termed the “cellular thiolstat” [2]. Such compounds may be useful to attack pathogenic microbes or to initiate apoptosis in sick cells whilst protecting healthy ones. The concept of RSS has not only stimulated the hunt for such sulfur ingredients in many culinary plants, such as garlic and mustard, it has also fuelled comprehensive investigations into the underlying mode(s) of action using the methods of “intracellular diagnostics” [3]. These studies have revealed rather intricate mechanisms, whereby several, often opposing pathways may be triggered simultaneously and the eventual outcome may depend not only on the compound but also on the cell itself [4].

Not surprisingly, possible applications in Medicine and Agriculture, sophisticated substrate/enzyme activation and delivery systems as well as derivatives have been considered [5]. Whilst some of these promising leads require considerably more research, especially in the context of activity and bioavailability and in more complex organisms, some less demanding applications have already reached the fields of eco-friendly phytoprotectants. Similarly, more straight-forward applications also seem possible in the context of the control of the microbiome, or of topical infections. In contrast, less available materials, such as hardly soluble and poorly bioavailable materials may be nano-sized to unlock their biological activity and potential and hence to circumvent some of their inherent drawbacks [6].

Redox active secondary metabolites will surely feature prominently on any future menu, not only on a culinary one, but also in research and the “logistics” concerned with their production - possibly from refined side-products of food manufacture - and delivery.

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Proteasome Activators in Our Diet: a Promising Strategy against Aging and Aggregation

Niki Chondrogianni

National Hellenic Research Foundation, Institute of Biology, Medicinal Chemistry and Biotechnology, Athens, GREECE

Ageing is a natural process characterized by a gradual decline of physiological function and the eventual failure of organismal homeostasis. The proteasome, one of the major cellular proteolytic systems, is responsible for the degradation of normal and damaged/abnormal/misfolded proteins that tend to accumulate during ageing. Impaired proteasome function occurs during the progression of ageing both *in vivo* and *in vitro*. While, its partial inhibition triggers an irreversible p53-dependent premature senescence phenotype, its activation through genetic means or compound treatment confers cellular lifespan extension and extended maintenance of youthful morphological features. Using the nematode *Caenorhabditis elegans*, we have achieved proteasome activation at the organismal level thus promoting beneficial effects on the lifespan of the nematode. Moreover, proteasome activation seems to be a potential strategy to minimize protein homeostasis deficiencies underlying aggregation-related diseases such as Alzheimer's or Huntington's. Consequently, compounds with proteasome activating properties might be used in preventive or therapeutic approaches against such diseases. In conclusion, our results show the dynamic interconnection between the proteasome, the proteostatic mechanisms and the progression of ageing and age-related diseases.

Keywords: *ageing, aggregation, proteasome activation, proteolysis*

Dietary Antioxidant Intake and Physical Activity Level Among Metabolic Syndrome Elderly Patients: Preliminary Evidence in the Balearic Islands Subgroup of the Predimed-Plus Trial

Cristina Bouzas, Manuela Abbate, Laura Gallardo, Sandra Carreres, Sofia Montemayor, Alicia Julibert, Mar Bibiloni, Josep A. Tur.

[1]Research Group in Community Nutrition and Oxidative Stress, University of Balearic Islands, Palma de Mallorca and CIBEROBN (Networking Center for Physiopathology of Obesity and Nutrition CB12/03/30038) Instituto de Salud Carlos III, Madrid, Spain.

E-mail: nucox@uib.es

Introduction: Optimal nutrition is essential for general well-being, maintenance of both physical and functional capacities and prevention of disease in the elderly[1]. Physical activity induces oxidative stress and tissue damage[2]. Dietary antioxidants may help on the oxidative–antioxidative balance.

Objective: To assess the antioxidants dietary intake in metabolic syndrome elderly patients of PREDIMED-PLUS trial according to their physical activity level.

Methods: Analyses include 270 patients (148 men and 122 women aged respectively between 55-75 and 60-75 years). Intervention group (n=134) received energy-restricted Mediterranean diet and patients were advised with specific weight-loss goals, physical activity and behavioural intervention. Control group (n=136) received a low-intensity intervention and general but no specific advice on physical activity (health usual care). Dietary antioxidant intake (Mg and vitamins A, C, and D) was assessed by 137-items validated Food Frequency questionnaire. Physical activity levels were measured using RAPA questionnaire, which classifies volunteers into three levels: not active, moderately active and active. Both questionnaires were filled at the beginning of the study and after one year of intervention.

Results: At baseline, the antioxidant nutrient (Mg and vitamins A, C, and D) intakes were no different among intervention groups according to physical activity level. After one year, no significant differences among groups were found.

Conclusions: Physical activity levels did not influence dietary antioxidant intake after intervention. More time will be needed for more consistent results on physical activity habits; nevertheless checking preliminary results during the process are important.

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An Energy Restricted Mediterranean Diet Does Not Compromise Dietary Antioxidant Intake: Preliminary Evidence in the Balearic Islands Subgroup of the Predimed-Plus Trial

Manuela Abbate, Cristina Bouzas, Sandra Carreres, Sofia Montemayor, Laura Gallardo, Alicia Julibert, Mar Bibiloni, Josep A. Tur.

Research Group in Community Nutrition and Oxidative Stress, University of Balearic Islands, Palma de Mallorca and CIBEROBN (Networking Center for Physiopathology of Obesity and Nutrition CB12/03/30038) Instituto de Salud Carlos III, Madrid, Spain.

E-mail: nucox@uib.es

Introduction: The PREDIMED-PLUS trial aims at evaluating the effects of an energy-restricted (ER) Mediterranean diet (MD) and increased physical activity, in comparison with an energy-unrestricted (EU) MD and traditional health care, on CVD prevention. Low calories diets need to be well designed as besides providing a lower amount of calories to promote weight loss they also need to deliver sufficient amounts of micronutrients. Inadequate intakes, especially of antioxidants, might have a variety of negative health consequences starting from fatigue to metabolic disruptions including acceleration of degenerative diseases (1). The principal components of the MD include fruits and vegetable, extra virgin olive oil and nuts, or rather foods generally rich in antioxidants; nevertheless, it remains crucial to run checks on the patient's actual intake of nutrients in order to avoid safety issues.

Objective: To assess dietary antioxidant intake levels after one year of the PREDIMED-PLUS intervention trial, in the subgroup of the Balearic Islands (UIB recruiting centre).

Methods: Analyses include 270 patients (148 men and 122 women aged respectively between 55-75 and 60-75 years) randomized to either an ER (n=136) or an EU (n=134) MD. Changes in antioxidant dietary intake levels (vitamin A, C, E and Magnesium) were assessed between groups as well as within groups at three time periods (baseline, six months and 12 months).

Results: Intake levels of vit. A and E were comparable between groups at baseline. Vits. C and Magnesium on the other hand were both significantly higher in the ER group as compared to the EU group. At six and 24 months no differences in antioxidant intakes were observed between the two groups as the EU group introduced significantly higher levels of both vitamin C and Mg as compared to baseline. No changes in antioxidant intakes were observed in the ER group despite reducing calories, as demonstrated by the loss of body weight and abdominal circumference during the first six months of trial.

Conclusions: Dietary antioxidant intake was not reduced in the ER group despite calorie restriction and weight loss. The EU group improved antioxidant intake of vitamin C and magnesium between baseline and the first year of intervention.

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Effects of Vitamins on *In Vitro* Lymphocyte Proliferation, Cytokine Release and Oxidant/Antioxidant Status in Obese Aged Subjects: Impact on Telomere Length

H. Merzouk[1] , S. Bouamama[1] , A. Merzouk-Saidi[1], A. Medjdoub[1] , S.A. Merzouk[1], M. Elhabiri[2], S. Zahn[3]

[1]*Laboratory of Physiology, Physiopathology and Biochemistry of Nutrition, Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe, Abou-Bekr Belkaïd University, Tlemcen 13000, Algeria;* [2]*Laboratory of Bioorganic and Medicinal Chemistry, UMR 7509 CNRS-Unistra, Strasbourg, France;* [3]*Département of Ecology, Physiology and Ethology, IPHC, CNRS-Unistra, Strasbourg, France.*

Aging is accompanied by a progressive increase in pro-inflammatory cytokine status, as well as immune alterations. These alterations are related to increased cellular oxidative stress and micronutrients inadequacy. Obesity is a major concern in the aging population. Obesity is also associated with oxidative stress and immune abnormalities, accelerates the ageing process and can exacerbate the age related decline in physiological function and oxidative stress. Increasing oxidative stress and inflammation affect telomeres. Telomeres are specialized DNA-protein structures found at the ends of chromosomes and are markers of genomic integrity and age-related metabolic dysfunction. Antioxidant supplementation could improve these age–related abnormalities. The aim of this study was to determine in vitro effects of vitamin C and Vitamin E on T cell proliferation, cytokines release and cell redox status in obese aged subjects compared to young subjects. Telomere length was also determined.

Peripheral blood lymphocytes were isolated using a density gradient of Histopaque. They were in vitro cultured and stimulated by Con A in the presence or absence of vitamins. Cell proliferation was determined by MTT assay and interleukin-2 and interleukin -4 secretions. Cell oxidant/antioxidant balance was studied by assaying glutathione (GSH), malondialdehyde (MDA), carbonyl protein levels and catalase activity. DNA was extracted from lymphocytes and Telomere length was quantified using quantitative PCR (at Strasbourg University).

The present study demonstrated that proliferation rate of T-lymphocytes was decreased with aging resulting from alterations in cytokine secretion, GSH depletion and intracellular oxidative stress. These abnormalities were worsened in obese aged subjects. In the elderly, vitamin C and E improved significantly lymphocyte proliferation and mitigated cellular oxidative stress. These effects were more pronounced in obese aged subjects. Indeed, Vitamins C and E increased telomere length in young lymphocytes. Currently, we are testing their effects on lymphocytes from obese aged subjects and we are discussing implications and future research directions.

In conclusion, vitamin C, E supplementation improved T-lymphocytes immune response reducing oxidative stress and generating an anti-inflammatory profile in obesity and aging. They could be included in the prevention of age-related immune alterations.

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Keywords: *aging, obesity, lymphocytes, vitamins, cytokines, oxidant/antioxidant status, telomeres.*

Thiol Based Nitric Oxide Donors for Cardiovascular Diseases Treatment

C. Gaucher

Université de Lorraine, CITHEFOR EA 3452, Cibles Thérapeutiques, Formulation et Expertise Pré-Clinique du Médicament, Faculté de Pharmacie, 54000 Nancy

Nitric oxide, NO, a physiological gaseous messenger, is a key actor to maintain vascular homeostasis. However, in the cardiovascular system, two conditions may result in NO bioavailability decrease: its insufficient synthesis by endothelial NO synthase (endothelial dysfunction) or excessive NO degradation by oxidative stress or decreased antioxidant enzyme activity). NO deficiency plays an important role in many cardiovascular diseases development like pulmonary hypertension, atherosclerosis, ischemia and cardiac arrhythmia. The endogenous *S*-nitrosothiols (RSNOs), one of the main transport and storage forms of NO in the bloodstream and tissues are good candidates regarding to their therapeutic potential to restore NO physiological concentration. Many investigations related to the therapeutic potential of RSNO in the cardiovascular system have focused on *S*-nitrosoglutathione (GSNO), which is a powerful antiplatelet agent with arterioselective vasodilator effects and also with well-documented antithrombotic effects. NO delivery from these RSNOs depends on redox enzymes like thioredoxins, proteins disulfide isomerase, gamma-glutamyl transferase activities[1,2]. We showed that the kinetic of NO release from RSNOs depending on their susceptibility to denitrosating enzymes and produced distinct efficiency to relax smooth muscle cells for example. We also demonstrated that RSNO is able to *S*-nitrosated different classes of proteins[3] modifying blood vessel vasomotricity[4] and are currently exploring the impact of this modification on their activities and location. Indeed, even if proteins *S*-nitrosation is studied from decades, everything is not known on targeted proteins and the impact of *S*-nitrosation on proteins activity.

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Identification of the c-Fos and IL8-Encoding Genes as Delayed Response Genes Following Resveratrol Stimulation

Gerald Thiel

Saarland University, Department of Medical Biochemistry and Molecular Biology, Homburg, Germany

Email: gerald.thiel@uks.eu

Stimulation with the phytoalexin resveratrol triggers the activation of stimulus-responsive transcription factors, leading to the transcription of delayed response genes. As part of a search for delayed response genes of resveratrol signaling, the gene encoding c-Fos was analyzed that encodes a basic region leucine zipper transcription factor. The results show that mutation of the binding sites for the transcription factors serum response factor (SRF), AP-1 and CREB within the c-Fos promoter significantly reduced reporter gene transcription in resveratrol-stimulated cells, indicating that resveratrol-induced upregulation of c-Fos gene transcription requires three genetic elements, the cAMP response element and the binding sites for SRF and AP-1, that function as independent resveratrol-responsive elements. In addition, the expression of interleukin-8 (IL-8) in resveratrol-stimulated cells was analyzed, because IL-8 expression is induced by a variety of stimuli, including cytokines, growth factors, and bacterial endotoxins. IL-8 is a proinflammatory cytokine that functions as a potent chemoattractant and neutrophil activator. The binding sites for the transcription factor NF-κB within the IL-8 gene promoter is identified to be essential for connecting resveratrol stimulation with IL-8 gene transcription. Thus, the κB site functions as resveratrol responsive element. This observation was corroborated by an experiment showing that resveratrol treatment significantly increased transcription of an NF-κB-responsive reporter gene. Accordingly, pharmacological inhibition of nuclear translocation of NF-κB attenuated the resveratrol-triggered increase in IL-8 promoter activity. Together, we conclude that the c-Fos and the IL-8 genes are delayed response genes of the resveratrol-induced signaling cascade using distinct stimulus-responsive transcription factors.

Functional Effects of Dietary Inorganic Nitrate in Humans

Paul G. Winyard, Mark Gilchrist and Nigel Benjamin

University of Exeter *Medical* School, St Luke's Campus, Exeter EX1 2LU, U.K.

A diet rich in green leafy vegetables lowers the risk of human cardiovascular disease. Leafy green vegetables contain relatively high concentrations of inorganic nitrate. According to the entero-salivary pathway, ingested inorganic nitrate is absorbed from the stomach and proximal small intestine into the bloodstream, and actively concentrated by the salivary glands. Nitrate is then released into the mouth as salivary nitrate at relatively high concentrations. Nitrate (NO_3^-) is converted to nitrite (NO_2^-) by bacteria present in the human oral cavity. Nitrite is absorbed from the stomach, and/or converted to nitric oxide (NO) and S-nitrosothiols. Oral inorganic nitrate lowers diastolic/systolic blood pressure and the oxygen-cost of exercise, presumably via nitric NO formation. This involves the chemical reduction - in human tissues such as the blood vessels - of nitrite and S-nitrosothiols to NO, perhaps involving xanthine oxidase-catalysed reduction of nitrite.

The observed physiological effects of inorganic nitrate have stimulated clinical dietary intervention studies, using high-nitrate foodstuffs such as beetroot juice. To determine the effect of nitrate in the human diet on blood plasma concentrations of nitrate, nitrite, S-nitrosothiols, blood pressure, exercise capacity and other outcomes, we used beetroot juice as a source of a known quantity of nitrate. Unfortunately, human dietary intervention studies are confounded by numerous (often unidentified) factors, such as compliance problems and a lack of suitable placebo control. To overcome these issues, we developed a novel nitrate-depleted form of beetroot juice. This was achieved using an ion exchange resin, providing sufficient quantities of nitrate-depleted beetroot juice to facilitate double-blind, placebo-controlled, crossover, clinical studies. As will be discussed, our placebo beetroot juice preparation has been widely adopted in clinical studies by our research group, as well as others.

Keywords: *Nitrate, nitrite, nitric oxide, S-nitrosothiol, entero-salivary pathway, bacteria, nitrate reductase, human, blood plasma, clinical study, diet, beetroot, blood pressure, exercise capacity*

Intestinal absorption of nitric oxide donors

Justine Bonetti, Yi Zhou, Marianne Parent, Isabelle Fries, Philippe Giummelly, Pierre Leroy, Caroline Gaucher

CITHEFOR EA 3452 "Drug targets, formulation and preclinical assessment", Faculté de Pharmacie, Université de Lorraine, Nancy, France

Along physiological aging or cardiovascular pathologies development like atherosclerosis or angina, a decrease of nitric oxide (NO) bioavailability is observed. In order to restore a physiological NO concentration, the use of NO donors, especially *S*-nitrosothiols is considered. *S*-nitrosothiols are the storage and transport form of NO in the organism, ensuring a higher half-life than its free-radical form (< 0.5 s). Most of the cardiovascular pathologies need a chronic treatment for which the oral route is voted. The intestinal absorption and influencing parameters (efflux, pH), are one of the first factors to study in order to evaluate the oral bioavailability of these NO donors. We evaluated three different *S*-nitrosothiols: *S*-nitrosoglutathione (GSNO), *S*-nitroso-*N*-acetylcysteine (NACNO) and *S*-nitroso-*D*-penicillamine (SNAP), respectively classified from the most to the least hydrophilic one. These *S*-nitrosothiols are likely to be absorbed as molecular entities or inorganic anions like nitrite ions and nitrate ions, the NO-derived species stable in aqueous media. Intestinal absorption of *S*-nitrosothiols was studied from a differentiated intestinal epithelium model composed of Caco-2 cells, cultivated on a porous membrane, enabling selective absorption of molecules. Results showed higher absorption of NO-derived species than entire molecules, a global medium permeability as well as a passive absorption mechanism of these *S*-nitrosothiols whatever their hydrophilicity is. *S*-nitrosothiols can be classified upon medium permeability and high solubility between class I and class III of the Biopharmaceutics Classification System Drugs. As class I and II drugs present a good correlation between *in vivo*/*in vitro* results, our results open a new possibility for *S*-nitrosothiols oral administration for cardiovascular disease treatment.

Keywords: *Nitric oxide, S-nitrosothiols, Caco-2, Passive absorption, Intestinal permeability*

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S-Nitrosoglutathione and Intestine Barrier Integrity on an *Ex Vivo* Rat Model of LPS-Induced Inflammation

Romain SCHMITT[a], Hui MING[a], Franck HANSMANNEL[b], Caroline GAUCHER[a], Isabelle LARTAUD[a], Pierre LEROY[a], Anne SAPIN[a]

a) CITHEFOR EA3452, Université de Lorraine, 5 rue Albert Lebrun, 54000 Nancy, France
b) NGERE UMRS954, Université de Lorraine, 9 avenue de la Forêt de Haye, 54000 Nancy, France

E-mail: romain.schmitt@univ-lorraine.fr

Inflammatory bowel diseases (IBD) such as Crohn's Disease and Ulcerative Colitis, are disabling pathologies affecting young patients. They undergo high recurrence of acute inflammatory episodes all over their life, and this may finally lead to colorectal cancer. Most of the current therapeutics have a low efficiency/cost ratio, act by down regulating chronic inflammation in the intestine mucosa but cannot cure the disease. The main shortcoming responsible for chronic inflammation seems to be failure of the gut barrier integrity. Nitric oxide secreted by enteric glial cells plays a pivotal role to maintain integrity of the intestine barrier [1,2]. Therefore, an innovative chronic treatment of IBD – in order to decrease the occurrence of inflammation, to improve patient quality of life and to decrease the risk of cancers - may rely on supplementation of small amounts of nitric oxide to preserve the intestine barrier integrity. As nitric oxide is an unstable radical, *S*-nitrosothiols, especially the endogenously produced *S*-nitrosoglutathione (GSNO), may be used as nitric oxide donors.

In this preliminary pre-clinical study, impacts of GSNO on epithelial cells junctions, inflammation and barrier permeability were evaluated, using an *ex vivo* model. The Ussing chamber model was composed of an oriented rat intestine tissue (0.25 cm^2, ileum segment) separating two compartments (luminal and mucosal). Intestinal barrier integrity was evaluated by measuring apparent permeability (Papp) of sodium fluorescein (NaFlu, medium permeability, paracellular transport). Different concentrations of GSNO (0.1 µM and 100 µM, above physiological conditions [3]) were pre-incubated with the intestine. Experiments were conducted in absence or presence of lipopolysaccharides (LPS, 100 µg/mL) in the luminal compartment to mimic inflammation [4]. Western blotting was also performed on rat intestine to check the expression of cell junction proteins (occludin, claudin-1, E-cadherin, β-catenin) and the expression of pro-inflammatory cytokines (IL-1β, TNF-α).

In healthy tissue (absence of LPS), high concentration of GSNO (100 µM) increased the NaFlu permeability without modification of cell junction proteins and inflammation markers expression. No modification of permeability was observed with low concentrations of GSNO. Furthermore, in stressed tissue (LPS added to the luminal compartment), preventing treatment with 0.1 µM GSNO during 1 h induced a decrease of NaFlu permeability when compared to control (without preventing treatment): GSNO seemed to improve barrier integrity. Expression of occludin and β-catenin decreased after LPS exposure, but this decrease was attenuated following a GSNO pre-incubation. However, there was no change in IL-1β and TNF-α expressions.

In conclusion, GSNO may be a new drug candidate to prevent barrier disruption and IBD induction.

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Keywords: *S-nitrosoglutathione, inflammation prevention, barrier disruption, Ussing chamber*

Lipid Peroxidation, Lipohormesis and Lipotoxicity in Insulin Secreting Beta Cells

Shlomo Sasson

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

Little is known about the impact of phospholipid turnover and metabolism on the function of insulin secreting pancreatic beta cells. We have discovered that increasing levels of glucose and saturated fatty acids (SFA, e.g., palmitic acid) activate the enzyme cPLA2, which remodels quickly phospholipids in membranes and releases polyunsaturated fatty acid (PUFA). Concomitantly, the enzyme Δ9-desaturase (SCD1), which transforms SFA into monounsaturated fatty acids (MUFA), increases the abundance of palmitoleic acid in the cells. These complex remodelling interactions profoundly alter the fluidity of the membranes of insulin granules in the cells, and thereby their function. We have also discovered that nutrient overload affects glucose-stimulated insulin secretion and cell viability in a biphasic manner: non-cytotoxic levels of glucose and palmitic acid induce a hormetic response that augments insulin secretion, which in turn can increase the rate of glucose and fatty acids disposal from the circulation to storage organs. Our studies show that this hormetic response is tightly linked to free radical-driven peroxidation of the released PUFA and their transformation to 4-hydroxyalkenals (e.g., 4-hydroxynonenal), which act at low levels as activating ligands of PPARδ. The latter augments the capacity of the cells to synthesize and secrete insulin in a regulated manner. Nonetheless, at higher levels 4-hydroxyalkenals become cytotoxic and induce beta cell dysfunction and death. Equally important, monounsaturated fatty acids (i.e., oleic acid and palmitoleic acid), products of desaturation of SFA by SCD1, protect beta cells against lipotoxic effects of SFA by enhancing their incorporation into triglycerides and their sequestration into newly formed lipid droplets. Collectively, our studies reveal the central role fatty acid metabolism and phospholipid turnover play in maintaining adequate beta cell function amid nutrient overload conditions and how this protective mechanism collapses under extreme nutritional conditions.

Keywords: *Beta cells; diabetes; fatty acids; lipohormesis; lipotoxcity; peroxidation.*

Evidence for Accelerated Ageing in Type 2-Diabetes: RNA Oxidation Predicts Survival in Newly Diagnosed as well as in Manifest Type 2-Diabetes

Henrik Enghusen Poulsen MD, Laura Kofoed Kjær MD, Vanja Cejvanovic MD, Trine Henriksen, Torben Hansen MD, Oluf Pedersen MD, Cramer Kjeldahl Christensen MD, Christian Torp-Pedersen MD, DMSc, Thomas Alexander Gerds, Ivan Brandslund MD, DMSc

Bispebjerg Frederiksberg Hospital, Capital Region of Copenhagen, Institute of Clinical Medicine, Health Science Faculty, University of Copenhagen

Efficacy has been demonstrated for microvascular complications for glycose lowering drugs, lipid control and for BT control in type 2-diabetes (T2D). The major events, however, are macrovascular complications. High quality controlled clinical trials (hard primary endpoints) for glucose lowering drugs are practically absent. Intensive glucose control trials indicate negative effects, consistent with a U-shaped relationship between HbA1c and mortality.

It is well documented that mitochondrial respiration is dysfunctional in T2D and leads to surplus production of reactive oxygen species. The oxidation of important macromolecules, cellular disturbances, dysfunction and cell death, is believed to follow, leading to disease and death. A biomarker for such events is the urinary excretion of the oxidized Guanine moiety of RNA, the oxidized nucleotide 8oxoGuanosine.

Obese males are high oxidizers (high urinary excretion of 8oxoGuo) compared with matched normal weight males. Newly diagnosed T2D patients, followed for 18 years, have a HR of dying of about 2 after adjusting for all known risk factors, including HbA1c based on their oxidized status. Their change in oxidizer status over 6 years (e.g. high quartile to low quartile) gives a HR of dying of 0.3.

In a cohort of manifest type 2-diabetes, a similar HR for death was found, and furthermore this HR was also significant for death from macrovascular complications.

Comparison of oxidizer status measured by RNA oxidation in type 2-diabetes (n=2738) and a non- diabetic control group (n=4796) showed higher RNA oxidation in type 2-diabetes than in controls, but also that for the same oxidizer status HR for dying was higher for type 2-diabetes.

Diet intervention with e.g. olive oil, and intervention with non-diabetes drugs seems to reduce oxidative stress to nucleic acids, but the effects are not clear and well defined.

These data support that oxidative stress is a mechanism for ageing, and that type 2-diabetes can be characterized as a disease with accelerated ageing.

Furthermore, oxidizer status is suggested as a novel clinical biomarker superior to blood glucose control markers such as HbA1c, because it reflects metabolic intracellular derangement as opposed to extracellular/plasma derangement.

Keywords: *oxidative stress, type 2-diabetes, ageing, RNA oxidation*

Nutrigenomics of Vitamin D: the Personalized Vitamin D Response Index

Carsten Carlberg

School of Medicine, Institute of Biomedicine, University of Eastern Finland, Kuopio, Finland

Monocytes are a human cell type that is well responsive to 1,25-dihydroxyvitamin D_3 (1,25D) and serve a reference for other human cell types and our human *in vivo* investigations. Triplicate RNA-seq (RNA expression), FAIRE-seq (accessible chromatin) and ChIP-seq (genomic association of the proteins VDR, PU.1 and CTCF, histone markers H3K27 and H3K4me3) datasets describe the response of the THP-1 monocyte cell culture model to stimulation with 1,25D. Within 24 h the transcription of more than 500 genes is significantly modulated by several thousand genomic VDR binding sites in concert with the pioneer transcription factor PU.1 and the 3D-chromatin organizer CTCF. At present we extrapolate the models created on the basis of these data to the *in vivo* response of human subjects to vitamin D supplementation. Based on our long-term (VitDmet, NCT01479933) and short-term (VitDbol, NCT02063334) vitamin D intervention studies we found that individuals can be distinguished into high, mid and low responders to vitamin D, i.e. that humans have personalized vitamin D response index. The group low responders comprises approximately 25% of the population; they will suffer most from an insufficient vitamin D status. In order to understand the molecular basis of the individual vitamin D responsiveness, we used RNA and chromatin was immediately prepared from peripheral blood mononuclear cells (PBMCs) of the VitDbol subjects, i.e. the cells were not further cultured or manipulated *ex vivo*. RNA-seq analysis indicated several hundred genes significantly responding 24 h after vitamin D supplementation. Moreover, FAIRE-seq demonstrated that within the same time frame a comparable number of chromatin sites increased in their accessibility. Computational tools, such as network analysis and self-organizing maps, indicated comparable principles of vitamin D signaling *in vivo* (PBMCs) and *in vitro* (THP-1) both on the level of target genes as well as on genomic VDR binding sites. In conclusion, long-term and short-term vitamin D supplementation studies allow monitoring different aspects of the vitamin D responsiveness of human individuals and represent new types of human *in vivo* vitamin D investigations. These studies demonstrate that vitamin D and its metabolites have a direct effect on the human epigenome and modulate the response of the transcriptome in a personalized fashion.

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Autophagy Modulates Survival during Mitotic Arrest by Involving p62 Protein in Colon Cancer

Esma Yagdi Efe[1], Claudia Cerella[1], Anthoula Gaigneaux[1], Patrick Chaimbeaux[2], Mario Dicato[1], Claus Jacob[3], Marc Diederich[4]

[1] *Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Hôpital Kirchberg, 9, rue Edward Steichen, L-2540 Luxembourg, Luxembourg*
[2] *Université de Lorraine, CNRS, UMR 7565, SRSMC, F-54506 Vandœuvre-lès-Nancy, France*
[3] *Department of Pharmacy, Saarland State University, D-66123 Saarbruecken, Germany*
[4] *Department of Pharmacy, College of Pharmacy, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea*

Colorectal cancer (CRC) is the second leading cause of cancer-related death worldwide. Epidemiological studies reported the chemopreventive effect of a diet rich in onions- and garlic-derived organic sulfur compounds (OSCs) in CRC. Our previous studies attributed the anticancer potential of OSCs to their ability to inhibit cell growth, thereby triggering apoptotic cell death [1-4]. We aim here to translate the molecular effects observed towards the treatment of CRC by understanding implication of cell cycle and autophagic flux in CRC survival and therapy.

Mass spectrometry analysis showed that DBTTS targets tubulin and interfere with microtubule network highly comparable to clinically used microtubule-disrupting drugs. DBTTS induces persistent mitotic arrest followed by apoptosis in HT-29, SW480 and SW620 CRC cell lines. As a potential anticancer drug candidate, DBTTS inhibited spheroids and colony formation in a concentration-dependent manner in CRC cell lines without triggering acute toxicity in Zebrafish.

Autophagy is a crucial cell survival mechanism in cancer. Monitoring autophagic flux in DBTTS-treated HT-29 cells with a green fluorescent protein-microtubule-associated protein light chain 3 (LC3) expression construct revealed the accumulation of autophagosomes, documented by transmission electron microscopy and further confirmed by Western blot of p62 accumulation and LC3-I to LC3-II conversion. Autophagy modulation occurred concomitantly with mitotic arrest in HT-29 cells accompanied by upregulation of antioxidant heme oxygenease-1 (HO-1) and p62 at mRNA levels, as assessed by real time PCR in HT-29 cells. In contrast, we observed no modulation of autophagy, HO-1 and p62 mRNA levels in SW480 and SW620 cells, which were faster committed to death.

Our results together with bioinformatics approach on the association between autophagy activation and the overall survival in 489 colorectal cancer samples from the TCGA dataset suggest that autophagy impairment may act as a survival modulator during mitotic arrest by potentially involving p62 scaffold protein and antioxidant heme oxygenase-1.

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Leptadenia Hastata Leaf Extracts Reduce Bodyweight Gain and Improve Insulin Sensitivity in Two Animal Models of Obesity and Insulin Resistance

Mohamed S. Zaibi[1], Aminu Chika[2], Małgorzata A. Kępczyńska[1], Edward T. Wargent[1], Claire J. Stocker[1], Suriyah Zardlikawi[1], Shaibu O. Bello[2] and David C. Hislop[1].

[1] *Buckingham Institute for Translational Medicine (BITM); Clore Laboratory, University of Buckingham, MK18 1EG, Buckingham, UK*
[2] *Department of Pharmacology and Therapeutics; College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria*

Background and aim: *Leptadenia hastata* (LH) is an edible vegetable and medicinal plant used traditionally in sub-Saharan African countries for various diseases. We have investigated the potential anti-obesity and anti-diabetic effects of aqueous and methanol leaf extracts of LH in high fat diet-induced obese mice and leptin-deficient (*ob/ob*) mice.

Methods: C57Bl/6 female mice fed 60% high fat diet (HFD), and leptin-deficient (*ob/ob*) male mice (fed chow diet) were treated for 6 weeks with 250 $mg.kg^{-1}$ of LH aqueous or methanol extracts. The time course of changes in food intake, body weight, body fat, energy expenditure, blood glucose and plasma levels of insulin and leptin (for HFD mice) were determined. *In vitro* effects of both extracts on lipolysis and lipogenesis were also investigated.

Results: In HFD animals, the treatment with aqueous or methanol extracts resulted in a significant reduction ($p<0.05$) in body weight (16.6% and 18.7%) and food intake (10% and 11%), with a significant increase in 24h energy expenditure (53.3 and 61.4%). These effects were coupled with a significant decrease in absolute and percentage fat mass ($p<0.05$), and in plasma leptin levels (2.8 and 3.5 fold change). Both extracts also improved ($p<0.05$) glucose tolerance and reduced fasted blood glucose and plasma insulin levels. Consequently, HOMA-IR was reduced by 65% (compared to control group). In *ob/ob* mice, the chronic treatment with methanol extract resulted in a significant reduction in cumulative body weight gain ($p<0.001$), an improvement in both oral glucose and insulin tolerance tests ($p<0.01$ and $p<0.001$, respectively) and a decrease in fasted plasma insulin by 64%. *In vitro*, the LH extracts decreased lipogenesis in human pre-adipocytes and increased lipolysis in mouse primary adipocytes.

Conclusions: LH would be beneficial as a dietary supplement in the treatment of obesity and insulin resistance related to high fat diet consumption, acting via a reduction of food intake and fat mass and an elevation of energy expenditure and improvement of insulin sensitivity.

Atypical Degradation of PARP-1 and Necrotic Cell Death Mediated by Methylated Indolequinone in Chronic Myeloid Leukemia Cells is Prevented by New Off Target Function of 3-Aminobenzamide

Barbora Orlikova[1,2], Claudia Cerella1,[2], Michael Schnekenburger[2], Mario Dicato[2], Marc Diederich[1]

[1]College of Pharmacy, Seoul National University, Republic of Korea
[2] Laboratoire de Biologie Moléculaire et Cellulaire du Cancer (LBMCC), Luxembourg

Indolequinone derivatives act as potent antitumor agents. So far, NAD(P)H quinone oxidoreductase 1 (NQO1) and thioredoxin reductase (TrxR) are considered as essential targets. Nevertheless, additional mechanisms implicated in the compound cytotoxic anticancer effects remain to be investigated.

We provide the evidence, that methylated indolequinone powerfully induces both caspase-dependent and –independent cell death mechanisms in different human leukemia cells. In K562 chronic myeloid leukemia cell line we witness induction of necrosis by caspase-independent cell death mechanisms resulting in non-apoptotic degradation of poly ADP ribose polymerase (PARP). Moreover, phosphorylation of Ser139 of histone H2AX indicates generation of DNA double strand breaks, another hallmark of this non-canonical cell death pathway (1). Additionally, we observed partial protective effects and a shift from necrotic to apoptotic cell death once cells were pre-treated with 3-aminobenzamide (3-ABA), an established PARP inhibitor. Surprisingly, we observed, that 3-ABA prevented also indolequinone-induced apoptosis in U937 cells, raising attention to the fact, that protective effects of 3-ABA are no linked to its PARP-inhibition properties. Docking analyses predicted that methylated indolequinone binds to the human PARP-1 protein with very high affinity. On the other hand a hypothetical interaction by Michael reaction between 3-ABA (Michael donor) and methylated indolequinone (Michael acceptor) showed high affinity binding to PARP-1 protein as well. We hypothesize that interaction with 3-ABA is responsible for inhibition of necrotic cell death in K562 cells as well as apoptotic cell death in U937 cells induced by methylated indolequinone. Interestingly, these findings provide important evidence about a new function of 3-ABA as an interaction partner with compounds susceptible to undergo Michael additions. Besides its well-known role as a PARP-inhibitor, binding options with other compound of interest resulting in alterations of compound-mediated mechanisms of action have to be considered and elucidated

Keywords: *Indolequinones, Cell death, PARP, 3-aminobenzamide*

Hdl Protein Composition as a Modulator of Lipoprotein Functionality: Evidence from Clinical Cases and Animals Studies

K. E. Kypreos[1], S. Filou[1], E. Zvintzou[1], P.I. Petropoulou[1], M. Lhomme[2], A. Kontush[3], E.A. Karavia[1]

(1) University of Patras Medical School, Patras, Greece (2) ICANalytics, ICAN, Paris, France (3) INSERM UMR_S 1166-ICAN, Faculté de Médecine Pitié-Salpêtrière, Paris, France

High density lipoprotein (HDL) has been for years an intriguing lipoprotein which attracted the attention of biomedical community, mainly because of its important role in atheroprotection. Even though HDL-cholesterol is usually referred to as the “good cholesterol”, certainly it is far more than just a “cholesterol”. HDL is a macromolecular assembly of proteins and lipids formed in the circulation as a result of a concerted action of apolipoproteins, lipid transporters and plasma enzymes. Studies in cell cultures as well as in experimental mice showed that biogenesis of classical Apoa1-containing HDL particles (Apoa1-HDL) involves lipid transporters ATP-binding cassette A1 (Abca1) and G1 (Abcg1) and plasma enzyme Lecithin:Cholesterol Acyl Transferase (Lcat).

Previously, we showed in mice that other apolipoproteins such as apolipoprotein E (APOE) and apolipoprotein CIII (APOC3) are also capable of promoting the *de novo* biogenesis of HDL in the absence of a functional APOA1. In addition to the studies in mice, we recently observed the existence of APOE-HDL and APOC3-HDL particles in the circulation of morbidly obese human subjects; analysis of HDL particle composition showed that rapid weight loss was associated with a significant switch from primarily APOE-HDL and APOC3-HDL to primarily APOA1-HDL displaying increased antioxidant capacity. In another clinical paradigm we observed that young asymptomatic subjects (≤35 years of age) who suffered an acute non-fatal myocardial infarction possessed elevated levels of plasma APOE-HDL and APOC3-HDL that correlated with reduced antioxidant potential. These clinical observations supported the hypothesis that variations in HDL apolipoprotein composition may set basis for its functional heterogeneity. Indeed, our more recent data support the contention that APOA1-HDLs are functionally distinct from APOE-HDL particles and that HDL proteome determines its lipidome.

The apparent differences in the HDL apolipoprotein content, lipidome and functionality between APOE3-HDL and APOA1-HDL that we identified through our preclinical and clinical studies reinforce the idea that not all HDL particles are equally active and that apolipoprotein composition is a key factor for defining HDL lipid content and particle functionality. Therefore, creation of effective pharmaceuticals that aim at improving HDL functionality requires deep understanding of the impact of apolipoprotein composition of HDL on its properties associated with protection from atherosclerosis and possibly from other metabolic disorders.

Keywords: *High density lipoprotein, antioxidant, antinflammatory, cholesterol efflux, functionality, proteome, lipidome*

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Cellular Reprogramming *via* Epi-CRISPRs-Induced Targeted DNA Methylation

Melita Vidaković[1], Marija Sinadinović[1], Jovana Rajić[1], Jelena Arambašić Jovanović[1], Nevena Grdović[1], Anja Tolić[1], Miloš Đorđević[1], Mirjana Mihailović[1], Aleksandra Uskoković[1], Goran Poznanović[1], Svetlana Dinić[1], Tomasz P Jurkowski[2]

[1]Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia; [2]Institute for Biochemistry, University of Stuttgart, Stuttgart, Germany

Diabetes is the perfect candidate for cell replacement therapy, since it is caused by either an absolute (type 1 diabetes) or relative (type 2 diabetes) defect of insulin-producing pancreatic beta cells. Our research is focused on applying a novel synthetic epigenetic tool (Epi-CRISPRs) for straightforward, one-step mouse pancreatic alpha (a-cells) to beta cell transdifferentiation by targeted DNA methylation and suppression of genes essential for maintaining pancreatic cell identity (homeobox Arx gene (Arx)). Up to now, we succeeded to transiently transfect a-cells with Epi-CRISPR constructs and 275 gRNA or mix gRNA. The suppression of Arx in a-cells was confirmed on day 5 and 8 post-transfection. The reduction of glucagon synthesis and beginning of insulin production in transfected a-cell was confirmed and visualised by immunostaining. DNA methylation-mediated suppression of Arx in a-cells leads to their transdifferentiation to insulin-producing beta cells will be confirmed by bisulfite sequencing (undergoing experiments).

Furthermore, we are also investigating an epithelial-mesenchymal transition (ETM), the mechanism which underlies the progressive decline in organ functioning in diabetes, such as the development of kidney and liver fibrosis. ETM is a process of reprogramming epithelial cells from a fully differentiated epithelial state to a more mesenchymal state. Our aim is to analyse the DNA methylation profile and gene expression of either epithelial (E-cadherin) or mesenchymal markers (α-smooth muscle actin and fibronectin) whose differential methylation and gene suppression could lead to more epithelial-like or mesenchyme-like phenotype. This will be accomplished using an *in vitro* model system based on epithelial cells treated with TGF-β1 and 2. The obtained data should enable ETM reversal and stop fibrosis in diabetes and other pathologies using different compounds that act as DNA methylating/demethylating agents or using Epi-CRISPRs-based targeted DNA methylation/demethylation in future.

We are on the way to develop a clear-cut technology able to provide a perfect delivery system for increase of insulin-producing cells *in vitro*. This system will allow for targeted gene silencing via increased DNA methylation of gene of interest. In addition, we are able to test if any compound used for treatment in different pathological conditions affects the DNA methylation profile of the examined cells. On the other hand, there is a great need for chemical compounds able to act as DNA hypo or hypermethylated agents.

***Keywords**: DNA methylation and demethylation, diabetes, Epi-CRISPRs, epithelial-mesenchymal transition, genome editing, pancreatic alpha cells.*

The Protective Role of Metabolic Antioxidant Alpha Lipoic Acid and Exercise Training in Diabetes

Mustafa Atalay

School of Medicine, Institute of Biomedicine, Physiology, University of Eastern Finland, Kuopio, Finland

While a basal level of reactive oxygen species (ROS) is required to drive redox signalling and numerous physiological processes, excess ROS production during exercise may have adverse implications on health and performance. Antioxidant nutrients may be helpful in that regard. On the other hand, prolonged mega-dose supplementation of micronutrients may attenuate the physiological exercise-related responses of tissues and blunt the training-induced adaptations.

Lipoic acid (thioctic acid or lipoate, LA) is a natural thiol antioxidant with metabolic effects. Early studies of our group confirmed insulin-mimetic functions of LA. Our studies in animal models indicated that LA is able to enhance tissue antioxidant defences by sparing glutathione levels. Notably, even at lower doses LA supplementation reduced the rate of free radical formation in skeletal muscle and simultaneously decreased the concentrations of oxidised protein and lipid peroxidation products at rest and in response to exercise.

In addition to physical exercise, heat shock proteins (stress proteins, HSPs) can be induced or modulated by pharmacological agents or dietary supplements. We demonstrated that although at high doses LA supplementation blunted exercise training-induced HSP levels, at lower loses LA enhanced skeletal muscle HSP response along with evidence for the induction of oxidative metabolism and attenuation of exercise-induced muscle damage. Moreover, our studies demonstrated that LA supplementation may contribute to the protection of other tissues including brain, kidney and liver in streptozotocin-induced diabetic rats.

Based on our results, it can be concluded that LA supplementation may be useful to enhance tissue protection and improve physical performance by inducing antioxidant protection, decreasing formation of free radicals and increasing the aerobic capacity and the rate of stress protein expression.

Gut Microbiota as a Central Mediator of the Metabolic Status of the Host

Isabel Moreno-Indias

Unidad de Gestión Clínica de Endocrinología y Nutrición, Hospital Virgen de la Victoria, Instituto de Investigación Biomédica de Málaga, Universidad de Málaga, 29010 Málaga, Spain; Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición 28029 Madrid, Spain

The idea that gut microbiota influence host health has gone from being a popular belief to being clearly referenced by the most prestigious scientists worldwide. In this manner, gut microbiota has been stated as a super-organ involved in multiple functions within the host. Many factors influence gut microbiota, and diet is one of the most important one. Western diets, mainly characterized by high fat amounts, have demonstrated to produce dysbiosis in gut microbiota, with a deleterious impact on the host's health. On the other hand, Mediterranean diet, rich in antioxidants, provides the necessary elements for its development. One example of a Mediterranean aliment is the red wine, which is rich in polyphenols. These polyphenols modulates gut microbiota [1]. Moreover, red wine polyphenols are able to ameliorate the parameters of the metabolic syndrome in obese patients [2]. But not only diet is able to affect gut microbiota ecology, other challenges like sex hormones in specific moments of the life, as an androgenisation or the retirement of the female hormones, are able to alter the gut microbiota profile and influence in the adult female phenotype and contribute to metabolic disturbances [3]. However, other disturbances should be taken into account for the complete understanding of this super-organ, such as the use of medications, such as the antibiotics, or the colonization patterns.

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Keywords: *gut microbiota, metabolic syndrome, Mediterranean Diet, Sex hormones, dysbiosis*

POSTERS COMMUNICATIONS

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miRNA Modulation by Resveratrol in Human Fibroblasts with Fatty Acid Oxidation-Deficiency as Consequence of Carnitine-Palmitoyl Transferase 2 (CPT2) Mutation.

[1,2]Virginie Aires, [1]Jean-Jacques Michaille, [1,2]Dominique Delmas, [3]Fatima Djouadi, [3]Jean Bastin, [1]Mustapha Cherkaoui Malki & [1]Norbert Latruffe

[1]Université de Burgundy, Laboratoire Bio-PeroxIL, Dijon, France
[2]INSERM UMR 866, Dijon, France
[3]INSERM U1124, Université Paris Descartes, Paris, France

Resveratrol is a well known phytophenol produced in huge amount by grape plant in response to biotic or a-biotic stresses. Related to its anti-oxidant properties, numerous papers established that the main targets of resveratrol are the transcription factors AP-1, NFκB, STAT-3 and COX genes. Interestingly, the effects of resveratrol depend on at least in part upon miRNAs whether they are anti- inflammatory (ex. miR-663), pro-inflammatory (ex. miR-155), tumor suppressors (ex. miR-663) or oncogenic (ex. miR-21). In addition to the above-mentioned functions, changes in miRNA levels are observed in mitochondrial fatty acid oxidation deficient-human fibroblasts harboring mutations in Carnitine-Palmitoyl Transferase 2 (CPT2) gene. Indeed, we reported that resveratrol is able to boost residual CPT2 activities in human fibroblasts derived from patients having CTP2 deficiencies and can restore normal fatty acids oxidation rates (V. Aires et al, *J Orphanet Rare Dis*, 2014). Changes are observed in mi-RNA expression in human fibroblast from subjects having (CPT2-deficient patient) or not (Control) a mitochondrial CPT2 deficiency. CPT2 deficiency either increases or decreases the expression of miRNAs. For instance, miR-301 was down-regulated by resveratrol in control cells, while miR-10b level was not modified either in control or in CPT2-deficient cells. In addition, resveratrol differently affected the levels of 193 miRNAs in CPT2-deficient cells as compared with control (WT) cells. For instance, following resveratrol treatment, the levels of miR-110a, miR-110b and miR-495 were respectively 2.9, 3.6 and 2.3 lower in CPT2-deficient fibroblasts as compared with control fibroblasts. Based on this study, miRNA changes depend on the cell genotype and are associated to the resveratrol-dependent stimulation of mitochondrial fatty acid oxidation in CPT2-deficient cells.

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The impact of Flawan-3-ols on Expression of Genes Related to Oxidative Stress and Antioxidant Defence

Monika Baranowska, Klaudia Suliborska, Wojciech Chrzanowski, Jacek Namieśnik, Agnieszka Bartoszek

Faculty of Chemistry, Gdansk University of Technology, Poland

Email: monbaran1@student.pg.gda.pl

Over the past three decades, the understanding of the role of diet derived polyphenolic antioxidant compounds in disease prevention has grown enormously. They were initially considered as simple ROS scavengers, because of their strong *in vitro* antioxidant activity, which was linked to prevention of diseases associated with oxidative stress mechanisms.

However, it must be taken into account that only small amounts of polyphenolic antioxidants are absorbed from alimentary tract and become bioavailable. The low concentration in body cells is not the only limiting factor. Their contribution to the total antioxidant activity of the body depends on a number of other factors among which redox properties are fundamental. Moreover, polyphenols and their derivatives arising after metabolism, are suggested to be not only redox active substances, but the molecules affecting signal transduction and gene expression as well.

Currently, there is no systematized knowledge about the relationship between redox properties of various antioxidant polyphenols and their biological behaviour in situation of ROS challenge. Furthermore, it is little known which genes related to oxidative stress and antioxidant defence are regulated by these antioxidants. Neither there is information if stimulation or inhibition of these genes is related rather to chemical structure or is associated with redox properties of polyphenols.

The aim of the study was to clarify the impact of structurally different compounds from flawan-3-ols group ((+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate) on expression of genes involved in redox homeostasis in human colon carcinoma HT29 cell line. For this purpose, real-time PCR array consisting of 84 genes involved in oxidative stress response and antioxidant defence was employed.

The results suggest the role of these polyphenols as gene expression modifiers, whose impact seems related to chemical structure. We try to combine the transcriptomic data with the information about redox properties, as well as biological activity assessed in the same cellular model to propose grounds for predictions of the chemopreventive value of investigated antioxidants.

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Keywords: *oxidative stress, antioxidant properties, gene expression, flawan-3-ols, real-time PCR*

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Estimation of Dietary Fatty Acid Intake in Mediterranean Old Adults

M.M. Bibiloni[1], J. Karam[2], A. Julibert[1], E. Argelich[1], J.M. Gámez[1], I. Llompart[1], A. Sureda[1], A. Pons[1], J.A. Tur[1]

[1]Research Group in Community Nutrition and Oxidative Stress, University of Balearic Islands, Palma de Mallorca and CIBEROBN (Fisiopatología de la Obesidad y la Nutrición CB12/03/30038) Instituto de Salud Carlos III, Madrid, Spain. [2]Faculty of Health Sciences, University of Balamand, Balamand Al Kurah, Lebanon.

E-mail: nucox@uib.es

Introduction: Fats provide food a unique flavor and texture, they are as well a major source of energy, help absorbing and transporting carotenoids and fat-soluble vitamins, are precursors of signaling molecules and are an essential component of cell membranes.

Objective: Assessment of the lipid dietary intake, fatty acids intake and food sources in a sample of Mediterranean old adults.

Methods: The study was conducted in 211 participants dwelling women (*n*= 112) and men (*n*= 99). Lipid and fatty acid intake were assessed using a Spanish database.

Results: The mean intake of lipids was 68.6 g/day (standard deviation, SD: 24.6; 34.4%, SD: 7.0 of total energy consumed). Men consumed more lipids than women. Younger participants and those with a higher education consumed more lipids than the other ones. The main sources of fat were MUFA (16.7%, SD: 4.1), followed by SFA (9.6%, SD: 2.6) and PUFA (5.0%, SD: 1.7). Oils & seeds were the major contributors in the lipid intake (38.8%, SD: 16.0), MUFA (53.9%, SD: 18.7) and PUFA (33.0%, SD: 16.4). The intake of the main classes of fatty acids did not abide by the International (PUFA) and Spanish recommendations (SFA, MUFA). The intake of ALA, EPA and DHA were lower than recommendations. The cholesterol intake (243.9 mg, SD: 140.4) was within the range of the Spanish recommendations.

Conclusions: Fat intake was assessed according to sex, age class and education level and results were shown to be statistically significant. The main dietary fat source was MUFA, abundantly found in the Mediterranean diet, and the most contributing food to lipid intake was oils & seeds. However, the fatty acids intake did not abide by the recommendations for all the observed ones.

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2,3-Dehydroflavonolignans as the Active Principle of Silymarin

VALENTOVÁ, Kateřina,[1] BIEDERMANN, David,[1] VRBA, Jiří,[2] ULRICHOVÁ, Jitka,[2] KŘEN Vladimír[1]

[1] Laboratory of Biotransformation, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic
[2]Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic

Email: biedermann@biomed.cas.cz

Introduction: *Silybum marianum* fruit extract silymarin displays antioxidant, anti-inflammatory, immunomodulatory and hepatoprotective properties. Silymarin contains over ten structurally related flavonolignans; main constituents[1] are silybin A, silybin B, isosilybin B, silychristin A, silydianin, taxifolin and polymers. Silybin is considered as the "major active principle" of silymarin because it is the most abundant and hence easily isolated[2]. 2,3-Dehydroflavonolignans occur as a result of bio-oxidation in the plant itself or due to oxidation during extraction and processing[3] and have been neglected for a long time, mainly due to their complicated isolation.

Material & Methods: 2,3-Dehydrosilybin, 2,3-dehydrosilychristin and 2,3-dehydrosilydianin were prepared from respective flavonolignans by optimized oxidative methods[3-4]. Radical-scavenging[3], cytotoxic[4] and cytoprotective[3] activities of all compounds were compared to those of parent flavonolignans.

Results: All compounds were successfully prepared in gram amounts. The 2,3-dehydroflavonolignans proved to be one to two orders of magnitude more active than respective parent compounds.

Conclusion: Although 2,3-dehydroflavonolignans occur as minorities, they may be responsible for the majority of biological activities of silymarin.

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Interactions of Divalent Minerals and Carotenoids during Digestion

Corte-Real J.[1,2,4], Guignard C.[2], Gatenbein M.[1], Bernard W.[3], Burgard K.[3], Hoffmann L.[2], Richling E[4], Bohn T.[1]

[1] Luxembourg Institute of Health, Population Health Department, 1A-B, rue Thomas Edison, L-1445 Strassen, Luxembourg. [2] Luxembourg Institute of Science and Technology, Environmental Research and Innovation Department, 41, rue du Brill, L-4422 Belvaux, Luxembourg. [3] Laboratoires Réunis Junglinster, 38, rue Hiehl, L-6131 Junglinster, Luxembourg. [4] Food Chemistry and Toxicology, Department of Chemistry, University of Kaiserslautern, Erwin-Schroedinger-Strasse 52, D-67663 Kaiserslautern, Germany.

Email: joana.corte-real@outlook.com

BACKGROUND: Previously we have shown that calcium (Ca^{2+}), and other divalent minerals (DM), have the ability to negatively affect the *in vitro* bioaccessibility of pure and dietary carotenoid (1,2). At concentrations within the supplement range, Ca^{2+} prevented the micellarization of carotenoids to undetectable levels. We hypothesized that the negative effects of DM result from the formation of insoluble bile and fatty acids calcium salts and soaps, respectively, during intestinal digestion, preventing the formation of mixed micelles and hence, uptake of carotenoids.

OBJECTIVE: Our objective was to test this hypothesis *in vivo* by testing whether Ca^{2+} supplementation affected carotenoid bioavailability from a carotenoid rich meal.

DESIGN: Twenty-four healthy and free living males, 19 to 50 years of age, completed a randomized and double blind placebo controlled cross-over postprandial trial, testing the effect of 3 supplementary Ca^{2+} doses (0mg, 500mg and 1000mg) on the bioavailability of carotenoids from a spinach meal. Each participant completed 3 full day clinical visits, during which he consumed for breakfast a 270g spinach meal (c.a. 23mg of carotenoids) seasoned with 18mL of rapeseed oil, and was given a randomly assigned Ca^{2+}supplement. Post-prandial blood samples were collected throughout the day (over a 10h period) and bioavailability was assessed as the Area-Under-the-Curve (AUC) of time vs. concentration of carotenoids extracted from the plasma triacylglycerol-rich-lipoprotein (TRL) fractions (reflecting newly absorbed carotenoids).

RESULTS: Statistical analysis of the baseline corrected carotenoids' AUC, between different meal groups, showed no significant differences regarding the effect of the Ca^{2+} doses. However, another recent study reported a significant decreased of lycopene's plasma concentration following the intake of 500mg of Ca^{2+} (3). Hence, we propose that these contradictory results relative to the hypothesis reflect a matrix-related effect, dose effect, or specific carotenoid effects. The effect of DM is worth being taken into consideration in future human trials targeting carotenoid bioavailability.

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Keywords: *absorption, divalent minerals, lutein, β-carotene, cross-over trial*

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The Protective Effect of *Opuntia Ficus Indica* Seeds against the Cytotoxicity Induced by Iron Overload: Chemical Analyses and *In Vitro* Studies in the Protozoan *Tetrahymena Pyriformis*.

Karym EL-Mostafa[1, 2], Bouchab Habiba [1, 2] , Badreddine Asma[1, 2], Thomas Nury[2], Abdelkhalid Essamadi[1], Khadija Moustaid[3], Pierre Andreoletti[2], Mustapha Cherkaoui-Malki[2],Gérard Lizard[2], & Boubker Nasser [1].

[1]*Laboratoire de Biochimie et Neurosciences, Faculté des Sciences et Techniques, Université Hassan I, BP577, 26000 Settat, Morocco.*
[2]*Laboratoire Bio-PeroxIL (Biochimie du Peroxysome, Inflammation et Métabolisme Lipidique), EA7270 Univ. Burgundy Franche-Comté / Inserm, 21000, Dijon, France;*
[3]*Laboratoire de Chimie appliquée et Environnement, Faculté des Sciences et Techniques, Université Hassan I, BP577, 26000 Settat, Morocco.*

Iron is a vital nutrient in human life. However, iron can also trigger oxidative stress. An abnormal accumulation of iron is reported in the development of tumors and in neurodegenerative diseases. Thus, the use of molecules with antioxidant and iron-chelation properties could be an effective approach for cancer prevention and neuroprotection. Here, we aimed at evaluating the protective effect of *Opuntia ficus indica* seeds against iron toxicity using the protozoa *Tetrahymena pyriformis* as biological model. The chemical evaluation of the antioxidant and the iron-chelation properties of cactus was carried out with the FRAP (Ferric Reduction/Antioxidant Potential) test and the Ferrozine test. Such chemical analyses revealed the presence of significant antioxidant properties and iron-chelation activities in *Opuntia ficus indica* seeds. This may be related to the polyphenols and flavonoids contents. Using Folin-Ciocalteu and $AlCl_3$ tests as well as HPLC analysis, we estimated seed polyphenols content. We identified interesting levels of catechin (54%), rutin (21%), quercetin (7%), dihydroxycinnamic acid (6%) and gallic acid (2%). The *in vitro* study with *Tetrahymena pyriformis* culture showed that the aqueous seed extract attenuates the toxic effects of iron: increased viability and cellular activity, decreased iron accumulation and normalized oxidative stress markers. These protective effects of *Opuntia ficus indica* seeds against the deleterious consequences associated with the abnormal iron accumulation might open new pharmacological perspectives in their use in the human healthcare.

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Mediterranean Plant Extracts Ameliorate Cellular and Animal Models of Neurodegenerative Diseases

Mario Caruana[1,2], Neville Vassallo[1,2], Ruben J.Cauchi[1,2], Michelle Briffa[1,2,3], Stephanie Ghio[1,2], Johanna Neuner[2], Alison J.Gauci[2], Rebecca Cacciottolo[1,2], Christelle Marchal[3], Christopher Cullin[3]

[1]Centre for Molecular Medicine and Biobanking, Biomedical Sciences Building, University of Malta, Msida, Malta.
[2]Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Msida, Malta.
[3]Institute de Chimie et de Biologie des Membranes et des Nano-Objets, CNRS-UMR5248, Université Bordeaux, Pessac, France.

A signature feature of age-related neurodegenerative proteinopathies is the misfolding and aggregation of proteins, typically amyloid-β(A β) in Alzheimer's disease (AD) and α-synuclein (α-syn) in Parkinson's disease (PD), into soluble oligomeric structures that are highly neurotoxic. Cellular and animal models that faithfully replicate the hallmark features of these disorders are being increasing exploited to identify disease-modifying compounds. Natural compounds have been identified as a useful source of bioactive molecules with promising neuroprotective capabilities. In the present report, we investigated whether extracts derived from two ubiquitous Mediterranean plants namely, the prickly pear *Opuntia ficus-indica* (EOFI) and the brown alga *Padina pavonica* (EPP) alleviate neurodegenerative phenotypes in yeast (*Saccharomyces cerevisiae*) and fly (*Drosophila melanogaster*) models of AD and PD. Pre-treatment with EPP or EOFI in the culture medium significantly improved the viability of yeast expressing the Arctic Aβ42 (E22G) mutant. Supplementing food with EOFI or EPP dramatically ameliorated lifespan and behavioural signs of flies with brain-specific expression of wild-type Aβ42 (model of late-onset AD) or the Arctic Aβ42 variant (model of early-onset AD). Additionally, we show that either extract prolonged the survival of a PD fly model based on transgenic expression of the human α-syn A53T mutant. Taken together, our findings suggest that the plant-derived extracts interfere with shared mechanisms of neurodegeneration in AD and PD. This notion is strengthened by evidence demonstrating that EOFI and to a greater extent EPP, while strongly inhibiting the fibrillogenesis of both Aβ42 and α-syn, accumulate remodelled oligomeric aggregates that are less effective at disrupting lipid membrane integrity. Our work therefore opens new avenues for developing therapeutic applications of these natural plant extracts in the treatment of amyloidogenic neurodegenerative disorders.

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Chemopreventive Role of Quercetin and Vitamin E on Bonny Light Crude Oil-Induced Redox Alterations in Testicular Function of Wistar Rats

Azubuike P. Ebokaiwe

Department of Chemistry/Biochemistry and Molecular Biology, Federal University, Ndufu-Alike, Ikwo (FUNAI), Nigeria

Email: petazk@yahoo.com, azubuike.peter@funai.edu.ng

Quercetin, is a member of the flavonoid family and prominent dietary antioxidant. It is ubiquitously present in foods which includes vegetables, fruit, tea and wine as well as countless food supplements and is claimed to exert beneficial health effects. Similarly, vitamin E acts as antioxidant, by helping to protect cells from aging and damage caused by free radicals and also appears to boost immune system to fight off invading bacteria and viruses. Whilst the preventive or therapeutic actions of such antioxidants is still controversial, under more extreme conditions, such as in areas of heavy environmental pollution, the attenuating role of these prominent nutritional agents against deleterious effect of environmental pollutants via control of redox homeostasis has received a robust scientific support in recent times. This is witnessed in many developing countries, where nutritional redox protection especially pertains to testicular function – an aspect combining traditional believe with modern day problems caused by environmental pollution by crude oil and other chemicals.

The protective role of quercetin and vitamin E on Bonny Light crude oil (BLCO)-induced testicular alterations in stress proteins, apoptosis, and steroidogenesis has therefore been investigated. Experimental rats were divided into four groups, orally administered 2 ml/kg corn oil (control: group 1), BLCO-800 mg/kg body weight alone, simultaneously with 10 mg/kg quercetin or 50 mg/kg vitamin E for 7 d. Protein levels of caspase 3, FasL, NF-kB, Star protein and stress response proteins were analyzed by SDS-PAGE. Immunofluorescence staining was used to quantify the expression of caspase 3, FasL and NF-kB. Apoptosis was quantified by TUNEL assay.

Administration of BLCO resulted in a significant increase in the levels of stress response proteins and apoptosis-related proteins by 50% and above after 7 d and a concomitant increase in caspase 3, FasL and NF-kB. A significant increase in TUNEL positive cells was observed. Co-administration with quercetin or vitamin E reversed apoptosis and levels of stress proteins, relative to control. These findings suggest that quercetin and vitamin E may confer protection against testicular dysfunction, an issue which may be of particular importance for people working with BLCO or living in areas polluted by mineral oils.

Keywords: *Quercetin; Vitamin E; Redox homeostasis; Apoptosis; Steroidogenesis; Rats*

African Plants with Redox Modulatory Properties Relevant in Medicine and Agriculture

Chukwunonso ECC Ejike

Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University, Ndufu-Alike, Ikwo, Ebonyi State, Nigeria

The role of oxidative stress in the pathogenesis of several chronic diseases, including obesity, diabetes, hypertension, cancers, etc., is established in the scientific literature. Consequently, there is evidence accumulating around the hypothesis that antioxidants may play critical roles in the prevention and management of such diseases. Given the growing demand for "green" medicinal and agricultural agents, and the reports of adverse effects due to synthetic (human and veterinary) pharmacologic agents, there is currently a lot of scientific research attention being given to resources from plants with health-promoting properties. Africa as a continent, and particularly sub-Saharan Africa, is blessed with a rich diversity of plant species with documented (and yet to be documented) medical and agricultural benefits. Here, plants with redox modulatory constituents appear to be "goldmines" for these "green" antioxidant agents. This paper examines the redox modulatory properties of such African plants as *Vernonia amygdalina, Occimum gratissimum, Gongronema latifolium,* and *Telfairia occidentalis*. The methods for their exploitation and prospects for the future are also explored. The paper concludes that with appropriate investments in research and development, the green forests of Africa may well harbor the "Chuck Norris" against prevalent hunger and disease in the continent.

Keywords: *African plants, agriculture, antioxidants, redox modulation, medicine*

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Multifunctional Supplement is that the Key to Modulate Redox Signaling in Metabolic Syndrome Volunteers: Cellular Metabolic Index.

Bruno Fink[1], Coy Brunßen[2], Henning Morawietz[2], Alexander Rabovsky[3]

[1]*Noxygen Science Transfer & Diagnostics GmbH, Lindenmatte 42, 79215 Elzach, Germany*
[2]*Division of Vascular Endothelium and Microcirculation, Department of Medicine III, University Hospital Carl Gustav Carus Dresden, Dresden, Germany*
[3]*Research & Development, Melaleuca Inc, 4609 West 65th South, Idaho Falls, ID 83402, USA*

Scientists around the world concur that maintaining proper levels of redox signaling, which often will mistakenly interpret as oxidative and nitrosative stress. So, identifying the roots of such condition allowed us to design a strategy of nutritional intervention. Utilizing this approach, we performed a randomized, controlled, long-term study of the effect of a multifunctional nutritional supplement on redox signaling and health markers in 48 healthy and pre-metabolic syndrome volunteers.

The measurement of extended cellular metabolic index based on cellular and mitochondrial dependent ROS formation and respiratory activity was performed using electron spin resonance spectrometer NOXYSCAN. Spin probe 1-hydroxy-3-methoxycarbonyl-2.2.5.5-tetramethylpyrrolidine (CMH) and oxygen label NOX-14.1 were used. Health parameters (blood pressure, heart rate, lipid and glucose profile, bioavailability of circulating NO was analyzed at control day, after 6 and 12 weeks of twice daily supplementation.

Long-term supplementation resulted in significant inhibition of metabolic activity (20.7% cellular and 42.6% mitochondrial). We also observed significant inhibition of extracellular NADPH oxidase dependent generation of superoxide, nearly complete inhibition of extracellular H2O2 formation as well as a 2.5 times increase in bioavailable NO. Evaluated health parameters were improved on healthy people with even greater effects on those with metabolic syndrome: significant improvements in lipid profiles (TC, LDL, HDL, VLDL and TG), glucose metabolism (glucose, insulin, HbA1c, HOMA, postprandial hyperglycemia), inflammatory response as well as expression of redox sensitive proteins as NRF2, SOD1, eNOS were shown.

For the first time, we demonstrate the effects of a multifunctional nutritional supplement containing minerals, vitamins, antioxidant and anti-inflammatory phytonutrients on cellular redox signaling in pre-metabolic syndrome volunteers. We observed strong correlation of extended cellular metabolic index with more than 25 markers and the indicators of “optimal health” as lipid-, glucose-, inflammatory profile, redox sensitive protein expression and other functional cardiovascular parameters.

Keywords: *Cellular metabolic index, redox signaling, dietary supplement, electron spin resonance spectroscopy, inflammatory resistance, RNS, ROS, metabolic syndrome.*

Is the Future of Human Diet Green: a Genome Damage Study

Marko Gerić, Goran Gajski, Vera Garaj-Vrhovac

Institute for Medical Research and Occupational Health, Zagreb, Croatia

People usually turn to vegetarian diet due to ethical issues toward animals or due to improving their health. Avoidance of animal originated food and high intake of vitamin-, antioxidant-, and micronutrient-rich food is believed to improve human health status and to promote longevity. However, the beneficial properties of vegetarian and vegan nutrition are still a matter of debate, without clear scientific consensus. Therefore we conducted a pilot study on 40 volunteers (20 per group) to screen baseline DNA damage between vegetarian and omnivorous group. Our study groups were matched by age (32.05±7.33 vs 32.21±8.02 years), gender (20 male:20 female each group), and active smokers (30% each group). The vegetarianism was defined as avoiding consumption of any meat product for at least 3 years. Average duration of consuming vegetarian food was 9.95±5.17 years. The results of this pilot study shown that baseline DNA damage in vegetarian group was higher using the micronucleus test (total number of micronuclei was 7.35±3.57 vs 3.89±1.71) and the comet assay (tail intensity was 2.52±0.91 vs 1.39±0.58) at statistical level $p<0.05$. Since the DNA damage is critical in cancer initiation, and micronucleus test has cancer predictive properties, such results imply that omnivorous diet might be more appropriate for human consumption in the context of human genome instability. Still, it has to be taken into account that most of our omnivorous volunteers shown habits that follow Mediterranean type of diet that is rich in fruits and vegetables. Further studies are needed that will include more biomedical biomarkers and that will be performed on greater number of volunteers to draw more reliable conclusions.

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Influence of Proteins on Carotenoid Digestion and Aspects of Bioavailability

Iddir M.[1,4], Devaux Y.[1], Guignard C.[2], Appenzeller B.[1], Borel P.[3], Larondelle Y.[4], Bohn T.[1].

[1]*Department of Population Health, Luxembourg Institute of Health, L-1445 Strassen, Luxembourg.*
[2]*ERIN Department, Luxembourg Institute of Science and Technology, L-4422 Belvaux, Luxembourg.*
[3]*NORT, INRA, INSERM, Aix-Marseille Université, F-13005 Marseille, France.*
[4]*Institut des Sciences de la Vie, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium.*

BACKGROUND: Dietary intake and plasma levels of carotenoids have been associated with many health benefits such as reduced risk of a**ge-related macular degeneration** (AMD), **cataract**, **cardiovascular disease**, **type 2 diabetes** and **certain types of cancer,** and some specific carotenoids act as **vitamin A precursors.** The primary health benefits of carotenoids can be explained by their **anti-oxidant effects** and their **anti-inflammatory properties** [1]. However, carotenoid absorption depends on several host [2] and dietary factors, including lipids and divalent minerals [3]. One factor that has never been systematically studied is the influence of co-digested proteins on carotenoid solubility/bioaccessibility. Several proteins have exhibited emulsifying properties during digestion, stabilizing oil-in-water emulsions.

OBJECTIVE: To i) elucidate the current knowledge regarding interactions between dietary proteins and carotenoids, ii) to study effects of co-digested proteins on carotenoids *in vitro*, and iii) to determine the impact of protein co-ingestion on carotenoid bioavailability *in vivo*.

DESIGN: A literature survey is carried out on the influence of proteins on the bioaccessibility of lipophilic dietary constituents. For studying protein-carotenoid interactions during simulated gastro-intestinal (GI) digestion, several proteins (whey protein isolate, soy protein isolate, gelatin etc.) are evaluated. Bioaccessibility of pure carotenoids (β-carotene, lycopene, and lutein) and from solid and liquid food matrices will be studied, as well as additional parameters (rheology, micelle size). Colonic recovery will be studied following fermentation, and potential carotenoid degradation products/metabolites will be investigated. For the human trial, 24 healthy adults will be recruited and served a protein and carotenoid-rich meal, with plasma concentrations and triacylglycerol-rich lipoprotein fractions as the observed outcomes.

RESULTS: Proteins may be involved in **stabilizing lipid droplets in oil-in-water emulsions** in the GI tract, forming a coating around droplets [4]. The adsorption of proteins into lipid droplet surfaces may **prevent their aggregation**, also forming **protective surfaces**. Results from micro- and nano-encapsulation with e.g. caseins have shown **improved bioaccessibility** [5]. Proteins are expected to **enhance the solubilization/ digestion** of lipid droplets, **reduce their size**, and **foster the transition** of lipid droplets to mixed micelles, resulting in **improved carotenoid bioaccessibility/bioavailability**.

CONCLUSION: Proteins are likely modifiers of the solubilization of liposoluble dietary constituents during digestion. The results should demonstrate an influence of dietary proteins on carotenoid bioavailability. This would have consequences also for other liposoluble molecules such as fat-soluble vitamins, and would be relevant for the public health sector, as well as food and pharmaceutical industries.

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Keywords: *Emulsions, bioaccessibility, in-vitro digestion, absorption, protein sources*

Targeting Redox Signaling by Phytophenols in Chondrocytes Isolated from Human Osteoarthritic Articular Cartilage in vitro

Zübeyir Elmazoğlu, Sait Yarayıcı, Cem Nuri Aytekin, Berivan Bitik, Berna Göker, Çimen Karasu

Cellular Stress Response & Signal Transduction Research Laboratory, Department of Medical Pharmacology, Gazi University, Faculty of Medicine, Beşevler, Ankara, Turkey. Farmasens R&D Co., Ltd. Ankara, Turkey. LongAgeHealth Consulting Co., Ltd. Ankara, Turkey.

Osteoarthritis (OA) is an age-dependent progressive destruction of joints related to articular cartilage damage, synovial lining thickening, subchondral bone alterations, and osteophyte formation at the joint margin and is supported by elevated inflammatory and catabolic responses, which finally result in a loss of joint architecture and deformity. The treatment of OA currently includes systemic or topical analgesic nonsteroidal anti-inflammatory drugs and intraarticular glucocorticoid injections as well as supportive agents such as chondroitin sulphate. These agents are not effective in the intracellular pathogenetic pathways of OA and only partially effective in easing some symptoms of it. Moreover, neither reconstructive surgery nor tissue engineering, biomaterial injections, stem cell or gene transfer has been widely applicable secondary to their invasive nature or cost issues and their long term efficacy are controversial. Therefore, there is an urgent need for systemic agents targeting a variety of molecular cascades in intracellular pathology of OA and stimulating regeneration while protecting cartilage from degeneration.

Inflammation and oxidative stress have been increasingly recognized as being closely integrated in OA pathology, however there are no pharmaceuticals targeting pathways associated with redox stress against OA. Thus, the regulation of redox homeostasis at the cellular level can decrease the severity of OA and limit disease progression (1). In this respect, as a master regulator of cellular redox homeostasis, NRf2 induction has been suggested to play a key role in OA pathophysiology via regulating chondrocyte apoptosis, senescence and inflammatory processes (1,2). Recently, several studies have revealed the essential role of plant-derived antioxidants in preventing pathophysiological events in joint diseases via modulating NRf2 expression and redox signaling (3-5).

We have previously demonstrated that ZeyEX® (TR2013 15662 A2), an olive leaf extract standardized with respect to major polyphenolic constituents protect a variety of cellular types against redox stress associated damage (6,7). In the present study we tested the effects and action mechanisms of ZeyEX® and its major polyphenolic components (oleuropein, hidroksitirozol, verbaskozid, kuersetin, luteolin) on the viability and redox modulatory pathways in cultures of primary chondrocytes obtained from knees of patients with grade 3-4 OA. Cell counts and alterations in the viability and proliferations were determined (MTT; RTCA-iCELLigence System), intracellular pathways effected by the compounds were scanned by using PathScan® Intracellular Signaling Array Kit. Findings were compared with effects of ibuprofen, a nonsteroidal anti-inflamatory agent. The chondrocytes differentiation were evaluated for each cellular passage and the passages 1-6 condrocytes were accepted as pathologic. Primary chondrocytes passaged >6 resulted in reversion of their phenotype towards articular chondrocytes. ZeyEX® polyphenols increases viability of OA chondrocytes and blocks inflammation. Co-treatment of ZeyEX® polyphenols with H_2O_2 decreases growth inhibition that was prevented especially by oleuropein. The study provides novel insights into the development of olive leaf polyphenols as promising candidates and ZeyEX® as a therapeutic agent for the management of OA.

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***Keywords:** Osteoarthritis, Chondrocyte, Oxidative stress, Redox modulation, phytophenol, ZeyEX®, Oleuropein, Hydroxytyrosol, Verbascoside, Quercetin, Luteolin, Ibuprofen.*

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Incorporation of Vitamins in DPPC Nanoparticles as Novel Approach for Drug Delivery to Tumorous MDCK Cells

M. Khvedelidze[1,2], T. Mdzinarashvili[1,2], E. Shekiladze[1,2]

[1] *Faculty of Exact and Natural Sciences, I. Javakhishvili Tbilisi State University, Tbilisi 0179, Georgia*
[2] *Institute of Medical and Applied Biophysics, I. Javakhishvili Tbilisi State University, Tbilisi 0179, Georgia*

Modern nanotechnology was used to encapsulate vitamins (C, E) in DPPC liposomes. Proportions of vitamins and DPPC lipids were selected so that, in average, it matched one vitamin molecule for one lipid. In liposome, hydrophobic vitamin E was placed in structural hydrophobic part of liposome and hydrophilic vitamin C - on a surface of a bilayer of liposomes' membrane. The formation of the complex is confirmed with either calorimetric or biological experiments. In particular, calorimetric curve of the complex differs considerably from calorimetric peak profiles of the pure DPPC liposomes, that further emphasize the existence of vitamins and liposomes complex. Thanks to the biological experiments, behavior of the complex nanoparticles (vitamin E and DPPC lipid complex) against tumorous MDCK cells became more effective than adding the same amount of pure vitamin E, which is a quite significant result.

NutriOX Related "Whos and Wheres" from Latvia

Maija Dambrova[1], Andrejs Skesters[2], Janis Liepins[3]

[1] *Latvian Institute of Organic synthesis,*
[2] *Laboratory of Biochemistry, Riga Stradins University,*
[3] *Institute of Microbiology and biotechnology, University of Latvia*

The aim of the poster is to introduce Latvian academic institutions, their activities and projects related to COST NutriOX activities. The Latvian Institute of Organic Synthesis (LIOS) is an academic research institute with extensive competence in synthesis and activity analysis of new drugs. This includes drug discovery and structure analyses, medicinal chemistry, drug and preclinical drug development. Cardiovascular diseases, CNS disorders and cancer are the clinical areas of LIOS interest. Future focus area of the LIOS is natural product based drug discovery that includes ethno-pharmacology and phytochemistry.

Riga Stradins University (RSU) is the largest academic institution for medical education and research in Latvia. Laboratory of Biochemistry of RSU is certified laboratory with following scientific expertise: analyses of clinically relevant ageing factors, analysis of public health determinants and cancer research, including early diagnosis and improvement of treatment strategies. One of the focuses of the laboratory is development of new methods and technologies for nutrition studies.

University of Latvia (UL) has expertise in vitro cell based assays to test antioxidative agents. Test systems include mammal cell lines, microorganisms (bacteria and yeasts).

Keywords: *cell based assays, nutrient antioxidative activity, drug development, aging.*

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Physical-Chemical Factors Influence on Bacteria Behavior

T. Mdzinarashvili[1,2], M. Khvedelidze[1,2], N. Turkadze[1,2], I. Papukashvili[2], E. Lomadze[1,2]

[1] *Faculty of Exact and Natural Sciences, I. Javakhishvili Tbilisi State University, Tbilisi 0179, Georgia*
[2] *Institute of Medical and Applied Biophysics, I. Javakhishvili Tbilisi State University, Tbilisi 0179, Georgia*

Nowadays, studying antibiotics, germs and their resistance has reached the highest level in the scientific world. Because around us an amount of microbes are huge, so their influence on living system are very important. Not only health depends on them, but also a creation of sterility area, for exemple food produced stuffs. After many various experiments and measurements scientists predicted, that using antibiotics (regarded as "world savers") in a couple of years, will be totally in vain, because germs will gain resistance towards antibiotics. As it seems, without any doubt, dilemma may cause lethal and fatal results for the whole humanity, but there is always a hope, isn't there?!

The experiments held by us concerns the dependence of bacteria E. coli proliferation on the miscellaneous external conditions. The roles of these conditions were played by various antibiotics, particularly by Ampiox and Gentamycin. We used to conduct our experiments on E.coli C bacteria. The effect of these antibiotics' action was determined according to bacterial proliferation. It was found out that bacterial proliferation depends on the amount of antibiotics, more precisely the bacterial proliferation is ceased at certain ratio between bacterial cells and antibiotics' molecules, and this ratio in microbiology has got a special term- minimum inhibitory concentration, MIC. It was used the turbidimeter in our experiments that enabled us to observe the variability of bacteria by means of measuring the turbidity of the solution real-time mode and exactly this method enabled us to define the MIC for these both antibiotics. In addition, by means of using the turbidimeter we observed a very important case of interaction between bacteria and antibiotic such as acquired resistance when adding the same antibiotic the second times to bacteria which could not affect the bacterial proliferation any more. Moreover, this methods enabled us ascertain that the autocrine signaling is responsible for this acquired resistance. It is interesting that turbidimeter also showed that heating the solution by 20 degrees forced this resistance to vanish.

Continuous real-time observation of bacterial growth has a great advantage for studying the mechanisms of various compounds interactions with the bacterial cell membrane. Using the turbidimetry method, we showed that bacterial growth pattern is influenced not only by the presence of the antibiotics and phages, but also on the concentration of them in the medium. We also showed that, the pattern and the speed of bacterial growth depends on the concentration of the liquid media. The concentration of antibiotics and bacteriophages in media are not always directly correlated to the inhibition of bacterial growth. Conversely, it is shown, that their very small amount is practically incapable to inhibit the growth process. According to our results, receptor proteins on the bacterial cell membrane are not saturated with antibiotics or bacteriophages fully and there are free unbound membrane receptors, which we hypothesize is the reason for uninhibited bacterial growth. Only after the majority receptors are occupied, bacteriophage starts the injection process of DNA into the bacterial cytoplasm. According to our research, the biological method for enumeration of viable phage is not equal to the number of phage plaques on the Petri plate and the real quantity is several degrees higher.

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Impact of Dietary Oxidized Phospholipids on Human Health and Methods of their Profiling and Qualitative Assessment in Foods

Karol Parchem, Agnieszka Bartoszek

Gdansk University of Technology, Department of Food Chemistry, Technology and Biotechnology, Gabriela Narutowicza 11/12 Street, 80-233 Gdansk, Poland

E-mail: parchem.karol@gmail.com

The results of numerous epidemiological studies indicate that the type, quality and intake of food-delivered lipids contribute to both to the promotion and prevention of nutrition related and metabolic diseases such as obesity, atherosclerosis or diabetes. The growing interest in assessment of the impact of dietary lipids on human health requires their characterisation in foods. Among the bioactive lipids, phospholipids (PLs) attract increasing attention due to their high nutritional value and functional properties. PLs, especially those containing essential fatty acids (FAs) exhibit a number of key biological activities. On the other hand, polyunsaturated FAs built into PLs structure are susceptible to oxidation during processing, storage and final preparation of foods. Oxidized phospholipids (OxPLs) may be formed in three ways, including non-enzymatic (autooxidation and photooxidation) and enzymatic mechanisms.

OxPLs are potentially toxic compounds to the digestive tract cells, which are directly exposed on contact with chyme lipid emulsion. Accumulation of OxPLs can lead to gut pathologies, such as inflammation and cancer, as a result of cell membrane modifications, as well as DNA and protein damage. During intestinal digestion, native and OxPLs are hydrolysed by pancreatic phospholipase A_2 and products of this reaction are absorbed through the epithelial cells of the small intestine. After PLs resynthesis, native and OxPLs are released into the bloodstream, where OxPLs can contribute to the development of atherosclerosis. In addition, OxPLs, as opposed to native PLs, are recognized by the innate immune receptors including scavenger receptors, antibodies or C-reactive protein leading to the induction of cellular apoptosis.

In the our work, we characterised products of thermal and enzymatic oxidation of PLs isolated from hen egg yolk using *offline* two-dimensional liquid chromatography coupled with DAD, CAD and MS detection.

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Ability of Different *S*-Nitrosothiols to Induce Vascular Storage of Nitric Oxide and to Decrease Vasoconstrictive Capacities of Isolated Rat Aortae

Perrin-Sarrado C., Salgues V., Giummelly P., Leroy P., Lartaud I.

EA 3452 Cibles thérapeutiques, Formulation et Expertise Préclinique du Médicament, Faculté de Pharmacie, Université de Lorraine, Nancy, France

S-nitrosothiols are an emerging class of •NO donors with a potential use in cardiovascular disorders associated with a decreased bioavailability of nitric oxide (•NO).

The aim of this study was to compare the ability of three *S*-nitrosothiols: *S*-nitrosoglutathion (GSNO), *S*-nitroso-*N*-acetylcysteine (NACNO) and *S*-nitroso-*N*-acetylpenicillamine (SNAP) to induce vascular storage of •NO and to decrease vasoconstrictive capacities.

The impact of each *S*-nitrosothiol on vasoreactivity was evaluated on aortic rings isolated from 12 week-old, male, normotensive Wistar rats. Aortic rings were exposed for 30 min to GSNO, SNAP or NACNO (2 µM) in order to form *S*-nitrosocysteine residues in peptides and proteins, followed by 1-h washing with saline solution to remove the excess of RSNO. Then, aortae were submitted to two different protocols. First, •NO storage in the aorta was evaluated by measuring the vasorelaxation after addition of *N*-acetylcysteine (NAC) which will displace •NO from cysteine-NO residue. Aortic rings (n = 8-10 per group, from 3-8 different rats in each group) were preconstricted with 10^{-6} M phenylephrine, then NAC (10^{-5} M and 10^{-4} M) was added. Second, cumulative concentration response curves to phenylephrine (3×10^{-10} M to 3×10^{-5} M, n = 4-19 per group, from 2-8 different rats in each group) were performed. The endothelium role was also investigated by performing experiments in either endothelium-intact or endothelium-removed aortae.

NAC at a concentration of 10^{-5} M induced vasorelaxation of endothelium-intact aortae pre-incubated with GSNO, NACNO or SNAP (27 ± 4%*, 43 ± 3%*# and 23 ± 4 %*, respectively, *p<0.05 *versus* control 1.8 ± 0.9 %, #p<0.05 *versus* GSNO and SNAP, one-way ANOVA). Concentration response curves to phenylephrine were shifted to the right (one log unit) with an increased half maximal effective concentration (3.3 ± 0.5, 5.4 ± 0.1, 3.1 ± 0.3 x 10^{-7}M, respectively, *versus* control 4.8 ± 0.2 x 10^{-8}M). In endothelium-removed aortae, NAC did not induce vasorelaxation nor changed the responses to phenylephrine compared to control.

In conclusion, *S*-nitrosothiols - NACNO in a better extent - are able to induce storage of •NO within the vessel wall, especially in isolated aortae with removed endothelium. This is associated with a hypo-responsiveness to the vasoconstrictive agent phenylephrine. Treatment with *S*-nitrosothiols could present a benefit to restore •NO-dependent functions in pathological states associated with injured endothelium.

Keywords: *S-nitrosothiols, nitric oxide, NO stores, vascular contraction, endothelium*

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The Radical Scavenging Activity of *Ajuga genevensis* L. *In Vitro* Culture

Sahakyan N., Petrosyan M., Trchounian A.

Department of Biochemistry, Microbiology and Biotechnology, Biology Faculty, Yerevan State University, 1 A. Manoogian Str., 0025 Yerevan, Armenia

Since the 1970s, oxidative stress has been evoked as a contributor to pathogenesis and thousands of studies have reported protective or therapeutic benefits of antioxidants in cellular and animal models of age related diseases. It is believed that two-third of the world's plant species have medicinal importance, and almost all of them have antioxidant potential. Armenian flora is very rich of many plants which can be used in traditional and modern medicine, food, cosmetics. Genus *Ajuga* includes over 50 species among which 4 species grow in Armenia. *A. genevensis* is of great interest for pharmacology due to it's high biological activity. But a great number of plants are considered to be endangered, scarce or included in Red books, therefore biotechnological methods of obtaining of valuable metabolites are also of interest. The aim of our investigation was to study the dependence of radical scavenging activity of *A. genevensis* callus culture extraction conditions and growing phase.

The *A.genevensis* callus culture was obtained and the further growth was supported on Murasige-Skoog (MS) nutrient medium, which contained 2 mg/l indole-3-acetic acid and 0.2 mg/l kinetine. The antiradical activity of callus extracts (ethanol, methanol, acetone (propanone), water, chlorophorm) was tested using DPPH-assay (2,2-diphenyl-1-picrylhydrazyl) and photochemiluminescent method. The water, methanol, acetone and chlorophorm extracts of 30th sub-cultivation callus cultures possess the ability to neutralize the DPPH free radicals, whereas 45th sub-cultivation cultures did not show any activity. The highest antiradical activity possesses the water extract. During the cultivation some quantitative and qualitative changes may occur in callus metabolism. This makes significant bases to study the metabolic features of plant cultures with medicinal purposes. Our investigations show that the prolonged cultivation of *A. genevensis* callus culture leads to inactivation of the synthesis of substances with antioxidant activity.

Keywords: *Ajuga genevensis, antioxidant activity, callus culture*

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Cysteine Triggers the Copper Transfer from Aβ$_{4-16}$ Peptide to Metallothionein-3

Alice Santoro[a], Nina Ewa Wezynfeld[b], Wojciech Bal[b], Peter Faller[a]

[a]*Laboratoire de Biometaux et Chimie Biologique,* UMR 7177, Universite de Strasbourg, *1 Rue Blaise Pascal, 6700, Strasbourg*
[b]*Institue of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5a, 02-106, Warsaw*

Email: alice.santoro@unistra.fr

Metallothionein-3 (MT-3) is highly expressed in the brain and belongs to the family of Metallothioneins (MTs). MTs are characterized by an elevated number of Cys and the binding of up to 10 metals in clusters. Unlike the other mammalian MTs (i.e. 1, 2 and 4), which mostly bind zinc, MT-3 plays a fundamental role, *in vivo*, in the metabolism of both zinc and copper atoms. [1]

From the last decade, interest arose on MT-3 interaction with copper-peptide complexes of amyloidogenic peptides [2, 3], in particular with Amyloid-beta, the peptide involved in Alzheimer's disease (AD). Indeed, MT-3 was found to be downregulated in AD. Zn_7MT3, in its native form, was shown to have a neuroprotective effect against copper mediated Cu(II)Aβ$_{1-40}$ toxicity, related to ROS production. The mechanism proposed to underlie such an activity was a copper-zinc swap between the two species, with formation of an oxygen-stable $Cu(I)_4Zn_4$MT-3 complex and Zn-amyloid-beta. [2]

More recently, another relevant species of Aβ peptide, the N-truncated fragment Aβ$_{4-16}$ (model for the Aβ$_{4-42}$ peptide), was studied in its interaction with MT-3. Aβ$_{4-42}$ is a major isoform of Aβ peptide, detected both in healthy individual brains and those affected by of AD. It has been shown to bind copper(II) stronger (about 3-4 orders of magnitude at pH 7.4) compared to the full-length Aβ$_{1-40/42,}$ through its ATCUN-like N-terminal Phe-Arg-His sequence. For the Cu-Aβ$_{4-16}$, no copper transfer to Zn_7MT3 has been observed, which led to the proposition that Aβ$_{4-42}$ can act as Cu^{II} scavenger in the synaptic cleft.

In this context, we are interested in molecules that could trigger the Cu transfer from Aβ$_{4-16}$ to MT3. Glutathione is the main thiol containing compound intracellularly, but also reduced cysteine is present. Extracellularly reduced cysteine is more abundant than reduced glutathione. Thus we investigated the impact of cysteine and glutathione on the copper transfer. Indeed both compounds are able to trigger the Cu transfer, but cysteine was more efficient. Thereby cysteine plays a dual role, acting both as reducing agents for Cu(II) as well as shuttles for Cu(I) .

Therefore by complementary use of Uv-Vis and Circular Dichroism Spectroscopy, we assess the release of Cu^{II} from Cu^{II}(Aβ$_{4-16}$) in the presence of Zn_7MT3. Besides we prove that in the reaction mixture Cu^{II}(Aβ$_{4-16}$) / Zn_7MT3 the extracted copper ions are transferred to Zn_7MT3 in form of Cu(I) bound to Cys/GSH, thus generating the species $Cu(I)_4Zn_4$MT-3, in which an air stable $Cu(I)_4$-thiolate cluster is present.

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Keywords: *Copper, Copper metabolism, Amyloid-β peptide, Metallothionein-3*

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Influence of Microencapsulated Probiotic Intake on Myeloperoxidase Activity in TNBS-Induced Colitis in Rats

Katarina Smilkov[1], Tanja Petreska Ivanovska[2], Tatjana Ruskovska[1], Kristina Mladenovska[2]

[1]*Faculty of Medical Sciences, Goce Delcev University, Stip, Republic of Macedonia*
[2]*Faculty of Pharmacy, University "Ss. Cyril and Methodius", Skopje, Republic of Macedonia*

Introduction: The hypothesis that the intestinal bacterial flora contributes to the pathogenesis of inflammatory bowel disease (IBD) has been supported by experimental and clinical evidence [1]. The dysbiosis present in this condition is related to dysregulation of mucosal immune response. One of the indicators of leukocyte infiltration at the sites of inflammation is the activity of myeloperoxidase (MPO) [2]. Numerous studies have been conducted in order to examine the effects of probiotic intake in IBD. However, during ingestion of probiotics, the harsh conditions which are present in the gastrointestinal (GI) tract often impair the delivery of viable microorganisms in the lower intestine. For this reason, probiotic (*Lactobacillus casei 01*) was incorporated in Ca-alginate-microparticles coated with whey protein [3] and the effects of the formulation were examined in rat model of TNBS (trinitrobenzene sulfonic acid) - derived colitis.

The objective of this work was to examine the lower intestine MPO activity after induction of TNBS colitis in rats, and to compare the effects of ingestion of microparticulate probiotic formulation *vs* non-encapsulated probiotic.

Materials and methods: The effect on MPO activity was assessed after oral administration of the microparticulate *L. casei* formulation (once daily during 21 days; probiotic viability 8,7 $\log_{10}$cfu/g) to Wistar rats in which inflammation was induced by intrarectal administration of TNBS (10 mg in 0.25 ml 50% ethanol). For comparison, a group of Wistar rats received the same amount of non-encapsulated *L.casei* (8,7 $\log_{10}$cfu/g). At the same time, a negative and a positive (TNBS) control group were also tested. The MPO activity was measured as described by Peran et al., 2007 [4].

Results: The obtained values of MPO confirmed the presence of inflammation in our rat model, with the highest activity noted in the positive (TNBS) control group. The activity of MPO was found to be lower in the group of rats that were administered a microparticulate probiotic formulation, in comparison to the group that was administered non-encapsulated probiotic. These results suggest that encapsulation of *L. casei* efficiently protects the probiotic during the GI transit, therefore resulting in better colonization of the lower intestine, which subsequently results in lower MPO activity. Still, other indicators of gut wall immune response should be examined in order to confirm and support the current finding that microencapsulated probiotic confer better effects than non-encapsulated one.

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Keywords: *microencapsulated probiotic, Lactobacillus casei, myeloperoxidase activity*

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Potentiometric Titration as a Reliable Method of Determination of Standard Reduction Potentials of Antioxidant Compounds on the Way to the of Antioxidant Power Series

Klaudia Suliborska[1], Monika Baranowska[2], Agnieszka Bartoszek[2], Claus Jacob[4], Jacek Namieśnik[3], Wojciech Chrzanowski[1]

[1] Department of Physical Chemistry, Faculty of Chemistry, Gdansk University of Technology, Poland
[2] Department of Food Chemistry, Technology and Biotechnology, Faculty of Chemistry, Gdansk University of Technology, Poland,
[3] Department of Analytical Chemistry, Faculty of Chemistry, Gdansk University of Technology, Poland,
[4] Department of Bioorganic Chemistry, Saarland University, Germany

The Antioxidant Power Series is proposed as a way to arrange antioxidants relevant for human health based on their reduction potentials in relation to glutathione, which is a major physiological antioxidant. The prerequisite for such a Series is a reliable method of determination of this physicochemical property.

In our study, the values of reduction potentials of antioxidant compounds were obtained by potentiometric titration. All measurements were carried out using an Ag/AgCl reference electrode and a Pt working electrode at 295.15 K, 298.15 K, 310.15 K, 314.15 K, 318.15 K in phosphate buffered saline (pH=7.4).

On the basis of titration curves received, the inflection point was read by the non-linear regression method (Marquardt-Levenberg algorithm) using the sigmoidal, 5-parameter model (with determination coefficient r^2 almost equal to 0.999). The results obtained were applied to calculate the values of standard reduction potentials (*vs*. the standard hydrogen electrode) and thermodynamic constants for oxidation reactions: standard Gibbs free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0). The results received for L-ascorbic acid served as a reference and were shown to be similar to the literature values determined by other methods.

Based on the thermodynamic constants calculated, two groups of antioxidants were identified. One that exhibited the straight line dependence, and the other that exhibited the parabola-shaped dependence $E^0=f(T)$, with minimum for the temperature near 310.15 K (37°C). Parabolic shape of $E^0=f(T)$ means that the reaction of oxidation at minimum point is the most exergonic, because the values of ΔG^0 are the lowest (most negative).

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Keywords: *Antioxidant Power Series, reduction potentials, thermodynamic constants, glutathione, catechins*

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A Mediterranean Diet Nutritional Intervention Lowers Blood Pressure and the Production of Reactive Oxygen Species by Immune Cells

Antoni Sureda[1], Carla Busquets-Cortés[1], Xavier Capó[1], Silvia Tejada[1,2], Alejandra Arenas[1], David Mateos[1], Antoni Pons[1], Josep A Tur[1]

[1]Research Group in Community Nutrition and Oxidative Stress, University of Balearic Islands, Palma de Mallorca and CIBEROBN (Fisiopatología de la Obesidad y la Nutrición CB12/03/30038) Instituto de Salud Carlos III, Madrid, Spain. [2]Experimental Laboratory, Research Unit, Son Llàtzer Hospital, IUNICS, E-07198, Palma de Mallorca, Balearic Islands, Spain.

E-mail: nucox@uib.es

Introduction: Obesity and metabolic syndrome are central health problems in developed countries. Low-grade chronic inflammation is directly associated with a cluster of disorders collectively known as the metabolic syndrome.

Objective: To evaluate the effects of a nutritional intervention based on Mediterranean Diet on peripheral blood mononuclear cells (PBMCs) and neutrophils capabilities to produce reactive oxygen species (ROS) after immune stimulation.

Methods: Subjects aged 55 to 75 years, with overweight/obesity and metabolic syndrome participated in the study. Subjects were divided in two groups: 1) Intensive lifestyle intervention with a hypocaloric Mediterranean diet, physical activity and behavioural therapy and 2) a control group assigned to non-intensive advice on healthy diet (also Mediterranean type) following habitual clinical practice.

Results: Anthropometrical and blood samples were obtained at the beginning and after 6 months follow-up in basal conditions. No significant differences were reported in the body max index, glucose, lipid profile or in markers of tissue damage (AST, ALT and GGT) after the 6th month of intervention. Systolic blood pressure was significantly reduced in both groups. ROS production decreased in both PBMCs and neutrophils after 6 months of intervention when stimulated with zymosan and lipopolyshaccaride, without significant differences between groups.

Conclusions: Although both dietary interventions reduced the production of ROS by immune cells, the analysis of a larger number of subjects and over a longer follow-up period is necessary to determine the efficacy of the most intensive intervention.

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Testing the Effects of Redox-Active Compounds on Thyroid Cells: a Call for Collaborations

Panos G. Ziros, Eleni Ntalampyra & Gerasimos P. Sykiotis

Service of Endocrinology, Diabetology and Metabolism, Lausanne University Hospital, Lausanne, Switzerland

Email: gerasimos.sykiotis@chuv.ch

Numerous redox-active natural substances present in foods can activate the transcription facotr Nrf2 (NFE2-related factor 2); well-studied examples include sulforaphane, curcumin, resveratrol, genistein and epigallocatechin-3-gallate. Interestingly, several of these substances or of other substances in the same chemical classes have been incriminated as causes or predisposing factors for thyroid disorders.

We have recently discovered that Nrf2 directly regulates the mRNA expression level and thereby the protein abundance of thyroglobulin (TG), the thyroid hormone precursor protein, by binding to two antioxidant response elements (AREs) in the upstream enhancer of the *TG* gene. The activation of Nrf2 via sulforaphane recapitulates these effects.

In the context of COST Action *NutRedOx «Personalized Nutrition in aging society: redox control of major age-related diseases»*, we have applied to the *Swiss National Science Foundation* for a research grant to characterize the effects of redox-active food-derived natural substances on the thyroid, with a particular focus on known and novel Nrf2 activators.

We hypothesize that, beyond sulforaphane, other redox-active natural substances present in foods will also have regulatory effects on TG levels via the activation or inhibition of Nrf2 activity. We propose to identify such substances via cell-based medium-throughput screens (MTS). We are also seeking collaborations with other members of the NutRedOx Action, in order to screen their substances of interest in our assays.

These studies will allow us to identify substances whose consumption may pose risks to the thyroid; such substances would be tested further in parallel with sulforaphane. Conversely, for substances that are not found to have an impact on TG levels in the MTS, there would be potentially less risk of thyroid toxicity; thus, both the positive hits and the negative results will be of scientific value.

While we have firmly established the impact of sulforaphane on TG gene expression and protein abundance, it remains unknown whether these effects translate into differences in thyroid anatomy and/or function in vivo.

Our preliminary data in mice show that genetic activation of Nrf2 leads to goiter and hypothyroidism, but whether this is also true for pharmacological activation of Nrf2 is not yet known. We thus propose to test this hypothesis in wild-type mice, with the expectation that treatment with sulforaphane may cause goiter and/or hypothyroidism. If these expectations are confirmed, we will then repeat the experiment in Nrf2 KO mice, to formally test whether the mechanism of the sulforaphane-induced thyroid anomalies involves the activation of Nrf2. Other redox-active substances would also be tested in the same experimental models.

Keywords: *thyroid, Nrf2, sulforaphane, Keap1, thyroglobulin*

Antioxidant Capacity of Plant Extracts

Zélie Triaux[1], Léa Briard[1], Odile Petit[1], Eric Marchioni[1], Diane Julien-David[1]

[1]*Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France.*

As part of a project on the diet of wild horses, the nutritive values of different plants were evaluated. In fact, in a previous study, eating habits of wild horses were observed. It was found out that wild horses had eating preferences for specific plants like for example meadowsweet (*Filipendula ulmaria*), different types of willows (*Salix*), hawthorn (*Crataegus*)… Leaves and sometimes branches of the different plants were harvested, dried and grinded into a small powder. Different studies were then conducted on the plant materials. In our case, we studied the antioxidant capacity of the plants. First, plant extracts were obtained using a pressurized liquid extraction (PLE). The extracts were made with different solvents (chloroform, ethyl acetate and eau/ethanol 50/50 v/v) in order to extract a large range of molecules. Then, the antioxidant capacity of the extracts was evaluated using two methods: TEAC (Trolox Equivalent Antioxidant Capacity) and ORAC (Oxygen Radical Absorbance Capacity). Those methods are indeed complementary as they are based on two different antioxidant mechanisms: one is a transfer of protons and the other of electrons. Some plant extracts showed high value of antioxidant activity such as the hydroalcoolic extract of the leaves of meadowsweet. Furthermore, differences of antioxidant activity were observed between the leaves and the branches of the same plant. Using a HPLC-antioxidant online system, some antioxidant compounds were identified in the plant extracts like chlorogenic acid for example.

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Contribution of Iron Overload and Cholesterol Oxidation in the Pathogenesis of Alzheimer's Disease

Karym E.M.[1,2], Badreddine A.[1,2], Nury T.[1], Cherkaoui-Malki M.[1], Nasser B.[2], Lizard G[1], Vejux A.[1]

[1] *University Burgundy Franche-Comté, Team 'Biochemistry of the Peroxisome, Inflammation and Lipid Metabolism' EA 7270 / Inserm, Dijon, France*
[2] *University Hassan I, Laboratory 'Biochemistry of Neuroscience', Settat, Morocco*

Introduction: Iron is an essential metal of human life. It is involved in important biological processes such as electron transport in mitochondria. Iron also catalyzes the Fenton reaction to produce reactive oxygen species (ROS). In the brain of Alzheimer patients, it is supposed that ROS overproduction favors lipid peroxidation processes leading to increased levels of 7-ketocholesterol (7KC). In addition, iron accumulation in brain cells, resulting either to abnormal iron metabolism or from diet habits, is also considered as a potential risk factor in dementia. Currently, iron and 7KC are recognized as important mediators of cell dysfunctions leading to various cytotoxic effects in a variety of major neurodegenerative diseases: Alzheimer's disease, Parkinson's disease and multiple sclerosis. The aim of the study consisted i) to evaluate the accumulation and the cytotoxicity of iron in neuronal cells, and ii) to determine whether iron was able to modulate 7KC-induced side effects.

Materials and Methods: Murine N2a and human SK-N-BE neuronal cells were exposed to iron (iron chloride: 1, 10, 50, 75, 100 µM) during 24 h without or with 7KC (0, 5, 10, 20 µM) introduced in the culture medium 2 h before iron. The cytotoxicity was evaluated by crystal violet and MTT tests allowing to evaluate the number of adherent cells and to measure mitochondrial succinate dehydrogenase activity (assessing cell metabolic activity), respectively. The effects on plasma membrane permeability, transmembrane mitochondrial potential (ΔΨm) and superoxide anion ($O_2^{\bullet-}$) production were quantified by flow cytometric methods after staining with propidium iodide (PI), $DiOC_6(3)$ and dihydroethidine (DHE), respectively. Iron accumulation was evaluated on cell deposits by Perls staining procedure: the formation of a blue precipitate inside the cells reflects the proportion of iron which has reacted with potassium ferrocyanide

Results: In nerve cells (N2a, SK-N-BE) cultured in the presence of iron, no iron accumulation were observed whatever the concentrations considered, and no cytotoxic effects were revealed compared to untreated cells: no change in cell adhesion, succinate dehydrogenase activity, membrane permeability and ROS production. Under the same experimental conditions, BV-2 murine microglial cells and 158N murine oligodendrocytes were capable of accumulating iron even in the absence of 7KC. Noteworthy, on N2a and SK-N-BE cells simultaneously treated with 7KC and iron, important iron incorporations were observed, and the cytotoxic effects of 7KC were increased: the loss of ΔΨm was higher; cell permeability to PI and ROS production measured with DHE were enhanced. Thus, comparatively to 7KC-treated cells, the percentage of PI positive cells and of DHE positive cells was around twice higher when N2a and SK-N-BE cells were simultaneously treated with 7KC (10 µM) and iron (100 µM).

Conclusion: Our data show a potentiation of 7KC-induced cytotoxic effects in the presence of iron. These data support the hypothesis that enhanced levels of iron in nerve cells (resulting either from abnormal iron metabolism or from environmental factors) in subjects with high 7KC levels in the brain might favor neuronal loss. So, it could be important to improve the management of neurodegenerative diseases by simultaneously carrying out the dosages of oxysterols produced by auto-oxidation such as 7KC and of metal ions such as iron.

Keywords: *Iron, 7-ketocholesterol, N2a cells, SK-N-BE cells, plasma membrane, oxidative stress, mitochondria, neurodegeneration.*

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List of Participants

PhD	**Abbate Manuela**	University of Balearic Islands	Palma de Mallorca	Spain	pep.tur@uib.es
PhD	**Abdin Yaman Ahmad**	University of Saarland	Saarbrücken	Germany	c.jacob@mx.uni-saarland.de
PhD	**Akhasan Rahma**	University of Saarland	Saarbrücken	Germany	c.jacob@mx.uni-saarland.de
PhD	**Alnahas Faez**	University of Saarland	Saarbrücken	Germany	c.jacob@mx.uni-saarland.de
Pr	**Andrejs Skesters**	Rīga Stradiņš University	Riga	Latvia	Andrejs.Skesters@rsu.lv
Ass. Pr.	**Andreoletti Pierre**	University of Burgundy	Dijon	France	Pierre.Andreoletti@u-bourgogne.fr
Pr	**Antohe Ileana**	University of Medicine and Pharmacy "Grigore T Popa"	Iasi	Romania	ileanaantohe@gmail.com
Pr	**Antosiewicz Jędrzej**	Medical University of Gdansk	Gdansk	Poland	jant@gumed.edu.pl
Pr	**Atalay Mustafa**	University of Eastern Finland	Kuopio	Finland	mustafa.atalay@uef.fi
PhD	**Baranowska Monika**	Gdansk University of Technology	Gdańsk	Poland	monbaran1@student.pg.gda.pl
Pr	**Bartoszek Agnieszka**	Gdansk University of Technology	Gdańsk	Poland	abartosz@pg.gda.pl
Dr	**Bibiloni Maria del Mar**	University of Balearic Islands	Palma de Mallorca	Spain	mar.bibiloni@uib.es
Dr	**Biedermann David**	Institute of Microbiology ASCR	Prague	Czech Republic	david.biedermann@gmail.com
Dr	**Bigovic Miljan**	Faculty of Natural Sciences and Mathematics	Podgorica	Montenegro	miljan@ac.me
Mr.	**Boettger Didier**	University of Strasbourg	Strasbourg	France	boettger@unistra.fr
Pr	**Bohn Torsten**	Luxembourg Institute of Health	Strassen	Luxembourg	Torsten.Bohn@lih.lu
PhD	**Bonetti Justine**	University of Lorraine	Nancy	France	justine.bonetti8@etu.univ-lorraine.fr
Dr	**Bouzas Cristina**	University of Balearic Islands	Palma de Mallorca	Spain	cristinabouvel@gmail.com
Pr	**Carlberg Carsten**	University of Eastern Finland	Kuopio	Finland	carsten.carlberg@gmail.com
Dr	**Caruana Mario**	University of Malta	Msida	Malta	mario.caruana@um.edu.mt
Pr	**Chaimbault Patrick**	University of Lorraine	Metz	France	patrick.chaimbault@univ-lorraine.fr
PhD	**Cherbonnel Angéline**	University of Strasbourg	Strasbourg	France	angeline.cherbonnel@etu.unistra.fr
Pr	**Cherkaoui-Malki Mustapha**	University of Burgundy	Dijon	France	malki@u-bourgogne.fr
Dr	**Chondrogianni Niki**	Institute of Biology, Medicinal Chemistry & Biotechnology	Athens	Greece	nikichon@eie.gr
PhD	**Corrochano Alberto**	Teagasc Food Research Centre	Fermoy, Co.Cork	Ireland	Alberto.Corrochano@teagasc.ie

Dr	**Cotoz-Munez Léandro**	University of Strasbourg	Strasbourg	France	cotosmunoz@unistra.fr
Dr	**Dadeshidze Inga**	COST Officer	Brussels	Belgium	inga.dadeshidze@cost.eu
Dr	**Davioud-Charvet Elisabeth**	Strasbourg University	Strasbourg	France	elisabeth.davioud@unistra.fr
Pr	**Di Costanzo Alfonso**	University of Molise	Campobasso	Italy	alfonso.dicostanzo@unimol.it
Pr	**Diederich Marc**	University of Luxembourg	Luxembourg	Luxembourg	marc.diederich@lbmcc.lu
Dr	**Dinić Svetlana**	University of Belgrade	Belgrade	Serbia	sdinic@ibiss.bg.ac.rs
Dr	**Ebokaiwe Azubuike Peter**	Federal University Ndufu Alike Ikwo	Abakaliki, Ebonyi	Nigeria	petazk@yahoo.com
Pr	**Eftimie-Totu Eugenia**	University Politehnica of Bucharest	Bucharest	Romania	eugenia_totu@yahoo.com
Dr	**Ejike Chukwunonso ECC**	Federal University Ndufu Alike Ikwo	Abakaliki, Ebonyi	Nigeria	nonsoejikeecc@yahoo.com
Dr	**Elhabiri Mourad**	Strasbourg University	Strasbourg	France	elhabiri@unistra.fr
Pr	**Faller Peter**	University of Strasbourg	Strasbourg	France	pfaller@unistra.fr
PhD	**Farooq Muhammad A.**	University of Strasbourg	Strasbourg	France	muhammad-akmal.farooq@unistra.fr
Dr	**Fernandes Ana**	Universidade Lusofona de Humanidades e Tecnologias	Lisboa	Portugal	ana.fernandes@ulusofona.pt
Dr	**Fink Bruno**	oxygen Science Transfer & Diagnostics	Elzach	Germany	bruno.fink@noxygen.de
Pr	**Gabbianelli Rosita**	University of Camerino	Camerino	Italy	rosita.gabbianelli@unicam.it
Pr	**Garaj Vrhovac Verica**	Institute for Medical Research and Occupational Health	Zagreb	Croatia	vgaraj@imi.hr
Ass. Pr.	**Gaucher Caroline**	University of Lorraine	Nancy	France	caroline.gaucher@univ-lorraine.fr
Dr	**Gerić Marko**	Institute for Medical Research and Occupational Health	Zagreb	Croatia	mgeric@imi.hr
Dr	**Giblin Linda**	Teagasc Food Research Centre	Fermoy, Co.Cork	Ireland	Linda.Giblin@teagasc.ie
PhD	**Gomes Andreia**	Instituto de Biologia Experimental e Tecnológica	Oeiras	Portugal	andreiagomes@itqb.unl.pt
Dr	**Grabez Milkica**	Department of Preventive Medicine, Faculty of Medicine	Banja Luka	Bosnia and Herzegovina	milkica_grabez@yahoo.com
PhD	**Griffin Sharoon**	University of Saarland	Saarbrücken	Germany	c.jacob@mx.uni-saarland.de
Pr	**Hermans Nina**	Universiteit Antwerpen	Wilrijk	Belgium	nina.hermans@uantwerpen.be
PhD	**Iddir mohammed**	University of Luxembourg	Luxembourg	Luxembourg	Mohammed.Iddir@lih.lu
Pr	**Jacob Claus**	University of Saarland	Saarbrücken	Germany	c.jacob@mx.uni-saarland.de
Pr	**Karasu Çimen**	Gazi University, Faculty of Medicine	Ankara	Turkey	cimenkrs@gmail.com
Dr	**Kepczynska Malgorzata Anna**	The University of Buckingham	Buckingham	U.K.	malgosia.kepczynska@buckingham.ac.uk
PhD	**Kharma Ammar**	University of Saarland	Saarbrücken	Germany	c.jacob@mx.uni-saarland.de

PhD	**Khobragade Vrushali**	University of Strasbourg	Strasbourg	France	khobragade@unistra.fr
Pr	**Khvedelidze Mariam**	Tbilisi State University	Tbilisi	Georgia	mariam.khvedelidze@tsu.ge
Dr	**Kypreos Kyriakos E.**	University of Patras	Rio Achaias	Greece	kkypreos@upatras.gr
Mrs.	**Konowalska Maja**	Rehabilitation & recreation Center "Dobry Brat"	Osiek	Poland	mkonowalska@gmail.com
Pr	**Latruffe Norbert**	University of Burgundy	Dijon	France	Norbert.Latruffe@u-bourgogne.fr
Dr	**Liepins Janis**	University of Latvia	Riga	Latvia	Janis.liepins@lu.lv
Dr	**Mackrill John**	University College Cork, BioScience Institute	Cork	Ireland	J.Mackrill@ucc.ie
Pr	**Mahajna Jamal**	MIGAL	Kiryat Shmona	Israel	jamalm@migal.org.il
Pr	**Marchioni Eric**	University of Strasbourg	Strasbourg	France	Eric.Marchioni@unistra.fr
Pr	**Marko Doris**	University of Vienna	Vienna	Austria	doris.marko@univie.ac.at
Dr	**McElhatton Anna**	University of Malta	Msida	Malta	anna.mcelhatton@um.edu.mt
Pr	**Mdzinarashvili Tamaz**	Tbilisi State University	Tbilisi	Georgia	tamaz.mdzinarashvili@tsu.ge
Dr	**Meiser Peter**	Ursapharm	Saarbrücken	Germany	peter.meiser@ursapharm.de
Pr	**Merzouk Hafida**	Tlemcen University	Tlemcen	Algeria	hafidamerzouk_2@hotmail.com
Dr	**Milev Misko**	Goce Delcev University	Stip	fYR Macedonia	misko.milev@ugd.edu.mk
Dr	**Moreno-Indias Isabel**	University Hospital of Malaga	Malaga	Spain	isabel_moreno_indias@hotmail.com
Pr	**Nasser Boubker**	University Hassan 1st	Settat	Morocco	boubker_nasser@hotmail.com
PhD	**Nassim Jawad**	University of Saarland	Saarbrücken	Germany	jawadnasimpk@yahoo.com
PhD	**Ney Yannick**	University of Saarland	Saarbrücken	Germany	yannick.ney@gmail.com
PhD	**Nury Thomas**	University of Burgundy	Dijon	France	thomas.nury@u-bourgogne.fr
Pr	**Oliveira Nuno**	University of Lisbon	Lisboa	Portugal	ngoliveira@ff.ulisboa.pt
Dr	**Orlikova barbora**	University of Luxembourg	Luxembourg	Luxembourg	barbora.orlikova@lbmcc.lu
PhD	**Parchem Karol**	Gdansk University of Technology	Gdańsk	Poland	parchem.karol@gmail.com
Dr	**Perecko Tomas**	Institute of Experimental Pharmacology and Toxicology	Bratislava	Slovakia	tomas.perecko@savba.sk
Ass. Pr.	**Perrin-Sarrado Caroline**	University of Lorraine	Nancy	France	Caroline.Perrin-Sarrado@univ-lorraine.fr
Dr	**Philippou Elena**	University of Nicosia	Nicosia	Cyprus	Ephilippou2@gmail.com
Pr	**Poulsen Henrik E.**	Rigshospitalet	Copenhagen	Danmark	Henrik.Enghusen.Poulsen.01@regionh.dk
Pr	**Radak Zsolt**	Semmelweis University	Budapest	Hungary	radak@tf.hu
PhD	**Rafla Mina**	University of Saarland	Saarbrücken	Germany	c.jacob@mx.uni-saarland.de
Dr	**Rai Dilip**	Teagasc Food Research Centre Ashtown	Dublin	Ireland	dilip.rai@teagasc.ie
Pr	**Richling**	University of Kaiserlautern	Kaiserslautern	Germany	richling@chemie.uni-kl.de
Dr	**Robajac Dragana**	Institute for the Application of Nuclear Energy	Belgrade-Zemun	Serbia	draganar@inep.co.rs
Pr	**Ruskovska Tatjana**	Goce Delcev University	Stip	fYR Macedonia	tatjana.ruskovska@ugd.edu.mk
PhD	**Saad Tamara**	University of Saarland	Saarbrücken	Germany	c.jacob@mx.uni-saarland.de

PhD	**Sahakyan Naira**	Yerevan State University	Yerevan	Armenia	sahakyannaira@yahoo.com
PhD	**Salafzoon Narsis**	University of Saarland	Saarbrücken	Germany	narsis.s1980@gmail.com
PhD	**Santoro Alice**	University of Strasbourg	Strasbourg	France	alice.santoro@unistra.fr
Dr	**Santos Claudia**	Instituto Biologia Experimental e Tecnologica	Oeiras	Portugal	csantos@itqb.unl.pt
PhD	**Sarfraz Muhammad**	University of Saarland	Saarbrücken	Germany	c.jacob@mx.uni-saarland.de
Pr	**Sasson Shlomo**	The Hebrew University of Jerusalem	Jerusalem	Israel	shlomo.sasson@mail.huji.ac.il
PhD	**Scensna Anna**	University of Saarland	Saarbrücken	Germany	c.jacob@mx.uni-saarland.de
PhD	**Schmitt Romain**	University of Lorraine	Nancy	France	romain.schmitt@univ-lorraine.fr
Pr	**Skesters Andrejs**	Riga Stradins University	Riga	Latvia	Andrejs.Skesters@rsu.lv
Pr	**Smilkov Katarina**	Goce Delcev University	Stip	fYR Macedonia	katarina.smilkov@ugd.edu.mk
Dr	**Smirnovas Vytautas**	Vilnius university	Vilnius	Lithuania	vytautas.smirnovas@bti.vu.lt
Pr	**Sobajic Sladjana**	University of Belgrade	Belgrade	Serbia	sobajic04@yahoo.com
PhD	**Suliborska Klaudia**	Gdansk University of Technology	Gdańsk	Poland	klaudiasuliborska@wp.pl
Dr	**Sunderic Milos**	Institute for the Application of Nuclear Energy-INEP	Belgrade	Serbia	milos@inep.co.rs
Dr	**Sureda Antoni**	University of Balearic Islands	Palma de Mallorca	Spain	antoni.sureda@uib.es
Dr	**Sykiotis Gerasimos**	University of Lausanne	Lausanne	Switzerland	gerasimos.sykiotis@chuv.ch
Dr	**Tadayyon Mohammad**	Pharmidex	London	U.K.	Moh.Tadayyon@gmail.com
Pr	**Thiel Gerald**	University of Saarland	Saarbrücken	Germany	gerald.thiel@uks.eu
Pr	**Trchounian Armen**	Yerevan State University	Yerevan	Armenia	Trchounian@ysu.am
PhD	**Triaux Zélie**	University of Strasbourg	Strasbourg	France	zelie.triaux@gmail.com
Pr	**Tur Mari Josep Antoni**	University of the Balearic Islands	Palma de Mallorca	Spain	pep.tur@uib.es
Dr	**Uskokovic Aleksandra**	Institute for Biological Research	Belgrade	Serbia	auskokovic@ibiss.bg.ac.rs
Pr	**Vanden Berghe Wim**	University of Antwerp	Wilrijk	Belgium	wimk.vandenberghe@gmail.com
Ass. Pr.	**Vejux Anne**	University of Burgundy	Dijon	France	anne.vejux@u-bourgogne.fr
Ass. Pr.	**Vervandier-Fasseur Dominique**	University of Burgundy	Dijon	France	dominique.vervandier-fasseur@u-bourgogne.fr
Pr	**Vidakovic Melita**	University of Belgrad	Belgrade	Serbia	melita@ibiss.bg.ac.rs
Pr	**Vidovic Bojana**	University of Belgrade	Belgrade	Serbia	vidovic.bojana@gmail.com
Dr	**Vucic Vesna**	Institute for Medical Research	Belgrade	Serbia	vesna.vucic.imr@gmail.com
Dr	**Wasfy Darwin**	University of Saarland	Saarbrücken	Germany	narsis.s1980@gmail.com
Pr	**Winyard Paul G.**	University of Exeter Medical School	Exeter	U.K.	P.G.Winyard@exeter.ac.uk
PhD	**Yagdi Efe Esma**	University of Luxembourg	Luxembourg	Luxembourg	esma.yagdi@lbmcc.lu
Mrs.	**Zahn Sandrine**	University of Strasbourg	Strasbourg	France	sandrine.zahn@iphc.cnrs.fr
Dr	**Zaibi Mohamed S.**	The University of Buckingham	Buckingham	U.K.	mohamed.zaibi@buckingham.ac.uk

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