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OCC 2015

OXYGEN CLUB OF CALIFORNIA

WORLD CONGRESS

"OXIDANTS AND ANTIOXIDANTS IN BIOLOGY"

VALENCIA, 24th-26th JUNE 2015

CONGRESS PALACE OF VALENCIA

OXYGEN CLUB OF CALIFORNIA O_2 ESTABLISHED 1994

GEIRLI GrupoEspañol de Investigación en Radicales Libres

Sociedade Portuguesa de Bioquímica SPB Portuguese Oxidative Stress Group

Société Française de Recherche sur les Radicaux Libres

Dear colleagues and friends,

You are most welcome to the "***Oxygen Club of California World Congress 2015 Oxidants and Antioxidants in Biology***" held in the Congress Palace of Valencia, Spain, on June 24–26, 2015. This is a **Joint Meeting** of the **Oxygen Club of California**, the **Spanish Group for Research on Free Radicals** (GEIRLI), the **Portuguese Group of Free Radicals**, and **Le Société Française de Recherches sur les Radicaux Libres**.

The major topics of this congress are Nutrition, Oxygen Biology and Medicine**,** Redox Signaling in Inflammation, Aging and Longevity, Redox Biology in Vascular Disease, and Redox Biology in Neurodegenerative Diseases. The scientific programme comprises **2 plenary keynote lectures, 28 lectures, 15 oral communications, and 199 posters**, which all will provide new and exciting research, being an exceptional opportunity to foster interdisciplinary and international research at a high level. The selection of oral communications after evaluation by an international committee has been hard due to the high quality of the research. We are very pleased to know that the international committee considered that all oral communication and posters show very interesting and remarkable findings in the field.

We thank you for your contribution to the success of the event and we do hope that you will enjoy this great scientific event as well as your stay in a beautiful and modern city close to the seaside such as Valencia.

With our best wishes and warm regards,

Juan Sastre, Josiane Cillard and João Laranjinha

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OXIDANTS AND ANTIOXIDANTS IN BIOLOGY

SCIENTIFIC PROGRAMME

JOINT MEETING OF

The Oxygen Club of California
The Spanish Group for Research on Free Radicals
The Portuguese Group of Free Radicals
Le Société Française de Recherches sur les Radicaux Libres

SCIENTIFIC PROGRAMME

24th June 2015

09:00 – 09:15 h. **OPENING CEREMONY WITH THE RECTOR OF THE UNIVERSITY OF VALENCIA**

09:15 – 10:15 h. **OPENING KEYNOTE LECTURE**

Chair: **Helmut Sies**, *Heinrich-Heine-Universitaet Duesseldorf, Germany*

- **Nitric oxide and Oxygen: actions and interactions in health and disease**
  Salvador Moncada, *Wolfson Institute for Biomedical Research University College London, UK*

10:15 – 11:00 h. **COFFEE BREAK AND POSTER SESSION I**

11:00 - 13:00 h. **SESSION I. NUTRIENTS, OXYGEN BIOLOGY, AND MEDICINE**

Chairs: **Leopold Flohé**, *University of Padova, Molecular Medicine, Italy*
Guillermo Sáez, *University of Valencia, Spain*

- **Prooxidant and anticancer potential of spice-derived nutraceuticals and their role in cancer**
  Bharat Aggarwal, *The University of Texas MD Anderson Cancer Center, Houston, USA*
- **Targeting diabetic nephropathy through podocyte-specific soluble epoxide hydrolase deficiency in mice**
  Fawaz Haj, *University of California Davis, USA*
- **Procyanidins interact with lipid rafts: mechanisms and biological consequences**
  Patricia Oteiza, *University of California at Davis, USA*
- **Benefits of alpha-lipoic acid and caloric restriction in obesity**
  Mulchand S. Patel, *University of Buffalo, Buffalo, New York, USA*

13:00 – 14:30 h. **LUNCH**

14:30 – 16:30 h. **SESSION II. REDOX SIGNALING IN INFLAMMATION**

Chairs: **César Fraga**, *University of Buenos Aires-CONICET, Argentina*
José María Mato, *CIC bioGUNE, Bizkaia, Spain*

- **Lipoic acid supplementation decreases biomarkers of inflammation and lipid peroxidation in overweight or obese adults: results of a randomized, double-blind, placebo-controlled study**
  Balz Frei, *Linus Pauling Institute, Oregon State University*
- **GSH in cholestatic liver injury**
  Shelly Lu, *Cedars-Sinai Medical Center, Los Angeles, USA*
- **Impaired mitophagy leads to stress-induced cellular senescence**
  Irfan Rahman, *University of Rochester Medical Center, New York, USA*
- **Redox regulation of FOXO transcription factors**
  Tobias B. Dansen, *University Medical Center Utrecht, The Netherlands*

16:30 – 17:00 h. **COFFEE BREAK**

17:00 – 18:30 h.

- **Oxidized phospholipids and cell signaling in inflammation**
  Corinne Spickett, *Aston University, Birmingham, UK*
- **Ca^2- independent biosynthesis of lipid mediators via oxygenation and hydrolysis of mitochondrial cardiolipins**
  Valerian E. Kagan, *University of Pittsburgh, USA*
- **Biochemical mechanisms of mitochondrial SOD nitration and inactivation: pathological relevance**
  Rafael Radi, *Universidad de la República, Montevideo, Uruguay*

18:30 – 19:30 h. **ORAL COMMUNICATIONS I**

Chairs: **Arlette Delamarche**, *University Rennes 2, France*
Jordi Muntané, *Hospital Universitario "Virgen del Rocío-Virgen Macarena", Sevilla, Spain*

O1 **Nitrite reductase activity of xanthine oxidase, xanthine dehydrogenase and aldehyde oxidase: evaluation of their contribution to NO formation *in vivo***
Maia, Luisa and Moura, José J. G. *Universidade Nova de Lisboa, Portugal*

O2 **Peroxynitrite-dependent inactivation of human glutamine synthetase: biochemical characterization and functional consequences"**
Bartesaghi, Silvina[1]; Campolo, Nicolás[1]; Ott, Christiane[2]; Hugo-Pereira, Martín[2]; Meinl, Walter[2]; Grune, Tilman[2]; and Radi, Rafael[1]. *[1]Universidad de la República Uruguay; and [2]German Institute of Human Nutrition, Germany.*

O3 **Chronic intake of the optimized EPA:DHA 6:1 formulation protects against angiotensin II-induced hypertension, oxidative stress and endothelial dysfunction in rats**
Zahid, Rasul; Grazielle C. , Silva; Thais, Porto Ribeiro; Faraj, Zgheel; Cyril, Auger; and Valérie B., Schini-Kerth. *University of Strasbourg, France.*

O4 **Effects of high versus low dietary intake of bioactive food compounds on biomarkers of oxidative stress and metabolic and vascular health in the BIOCLAIMS cohort**
Winklhofer-Roob, Brigitte M.; Faustmann, Gernot; Kieslinger, Petra; Walter, Natalie; Maimari, Theopisti; Hafner-Giessauf, Hildegard; Zelzer, Sieglinde; Sattler, Matteo C.; Meinitzer, Andreas; Öttl, Karl; Wonisch, Willibald; Obermayer-Pietsch, Barbara; Tiran, Beate; and Roob, Johannes M. *University of Graz, Austria.*

O5 **Pancreatic beta cell response to nutrient overload is triggered by phospholipid remodelling and lipid peroxidation**
Sasson, Shlomo (*The Hebrew University of Jerusalem (Faculty of Medicine), Institute for drug Research, Israel)*

19:30 h. **GENERAL ASSEMBLY OF THE SPANISH GROUP FOR RESEARCH ON FREE RADICALS (GEIRLI)**

25th June 2015

09:00 – 11:00 h. **SESSION III. AGING AND LONGEVITY**

Chairs: **Regina Brigelius**, *University of Potsdam, Germany*
Enrique Cadenas, *University of Southern California, Los Angeles, USA*

- **Redox biology: Where exposome meets genome**
  Dean P. Jones, *Emory University, Atlanta, USA*
- **Impact of mitochondrial fusion proteins on ROS handling**
  Antonio Zorzano, *Universitat de Barcelona, Spain*
- **The role of the mitochondrial sirtuin SIRT3 in aging and its retardation by caloric restriction**
  Tomas Prolla, *University of Wisconsin, Madison, USA*
- **Mitochondrial regulation of hematopoietic stem cell aging and rejuvenation**
  Danica Chen, *University of California, Berkeley, USA*

11:00 – 12:00 h. **COFFEE BREAK AND POSTER SESSION (II)**

12:00 – 13:00 h. **ORAL COMMUNICATIONS II**

Chairs: **Mª Begoña Ruiz Larrea**, *University of the Basque Country, UPV/EHU, Spain*
Fernando Antunes, *Universidade de Lisboa, Portugal*

O6 **Redox dependent selective targeting of mutant K-Ras expressing cancer cells**
Iskandar, Kartini; Rezlan, Majidah; Bellot, Gregory; Yadav, Sanjiv; Foo, Jonathan; and Pervaiz, Shazib. *National University of Singapore, Singapore.*

O7 **Repair of peroxidized membrane phospholipids: critical role of peroxiredoxin 6**
Fisher, Aron; and LI, Haito. *University of Pennsylvania, USA.*

O8 **Oxidant-induced cellular senescence is caused by alteration in telomere shelterin complex**
Tormos, Ana M.; Ahmed, Tanveer; Sundar, Isaac K.; Yao, Hongwei; and Rahman, Irfan. *University of Rochester, USA*

O9 **Complex I assembly into supercomplexes regulates mitochondrial ROS production in neurons and astrocytes.**
Lopez-Fabuel, I.[1]; Le Douce, J.[2]; Bonvento, G.[2]; James, A.M.[3]; Murphy, M.P.[3]; Almeida, A.[4]; and Bolanos, J.P.[1]. *[1]University of Salamanca-CSIC, Spain; [2]CNRS, France; [3]Medical Research Council Mitochondrial Biology Unit, United Kingdom; and [4]University Hospital of Salamanca, Spain*

O10 **Maintenance of mitochondrial function by site-specific ROS signalling extends animal lifespan**
Mallikarjun, Venkatesh[1]; Scialo, Filippo [1]; Sriram, Ashwin[1]; Gubina, Nina [2]; Lõhmus, Madis[3]; Nelson, Glyn [1]; Logan, Angela [4]; Cooper, Helen[3]; Enriquez, Jose Antonio[5]; Murphy, Michael[4]; and Sanz, Alberto[1]. *[1]Newcastle University; [2]The Institute of Theoretical and Experimental Biophysics RAS; [3]*Åbo *Akademi University; [4]MRC Mitochondrial Biology Unit; and [5]CNIC, Spain*

13:00 – 14:30 h. **LUNCH**

14:30 – 16:00 h.

Chairs: **Nesrin Kartal-Özer**, *Marmara University, Istanbul, Turkey*
Abraham Reznick, *Rappaport Faculty of Medicine, Haifa, Israel*

- **Aggregated lipids and proteins in aging, Alzheimer's disease, and diabetes**
  Tilman Grune, *German Institute of Human Nutrition, Nuthetal, Germany*
- **The aged proteome - impact of protein oxidative modifications on cellular aging**
  Bertrand Friguet, *Université Pierre et Marie Curie, Paris, France*
- **Clock genes regulate neuronal redox homeostasis in brain**
  Gaetano Serviddio, *University of Foggia, Italy*

16:00 – 16:30 h. **COFFEE BREAK**

16:30-17:30 h.

- **Frailty and sarcopenia**
  Leocadio Rodríguez-Mañas, *Hospital Universitario de Getafe, Madrid, Spain*

- **Reactive oxygen species production in skeletal muscle during contractile activity and aging**
  Malcolm Jackson, *University of Liverpool, UK*

17:30 – 18:30 h.	**ROUND TABLE DISCUSSION ON AGING AND LONGEVITY**
Discussion Leader:	**Michael Murphy**, *MRC, Mitochondrial Biology Unit, Cambridge, UK*
Panel Members:	Speakers of the Aging and Longevity session

20:00 h.	**GALA DINNER**

26th June 2015

09:00 – 10:30 h. SESSION IV. REDOX BIOLOGY IN VASCULAR DISEASE

Chairs: **Michael J. Davies**, *University of Copenhagen, Denmark*
Lisardo Boscá, *Institute of Biomedical Research Alberto Sols, CSIC-UAM, Madrid, Spain*

- **How mitochondria produce ROS during cardiac ischaemia-reperfusion injury**
  Michael Murphy, *MRC, Mitochondrial Biology Unit, Cambridge, UK*
- **Mechanisms of redox responses to endothelial shear stress: from peroxide to nitric oxide**
  Santiago Lamas, *Centro de Biología Molecular Severo Ochoa,CSIC, Madrid, Spain*
- **Vascular endothelial and smooth muscle cell redox signaling and function in health and disease**
  Giovanni Mann, *British Heart Foundation Centre of Research Excellence, King's College London, UK*

10:30 – 11:30 h. COFFEE BREAK AND POSTER SESSION (III)

11:30 – 12:30 h. ORAL COMMUNICATIONS III

Chairs: **Anne Negré Salvayre**, *University of Toulouse, France*
María Teresa Mitjavila, *University of Barcelona, Spain*

O11 **Regulation of endothelial function and angiogenesis by PGC-1α relies on ROS control of vascular stability**
García-Quintans, Nieves[1]; Sánchez-Ramos, Cristina[1]; Tierrez, Alberto[2]; Olmos, Yolanda[2]; Luque, Alfonso[2]; Arza, Elvira[2]; Alfranca, Arantzazu[2]; Redondo, Juan Miguel[2]; and Monsalve, Maria[1].*[1]CSIC (IIBm); and [2]CNIC, Spain).*

O12 **The transcription factor Nrf2 mediates UCP3 upregulation in response to 4-hydroxynonenal in mouse cardiomyocytes**
López-Bernardo, Elia[1]; Anedda, Andrea[2]; Sánchez-Pérez, Patricia[1]; Acosta-Iborra, Bárbara[2]; and Cadenas, Susana[1].*[1]Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Spain; and [2]Instituto de Investigación Sanitaria Princesa, Spain.*

O13 **Searching for biomarkers of Charcot-Marie-Tooth, a neuromuscular rare disease**

Seco-Cervera, Marta[1]; Ibáñez-Cabellos, Jose Santiago[2]; García-Giménez, Jose Luis[3]; Berenguer-Pascual, Ester[1]; Reyes-Palomares, Armando[4]; Rodríguez-López, Rocío[4]; Sánchez-Jiménez, Francisca[4]; Sevilla, Teresa[5]; Espinós, Carmen[6]; Palau, Francesc[7]; and Pallardó, Federico V.[1]. *[1]INCLIVA-University of Valencia,; [2]SISTEMAS GENÓMICOS S.L.; [3,6,7]CIBERER-UV ; [4]University of Malaga, Spain; [5]La Fe hospital, Spain.*

O14 **Oxidative stress and NOX4 up-regulation contribute to aortic wall injuries in Marfan syndrome aortic aneurysms**

Meirelles, Thayna[1]; Crosas-Molist, Eva [2]; Gorbenko del Blanco, Darya [1]; Hernández, Vanessa[1]; García-Calero, Carolina [3]; Condom, Enric[4]; Forteza, Alberto[5]; Rodríguez-Pascual, Fernando[6]; Brandes, Ralf[7]; Mas Stachurska, Aleksandra[8]; Sitges, Marta[8]; Sorolla , M. Alba[9]; Ros, Joaquim [9]; Laurindo, Francisco[10]; Fabregat, Isabel [2]; and Egea, Gustavo[1]. *[1.4]Universitat de Barcelona, Spain; [2]Institut d'Investigacions Biomèdiques de Bellvitge , Spain; [3]Hospital de Bellvitge-IDIBELL , Spain; [5]Hospital 12 de Octubre, Madrid , Spain; [6]Centro de Biologia Molecular Severo Ochoa, Madrid, Spain; [7]Goethe-University Frankfurt, Germany; [8]Hospital Clínic, IDIBAPS, Spain; [9]Universitat de Lleida, Spain; and [10] University of São Paulo, Brazil*

O15 **Nitrite/ascorbate redox interaction leads to nitric oxide production in the brain hippocampus, supporting neurovascular coupling. An in vivo study in real-time.**

Ferreira, Nuno R.; Lourenço, Cátia F.; Costa, Sérgio M.; Barbosa, Rui M.; and Laranjinha, João. *[1]University of Coimbra, Portugal*

12:30 – 14:00 h. **LUNCH**

14:00 – 15:30 h. **SESSION V. REDOX BIOLOGY IN NEURODEGENERATIVE DISEASES (Sponsored by the University of Valencia)**

Chairs: **Leonor Almeida**, *University of Coimbra, Portugal*
Giuseppe Valacchi, *University of Ferrara, Italy*

- **Amyloid-ß disrupts calcium and redox homeostasis in brain endothelial cells**
  Catarina Oliveira, *Center for Neuroscience and Cell Biology, University of Coimbra Portugal*

- **Possible role of oxysterols in the brain pathophysiology**
  Giuseppe Poli, *University of Turin at S. Luigi Gonzaga Hospital, Italy*
- **Alzheimer's Disease: from oxidative stress to ApoE- mediated therapeutics**
  José Viña, *University of Valencia, Spain*

15:30 – 16:00 h. **COFFEE BREAK**

16:00 – 17:00 h.

- **Neurotransmission-dependent metabolic and redox coupling between neurons and astrocytes**
  Juan Bolaños, *Institute of Functional Biology and Genomics, University of Salamanca-CSIC, Spain*
- **The role and mechanisms of mitochondrial cholesterol in neurodegeneration**
  José Carlos Fernández-Checa, I*nstituto Investigaciones Biomedicas de Barcelona (IIBB)-CSIC, Barcelona, Spain; and University of Southern California, Los Angeles, USA*

17:00 – 18:00 h. **CLOSING KEYNOTE LECTURE**

Chair: **Josiane Cillard**, *University of Rennes 1, France*

- **Impaired inducibility of the mitochondrial lon protease in ageing: an example of declining adaptive homeostasis**
  Kelvin Davies, *University of Southern California, Los Angeles, USA*

18:00 – 18:30 h.

- **Prizes and Awards**
  Oxygen Club of California and Jarrow Formulas Health Sciences Prize
  Young Investigator Awards:
  – Oxygen Club of California
  – ABB
  – Linus Pauling Institute
  – Society for Free Radical Research-Europe
- **Closing Remarks**
  Juan Sastre, Josiane Cillard, João Laranjinha

LECTURES

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L1

Nitric oxide and Oxygen: actions and interactions in health and disease

Professor Sir Salvador Moncada

Director of Cancer Sciences, University of Manchester, Manchester Cancer Research Centre, Wilmslow Road, Manchester M20 4QL

Nitric oxide (NO) inhibits cell respiration reversibly and in competition with O_2 through the inhibition of the mitochondrial cytochrome c oxidase (Complex IV). At concentrations lower than those required to inhibit respiration, endogenous NO enhances the reduction of the electron transport chain, thus enabling cells to maintain their O_2 consumption. This facilitates the release of superoxide anion, which initiates the transcriptional activation of NF-κB as an early signal of a stress response. Through free radical formation, long-term inhibition of mitochondrial respiration by NO leads to persistent inhibition of Complex I. This is dependent on the S-nitrosylation of a specific thiol in the active form of this protein. S-nitrosylation of Complex I might indicate the early stages of a pathological process.

Inhibition of mitochondrial respiration by low concentrations of NO at critical O_2 concentrations also leads to prevention of the stabilization of hypoxia-inducible factor-1α (HIF-1α) due to the redistribution of O_2 towards non-respiratory O_2-dependent targets. This prevents the cell from registering a state of hypoxia at low O_2 concentrations.

On the other hand, at higher concentrations NO increases the expression of HIF-1α by an action most probably involving a free radical mechanism. Full inhibition of respiration by NO leads to a situation in which in cells are unable to use O_2. We have named this state, "Metabolic Hypoxia. Cells respond to metabolic hypoxia by activating glycolysis as a defense mechanism or by initiating apoptotic death when unable to activate glycolysis as it happens in neurons.

Thus NO/O_2 interactions take place in a variety of situations which range from physiological regulation to cellular defense and pathophysiology indicating a significant role in health and in disease.

L2
Prooxidant and anticancer potential of spice-derived nutraceuticals and their role in cancer

Aggarwal, Bharat B.
Cytokine Research Laboratory, Department of Experimental Therapeutics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, U.S.A.

Chronic infections, obesity, alcohol, tobacco, radiation, environmental pollutants, and high-calorie diet have been recognized as major risk factors for the most common types of cancers. All these risk factors are linked to cancer through inflammation. While acute inflammation that persists for short-term mediates host defense against infections, chronic inflammation that lasts for long-term can predispose the host to various chronic illnesses, including cancer. Linkage between cancer and inflammation is indicated by numerous lines of evidence; first, transcription factors NF-κB and STAT3, two major pathways for inflammation, are activated by most cancer risk factors; second, an inflammatory condition precedes most cancers; third, NF-κB and STAT3 are constitutively active in most cancers; fourth, hypoxia and acidic conditions found in solid tumors activate NF-κB; fifth, chemotherapeutic agents and gamma irradiation activate NF-κB and lead to chemoresistance and radioresistance; sixth, most gene products linked to inflammation, survival, proliferation, invasion, angiogenesis, and metastasis are regulated by NF-κB and STAT3; seventh, suppression of NF-κB and STAT3 inhibits the proliferation and invasion of tumors; and eighth, most chemopreventive agents mediate their effects through inhibition of NF-κB and STAT3 activation pathways. Thus suppression of these proinflammatory pathways may provide opportunities for both prevention and treatment of cancer.

Extensive research within the past half-century has indicated that curcumin (diferuloylmethane), a yellow pigment in curry powder, exhibits antioxidant, anti-inflammatory, and proapoptotic activities. We investigated whether the anti-inflammatory and proapoptotic activities assigned to curcumin are mediated through its prooxidant/antioxidant mechanism. We found that TNF-mediated NF-kappaB activation was inhibited by curcumin; and glutathione reversed the inhibition. Similarly, suppression of TNF-induced AKT activation by curcumin was also abrogated by glutathione. The reducing agent also counteracted the inhibitory effects of curcumin on TNF-induced NF-kappaB-regulated antiapoptotic (Bcl-2, Bcl-xL, IAP1), proliferative (cyclin D1), and proinflammatory (COX-2, iNOS, and MMP-9) gene products. The suppression of TNF-induced AP-1 activation by curcumin was also reversed by glutathione. Also, the direct proapoptotic effects of curcumin were inhibited by glutathione and potentiated by depletion of intracellular glutathione by buthioninesulfoximine. Moreover, curcumin induced the production of reactive oxygen species and modulated intracellular GSH levels. Quenchers of hydroxyl radicals, however, were ineffective in inhibiting curcumin-mediated NF-kappaB suppression. Further, N-acetylcysteine partially reversed the effect of curcumin. Based on these results we conclude that curcumin mediates its apoptotic and anti-inflammatory activities through modulation of the redox status of the cell.

L3

Targeting diabetic nephropathy through podocyte-specific soluble epoxide hydrolase deficiency in mice

Haj, Fawaz[1]; Bettaieb, Ahmed[1]; and Hammock, Bruce[1].
[1]*University of California Davis.*

Diabetic mellitus (DM) nephropathy is a serious problem that causes renal failure. Soluble epoxide hydrolase (sEH; encoded by *Ephx2*) deficiency and pharmacological inhibition have beneficial effects in kidney function.In the current study, we assessed the physiological role of sEH in podocytes using the *Cre*-loxP system to achieve podocyte-specific deletion. sEH deletion efficiency and specificity was confirmed using biochemical and immunostaining approaches. Subsequently, control and pod-sEH KO mice were intraperitoneally injected with streptozotocin to generate DM model for each study group. Insulin tolerance tests (ITTs) and glucose tolerance tests (GTTs) were used to evaluate glucose homeostasis, and serum and urine components to determine kidney function. No significant metabolic differences were observed between control and pod-sEH KO mice under basal conditions. However, STZ-injected KO mice exhibited improved insulin sensitivity, as measured by ITT, compared with STZ controls in the absence of body weight difference. In addition, STZ KO mice displayed improved fasted, fed blood glucose, serum creatinine, urine albumin levels and maintained serum albumin levels compared with STZ control mice. Moreover, STZ KO mice exhibited improved blood pressure compared with controls. These were associated with decreased STZ-induced inflammatory response and endoplasmic reticulum stress signaling in kidneys of STZ KO mice compared with controls. Together, these findings establish sEH in podocytes as a contributor to systemic glucose homeostasis and kidney function.

L4

Procyanidins interact with lipid rafts: mechanisms and biological consequences.

Oteiza, Patricia
University of California at Davis, USA

Intestinal epithelial cells (IEC) membrane lipid rafts can be the target of bioactives that, although not absorbed by cells could exert biological actions through their interactions with the cell membrane. Lipid rafts compartmentalize select cellular processes including endocytosis, cell signaling, and in IEC their "cross-talk" with tight junctions (TJs) regulates intestinal permeability. Large procyanidins (PAC), oligomers of the flavan-3-ols (-)-epicatechin and ( )-catechin, are not absorbed by IEC but interact with lipid rafts and through these interactions promote beneficial biological effects. We observed that PAC interact with both lipid raft-like synthetic membranes (liposomes) and Caco-2 cell lipid rafts in a process dependent on raft cholesterol. PAC promoted Caco-2 cell membrane rigidification and dehydration, effects that were abolished upon cholesterol depletion. In Caco-2 cells differentiated into an intestinal epithelium, PAC prevented: i- lipid raft structure disruption and lipid raft-associated signaling induced by cholesterol depletion/ redistribution; ii- bile salt-induced raft disruption, signaling activation and TJs permeabilization. In proliferating Caco-2 cells, a model of colorectal cancer cells (CRC), PAC modulated signaling cascades initiated upon activation of the lipid raft-located epidermal growth factor (EGF) receptor (EGFR), a major player in oncogenesis. PAC promoted CRC apoptosis and cell cycle arrest in part through the inhibition of EGF-induced EGFR activation and of the downstream PI3K/Akt and Raf/MEK/ERK1/2 cascades. Characterization of the mechanisms underlying these actions indicates that in CRC: i) PAC did not act interacting with the EGFR ligand (EGF), ii) PAC did not affect EGFR localization at lipid rafts, iii) PAC affected lipid raft membrane fluidity (EGFR lipid environment); and iv) PAC promoted lipid raft/EGFR internalization. The above results will be discussed in terms of the relevance of these interactions on the capacity of PAC and their monomers to prevent CRC and mitigate metabolic diseases.

L5

Benefits of alpha-lipoic acid and caloric restriction in obesity

Patel, Mulchand[1]; and Rideout, Todd[2]

[1]University at Buffalo,The State University of New York (School of Medicine and Biomedical Sciences), Biochemistry, USA; and [2]University at Buffalo, The State University of New York (School of Public Health and Health Professions), Department of Exercise and Nutrition Sciences, USA.

The rapid increase in the prevalence of overweight and obesity among adults and children is a major public health concern in westernized societies. In addition to genetic predisposition to obesity, increased daily caloric intake combined with a sedentary life style also contributes to the development of obesity in adulthood. Furthermore, altered nutritional experiences during the critical periods of early development *in utero* and/or in childhood have been shown to play a role in metabolic programming resulting in adult-onset obesity.Using the artificial rearing of rat pups on a high-carbohydrate milk formula (HC), we have shown the development of hyperinsulinemia and malprogramming of the pancreatic beta-cells, the hypothalamic orexigenic and anorexigenic neurons, and the autonomic nervous system in HC rat pups. This phenotype persisted in adulthood causing obesity due to hyperphagia. Caloric restriction (by pair-feeding) in the postweaning period normalized the body weight gain and plasma hormonal profiles in HC adult rats but failed to reverse the program at the tissue levels, suggesting the irreversibility of the metabolic program by a moderate dietary restriction. A higher level of calorie restriction may be necessary for reversal of early life metabolic programming. In another experiment with adult Zucker male rats, dietary alpha-lipoic acid supplementation (LA, 0.25% w/w) protected against high fat diet-induced weight gain, aberrant responses in plasma lipid and lipoprotein profile, and hepatic triglyceride infiltration by altering gene expression that regulate fatty acid synthesis and oxidation. The findings show the importance of nutrition throughout the life course in programming early and adult-onset obesity and highlight the application of caloric restriction and LA supplementation as effective means to protect against obesity. [Supported by the NIH grant DK-61518 (MSP) and KO1 award from the Center for Complementary and Alternative Medicine (TCR)].

L6

Lipoic acid supplementation decreases biomarkers of inflammation and lipid peroxidation in overweight or obese adults: results of a randomized, double-blind, placebo-controlled study

Frei, Balz
Linus Pauling Institute, Oregon State Universit, USA

We have shown previously that α-lipoic acid inhibits atherosclerosis, body weight gain, and vascular inflammation in apoE$^{-/-}$ and apoE$^{-/-}$ LDLR$^{-/-}$ mice. Further, α-lipoic acid induces Nrf2-mediated gene expression of antioxidant enzymes and inhibits the age-related increase in lipid peroxidation in rats. We hypothesized that oral (*R*)-α-lipoic acid (R-LA) supplementation of overweight or obese adults decreases biomarkers of inflammation and lipid peroxidation. Eighty adults (46 women, 34 men; 21-60 years; BMI 25.6-52.0 kg/m^2; plasma triglycerides 100-401 mg/dL) were enrolled in a 24-week randomized, double-blind, placebo-controlled trial and assigned to either R-LA (600 mg/day) or matching placebo. The study was completed by 64 participants (31 receiving R-LA). Using an intention-to-treat analysis, 24-week R-LA supplementation compared to placebo decreased plasma concentrations of sICAM-1 (-6.3%; *P*=0.04), but increased TNFα (25%; *P*=0.003) and had no effect on hs-CRP (*P*=0.78). In women (n=35), R-LA treatment compared to placebo decreased plasma sE-selectin (-17%; *P*=0.01). In both males and females, mRNA levels of antioxidant enzymes in PBMCs tended to be higher in R-LA supplemented subjects (13%; *P*=0.10). Furthermore, lipid peroxidation was decreased by R-LA treatment, as assessed by urinary concentrations of total F_2-isoprostanes (-25%; *P*=0.02), as well as 5- and 15-series $PGF_{2\alpha}$ (-26%; *P*=0.05 and -29%; *P*=0.002, respectively), 8-iso-$PGF_{2\alpha}$ metabolites (-29%; *P*=0.002), and 2,3-dinor-5,6-dihydro-8-iso-$PGF_{2\alpha}$ (-20%; *P*=0.03). We conclude that R-LA supplementation of overweight or obese adults decreases plasma and urinary markers of inflammation and oxidative stress. In the same study, we found that R-LA supplementation decreases body weight and body fat mass in very obese subjects. Based on these observations and our preclinical studies, R-LA supplementation may decrease the risk of cardiovascular disease in obese adults. – This study was supported by NIH grants P01 AT002034 and UL1TR000128

L7
GSH in cholestatic liver injury

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Chronic cholestatic liver injury remains a major cause of chronic liver disease for which treatment options are limited. Ursodeoxycholic acid (UDCA) is the only FDA-approved medication for the treatment of primary biliary cirrhosis but it fails in 40% of these patients. GSH is synthesized in all mammalian cells via two enzymatic steps catalyzed by glutamate-cysteine ligase (GCL), the rate-limiting enzyme, and GSH synthetase. Liver has the highest concentration of GSH in all organs and determines systemic GSH availability. GSH is also secreted into bile and is a key determinant of bile acid-independent bile flow. Reduced hepatic GSH level facilitates fibrogenesis and inflammatory response to endotoxin. We found in mice that chronic cholestatic liver injury induced by either bile duct ligation (BDL) or feeding lithocholic acid (LCA) resulted in induction of c-Myc, MafG, c-Maf and suppression of Nrf2 and GCL subunits (catalytic and modifier subunits GCLC and GCLM, respectively). We originally speculated increased MafG and c-Maf may have formed complexes that displaced Nrf2 from ARE binding and repressed ARE-dependent genes. However, we subsequently found using biotinylated ARE pull-down assay and proteomics that there is a change in the abundance of proteins that bind to the ARE in chronic cholestasis (increased c-Myc and decreased prohibitin 1 (PHB1)). This led us to identify an interesting circuit that involves c-Myc, miR27 and PHB1. In chronic cholestasis, increased c-Myc expression results in induction of miR-27a and miR-27b, both of which target PHB1 and Nrf2 to lower their mRNA levels. In addition, c-Myc, PHB1 and Nrf2 can interact directly with each other and co-occupy ARE. Our data indicate that c-Myc serves as a co-repressor while PHB1 serves as a co-activator of Nrf2 for ARE binding and ARE-dependent gene expression. Therapeutically, both S-adenosylmethionine (SAMe) and UDCA are protective but work better when combined and target all of the key mechanisms.

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L8
Impaired mitophagy leads to stress-induced cellular senescence

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Oxidative stress, chronic inflammation and cellular senescence are intertwined in premature aging which is considered as an important contributing factor in driving chronic inflammatory diseases including cigarette smoke-induced Chronic Obstructive Pulmonary Disease (COPD). Cellular senescence (stress-induced) is a form of irreversible growth arrest. Sirtuin1 (SIRT1), an anti-aging protein, attenuates oxidant/cigarette smoke-induced lung inflammation and injury. SIRT1 regulates cellular senescence via regulating FOXO3, p53, and NF- κ B, oxidative stress (antioxidant genes) as well as various proteins/coactivators involved in DNA damage and repair. Oxidant/carbonyl stress reduces SIRT1 level and activity, which results in acetylation of target proteins, such as p53, RelA/p65 and FOXO3, thereby leading to increased inflammatory response and cellular senescence. We have recently shown that defective mitophagy and impaired mitochondrial biogenesis are associated with stress-induced cellular senescence. Impaired mitophagy and perinuclear accumulation of damaged mitochondria associated with cellular senescence occur in both human lung fibroblasts and small airway epithelial cells (SAECs) by cigarette smoke. Impaired mitophagy was attributed to reduced Parkin translocation to damaged mitochondria, which was due to cytoplasmic p53 accumulation and its interaction with Parkin. Primary SAECs from patients with COPD exhibited impaired mitophagy and increased cellular senescence via suborganellar signaling. Mitochondria-targeted antioxidant (Mito-Tempo) restored impaired mitophagy, decreased mitochondrial mass accumulation, and delayed cellular senescence in Parkin-overexpressing cells. In conclusion, defective mitophagy leads to cigarette smoke stress-induced lung cellular senescence, and restoring mitophagy delays cellular senescence, which provides a promising therapeutic intervention in chronic airway diseases.

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L9
Redox regulation of FOXO transcription factors

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The forkheadbox O (FOXO) family of transcription factors regulates a variety of cellular programs, including cell cycle arrest, reactive oxygen species (ROS) scavenging, and apoptosis, and are of key importance in the decision over cell fate. In animal model systems it has been shown that FOXO is involved in the regulation of long lifespan. FOXO activity is tightly controlled by the insulin signaling pathway and by a multitude of ROS-induced posttranslational modifications. Over the past years my lab has discovered that cysteines in FOXO transcription factors become oxidized in response to redox signaling and that this leads to the formation of highly specific intermolecular disulfide formation with a number of regulators of FOXO activity. We have shown that ROS-induced Lysine acetylation on FOXO depends on the formation of an intermolecular disulfide with the p300 or CBP acetyltransferases. Furthermore, nuclear shuttling of FOXO is triggered by a shift in the cellular redox state towards more oxidizing conditions and this depends on intermolecular disulfide formation between FOXO and nuclear import receptors. By comparing cysteines present in human FOXO3 and FOXO4 isoforms by a quantitative proteomics approach we have identified paralog-specific redox signaling. We speculate that intermolecular disulfide formation could be a more common phenomenon in redox signaling and that it could serve to stabilize protein-protein interactions that otherwise would be of low affinity. By this means specific signaling cascades can be reversibly activated for as long as the cellular redox state remains more oxidizing.

L10
Oxidized phospholipids and cell signalling in inflammation

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Oxidized phospholipids (oxPLs) can be produced by attack of free radicals and reactive oxidizing compounds on unsaturated lipids, for example in inflammatory processes where phagocytic cells are activated. Esterified oxPLs, as well as small reactive breakdown products derived from them, have a variety of biological effects, many of which are considered detrimental. These include enhancing ROS production, monocyte-endothelial adhesion, and proliferation and differentiation of SMCs. There has been much interest in defining the receptors and cell signalling mechanisms that contribute to these effects. oxPLs can be considered as "damage-associated molecular patterns" and interact with several immune receptors, including toll-like receptors. The outcomes can be inflammatory or anti-inflammatory, depending on the oxidized phospholipid, cell type and concentration. Therefore to understand the contributions of oxPLs, it is necessary to have sensitive and specific methods of identifying different oxidized species. Mass spectrometry is a powerful technique for analysing both native and oxidized phospholipids, but the large number of different species present in biological and clinical samples presents a substantial challenge. LDL contains more than 350 different native lipid species, and cell membranes are similarly complex; oxidation further increases the complexity. Targetted or semi-targetted MS approaches can facilitate identification of individual oxPLs or groups of oxidation products and have been applied to analyse phospholipid oxidation in chronic conditions such as diabetes and obesity. Products commonly observed included the reactive products POVPC and PONPC, which are able to generate adducts with proteins by lipoxidation. This is thought to be an additional mechanism by which oxPLs can influence cell signalling in pathophysiology.

L11

Ca^2-independent biosynthesis of lipid mediators via oxygenation and hydrolysis of mitochondrial cardiolipins

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The central role of mitochondria in metabolic bioenergetics, metabolic reactions and cell-death mechanisms requires sophisticated and diversified signaling. Essential in this signalling process is an array of lipid mediators derived from polyunsaturated fatty acids. However, the molecular machinery for the production of oxygenated polyunsaturated fatty acids is localized in the cytosol and their biosynthesis has not been identified in mitochondria. In this talk, we will present new data showing that a range of diversified polyunsaturated molecular species derived from a mitochondria-specific phospholipid, cardiolipin (CL), oxidized by an intermembrane-space haemoprotein, cytochrome c, can be formed. We will further show that these oxygenated CL species can undergo phospholipase A2-catalysed hydrolysis thus generating multiple oxygenated fatty acids, including well-known lipid mediators. This sequence of redox reactions represents a new biosynthetic pathway for lipid mediators. Finally, we will give examples of the utlity and activation of this pathway, which includes the oxidation of polyunsaturated CLs and accumulation of their hydrolysis products (oxygenated linoleic, arachidonic acids and monolysocardiolipins), as a signaling mechanism realized in vivo in acute tissue injury.

L12

Biochemical mechanisms of mitochondrial SOD nitration and inactivation: pathological relevance

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Mn-containing superoxide dismutase (MnSOD) is an essential antioxidant enzyme of the mitochondrial matrix in mammalian cells. Under inflammatory conditions that enhance nitric oxide ($^{\bullet}NO$) formation, MnSOD is tyrosine nitrated and inactivated *in vitro* and *in vivo*; this process facilitates a vicious cycle of alterations in mitochondrial redox homeostasis. The inactivation of MnSOD *via* tyrosine nitration requires the exclusive modification of only one out of nine tyrosine residues present in mammalian MnSOD, namely Tyr34. This tyrosine is located in the entrance channel to the active site, only 5 A away from the Mn atom. The selective nitration of Tyr34 is caused as a consequence of the fast reaction of peroxynitrite with the Mn center (*ca*. 10^5 $M^{-1}s^{-1}$), which generates nitrating species at the active site. The incorporation of a nitro group in Tyr34 creates steric and electrostatic restrictions that impede the diffusion of superoxide radical anion ($O_2^{\bullet-}$) to the active site Mn atom, therefore leading to enzyme inactivation. Of interest in the context of these biochemical studies, we have recently found that the mitochondrial FeSOD from *Trypanosoma cruzi* (that has a similar protein fold than MnSOD but contains an Fe atom in the active site) is also similarly nitrated and inactivated by peroxynitrite. However, the analogous *T. cruzi* cytosolic FeSOD showed remarkable resistance to peroxynitrite. The endurance of the cytosolic *T. cruzi* FeSOD to peroxynitrite-dependent nitration and inactivation depends on an intramolecular electron transfer mechanism from the solvent-exposed and low pK_{SH} cysteine (Cys83) to the tyrosyl radical (at Tyr35), which is reduced back to tyrosine. These data may provide clues for protein engineering of MnSOD variants resistant to peroxynitrite. Overall, nitration and inactivation of MnSOD reflect reactions mediated by peroxynitrite in mitochondria and represent a contributory molecular mechanism in mitochondrial dysfunction and apoptotic signaling in inflammation and other pathological processes.

L13
Redox biology: Where exposome meets genome

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Cumulative lifelong environmental exposures are defined as the exposome, a counterpart to the genome in defining health. The genome-exposome interface in humans has a redox character because metazoan evolution occurred in an O_2-rich environment. Helmut Sies and I recently presented The Redox Code as a set of principles to complement the epigenetic code in using the genome for organization, differentiation and adaptation to environment. The principles provide an underlying logic to bioenergetics, metabolic and structural organization, defense, and reproduction. Specifically, NAD and NADP systems function at near equilibrium with energy precursors to maintain bioenergetics, metabolic organization and defense. NAD and NADP are used to generate and metabolize H_2O_2, maintaining a kinetically controlled structural organization system linked to bioenergetics and metabolism through redox switches in the proteome. Activation/deactivation cycles of H_2O_2 generation support spatiotemporal sensing and signaling in molecular function, tissue differentiation and response to exposures. Importantly, metazoans evolved genetically encoded exposure memory systems to enhance survival and reproductive potential in the O_2-rich atmosphere by providing mechanisms for an individual to adjust during lifespan to environmental resources and challenges. Activation of differentiation and exposure memory systems with lasting impact on structure or function decreases subsequent adaptability. Thus, an organism decreases flexibility from conception onward due to differentiation and cumulative responses to environmental exposures. Based upon this, I recently concluded that aging is a decline in plasticity of genome-exposome interaction that occurs as a consequence of execution of differentiation and exposure memory systems. I term this the Redox Theory of Aging because it accounts for major hallmarks of aging and provides a foundation for new approaches to improve healthspan and longevity.

L14
Impact of mitochondrial fusion proteins on ROS handling

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Mitochondrial dynamics modulates mitochondrial metabolism, and signaling, and participates in the maintenance of mitochondrial DNA. Mitochondrial fusion is a key process in the mitochondrial dynamics that is catalyzed by Mitofusins and OPA1 proteins. Mitofusin 2 (Mfn2) participates in the fusion of the outer mitochondrial membrane. In addition, Mfn2 participates in the tethering of mitochondrial-endoplasmic reticulum (ER) contact sites.

Mfn2 expression is finely regulated in tissues. Mfn2 is negatively regulated by pro-inflammatory factors and lipid availability, and positively by MEF transcription factors and PGCs nuclear coactivators. Mutations in the MFN2 gene causes Charcot-Marie Tooth type 2A neuropathy. Mfn2 deficiency causes alterations in mitochondrial and ER morphologies. In addition, Mfn2 loss-of-function causes mitochondrial dysfunction, and a chronic activation of the unfolded protein response (UPR) in cells and in tissues.

The alterations in mitochondrial metabolism driven by Mfn2 deficiency are characterized by reduced respiration and enhanced ROS production. The antioxidant compound N-acetylcysteine ameliorated glucose tolerance and insulin signaling in liver-specific Mfn2 KO mice. This indicates that oxidative stress is responsible for, at least, some of the alterations linked to Mfn2 deficiency. Moreover, in Mfn2 knockout MEF cells, the alterations in mitochondrial metabolism are normalized upon induction of PERK deficiency, indicating that they are secondary to chronic ER stress and not due to direct alterations of Mfn2 on mitochondria.

L15

The role of the mitochondrial sirtuin Sirt3 in aging and its retardation by caloric restriction.

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Caloric restriction (CR) extends the life span and health span of a variety of species and slows the progression of several age-related diseases. We have reported that CR reduces oxidative DNA damage in multiple tissues and prevents age-related hearing loss (AHL) in wild-type mice but fails to modify these phenotypes in mice lacking the mitochondrial deacetylase Sirt3, a member of the sirtuin family. Sirt3 acts as an essential player in enhancing the mitochondrial glutathione antioxidant defense system during CR, suggesting that Sirt3-dependent mitochondrial adaptations may be a central mechanism of aging retardation in mammals. We have recently analyzed gene expression datasets from eight tissues of mice subjected to CR and identified a common transcriptional signature that includes functional categories of mitochondrial energy metabolism, inflammation and ribosomal structure. This signature is detected in flies, rats, and rhesus monkeys on CR, indicating aspects of CR that are evolutionarily conserved. Detection of the signature in mouse genetic models of slowed aging indicates that it is not unique to CR but rather a common aspect of extended longevity. Mice lacking Sirt3 fail to induce mitochondrial and anti-inflammatory elements of the signature in response to CR, suggesting a potential mechanism involving Sirt3. The inverse of this transcriptional signature is detected with consumption of a high fat diet, obesity and metabolic disease. We propose that this evolutionarily conserved, tissue-independent, transcriptional signature of delayed aging and reduced disease vulnerability is a promising target for developing therapies for age-related diseases.

L16
Mitochondrial regulation of hematopoietic stem cell aging and rejuvenation

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Deterioration of adult stem cells accounts for much of aging-associated compromised tissue maintenance. Our recent studies indicate that HSC maintenance is critically dependent on the sirtuin family of proteins that possess deacetylase activity. We find that sirtuins sense various metabolic stresses in HSCs and regulate diverse metabolic pathways to exit cell cycle and prevent cell death. SIRT7, a H3K18 deacetylase, functions at chromatin and controls a regulatory branch of mitochondrial unfolded protein response. SIRT3, a mitochondrial deacetylase, regulates the global acetylation landscape of mitochondrial proteins and reduces oxidative stress. The protective programs mediated by sirtuins are suppressed in HSCs at an older age, underlying aspects of aging-associated HSC deterioration. We demonstrate that sirtuins can be targeted to reverse HSC aging and improve tissue homeostasis. Together, these studies highlight novel mitochondrial pathways essential for HSC maintenance throughout the aging process and identify aging-associated mitochondrial metabolic dysregulation in HSCs that are reversible.

L17

Aggregated lipids and proteins in aging, Alzheimer's disease, and diabetes

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Proteins which are oxidatively modified are degraded by the 20S proteasome in an ATP- and ubiquitin-independent pathway. If the proteasomal system is overwhelmed oxidized proteins aggregate and form a hydrophobic yellow-brownish material that accumulates predominantly in lysosomes. Lipids and other cellular components are recruited and finally, a cross-linked high molecular weight aggregate is formed. This is readily taken up into lysosomes by macrophagy.

One of the dominant effects of cross-linked proteins is the inhibition of the proteasomal system, regardless whether the cross-linking of proteins was achieved directly by protein-protein cross-linking or via lipid peroxidation mediated cross-linking agents. The efficiency of proteasomal inhibition seems to be linked to a direct binding of the proteasome and is dependent on the binding efficiency. Soan uptake of cross-linked proteins into an autophagosome seems to be a protective mechanism, which reduces the aggregate mediated proteasome inhibition. Since proteasomal inhibition is accompanied by a modulation of several regulatory pathways, an efficient macroautophagy seems to be of utmost importance.

Originally it was thought, that these protein aggregates are merely waste products of cellular metabolism, but mounting evidence demonstrates an active participation of such protein aggregates in several physiological and pathophysiological cellular responses, relevant for aging and diseases. So we could demonstrate that aggregate-mediated proteasomal inhibition plays a role in Alzheimers disease, in the accelerated senescence of ß-cells and of course in the aging process itself.

Further studies will reveal the functional importance of proteasome inhibition and protein aggregate formation in diseases and aging.

L18

The aged proteome - impact of protein oxidative modifications on cellular aging

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A hallmark of aging both at the cellular and organismal level is the accumulation of damaged proteins that is believed to participate to the age-related decline in cellular function. We have previously shown that oxidized proteins as well as proteins modified by lipid peroxidation and glycoxidation adducts are accumulating in senescent human WI-38 fibroblasts. To better understand the mechanisms by which these damaged proteins build up and potentially affect cellular function during replicative senescence of WI-38 fibroblasts, proteins targeted by these modifications were identified using a bidimensional gel electrophoresis-based proteomic approach coupled with immunodetection of HNE-, AGE-modified and oxidized (carbonylated) proteins. Proteins targeted for either one of these modifications are involved in different cellular functions such as protein quality control, energy metabolism and cytoskeleton. These studies underscore the importance of performing proteomic analyses addressing different aspects, such as expression levels and modifications by carbonylation or glycoxidation, to have a broader view of the age-related changes affecting the cellular proteome. Changes in the proteome of human myoblasts (satellite cells) during replicative senescence and upon oxidative stress have been also analyzed. Proteins involved in protein quality control and glycolytic enzymes are the main targets of oxidation and modification with advanced glycation/lipid peroxidation end products during replicative senescence of satellite cells. To provide mechanistic insights into the role of oxidized proteins in the development of the senescent phenotype, untargeted metabolomic profiling was performed for young and senescent satellite cells. Metabolomic profiling and functional analyses indicated glucose metabolism impairment in senescent cells, although mitochondrial respiration remained unaffected. This study establishes a new connection between oxidative protein modifications and the altered cellular metabolism associated with the senescent phenotype.

L19

Clock genes regulate neuronal redox homeostasis in brain

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Ageing leads to functional deterioration of many brain systems, including the circadian clock that controls physiological processes such as metabolism, hormone secretion and cardiac function, all of which exhibit daily oscillation.

The circadian system also maintains the internal coordination of multiple oscillators within and between various organ systems in order to provide the most efficient response to the envoronment.

Whereas the effect of ageing on the performance of the circadian system has been known for many years, recent reports are suggesting that a dysfunctional circadian clock can in turn contribute to ageing and pathologies associated with old age.

The genes encoding clock proteins are expressed throughout the brain, though it is unknown whether these proteins modulate brain homeostasis.

Very recently, it has been demonstrated that reduction of the expression of Bmal1, one of the regulating clock gene, promotes neuronal death in primary cultures and in mice treated with a chemical inducer of oxidative injury indicating that clock genes regulate cerebral redox homeostasis and connects impaired clock gene function to neurodegeneration.

The link between core circadina clock, brain oxidative stress, aging and age-related neurodegenerative diseases is discussed.

L20
Frailty and sarcopenia

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The increased life expectancy has raised new challenges to be overcome by modern societies. One of these challenges is the increased prevalence of disability as the population ages. Being the main responsible the decrease in the functional reserve accompanying the aging process, the best strategy to avoid disability is preventing its debut by detecting and intervening on pre-disability conditions. In this pre-disability conditions there is still some functional reserve allowing the implementing of some strategies that will promote the recovery toward robustness. Frailty has become the most accepted pre-disability condition, gaining progressive relevance in the last decade. This status, that is the final outcome of the joint action of the aging process plus some chronic diseases and conditions, can now be detected and, for the first time, we have strong evidence about the effectiveness of interventions (mainly based on physical exercise).

In addition, we have also increased our knowledge about their pathophysiological mechanisms, which include inflammation, oxidative stress, hormonal dysregulations and vascular dysfunction among the main mechanisms involved. These mechanisms are organised in the so-called cycle of frailty and it is becoming increasingly clear that, by opposition with the fact in other syndromes or diseases where one or two mechanisms are able to explain the whole picture and its severity, the participation of several of these components is the differential characteristic of the frailty syndrome.

This cycle of frailty has different effectors that mediate the phenotypic (i.e. clinical) expression of the frailty syndrome. The most important of them is sarcopenia, a condition consisting in low muscle mass plus functional deficits. Sarcopenia mediates the impaired functional performance that is characteristic of frailty and, probably, the additional effect of chronic diseases (diabetes, ischemic heart disease, heart failure and some others) on diabetes.

L21

Reactive oxygen species production in skeletal muscle during contractile activity and aging

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It has been recognized for a considerable period of time that contracting skeletal muscle generates superoxide and recent data support a role for NADPH oxidase(s) in this process. The superoxide generated appears to act to stimulate some adaptive responses to contractile activity through redox signaling pathways. Such pathways appear to be come dysregulated during aging leading to attenuated adaptations of skeletal muscle to exercise. Some studies indicate that this dysregulation may occur through an increase in mitochondrial generation of hydrogen peroxide and other reactive oxygen species (ROS) that occurs in muscles from older animals. Our current research is examining the role of age-related changes in the innervation of individual fibers on muscle mitochondrial ROS generation and data indicate that denervated fibers may play a key role role in the age-related increase in muscle mitochondrial ROS generation.

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L22

How mitochondria produce ROS during cardiac ischaemia-reperfusion injury

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Mitochondrial ROS have long been known to contribute to damage in conditions such as ischaemia-reperfusion (IR) injury in heart attack and stroke, but methods to stop this ROS production were limited. Over the past few years we have developed a mitochondria-targeted S-nitrosating agent, called MitoSNO, that we showed was effective in preventing ROS formation in IR injury with therapeutic implications. In addition, the protection by this compound suggested that ROS production in IR injury was mainly coming from complex I. This led us to investigate the mechanism of the ROS production and using a metabolomic approach we found that the ROS production in IR injury came from the accumulation of succinate during ischaemia that then drove mitochondrial ROS production by reverse electron transport at complex I during reperfusion. This surprising mechanism led up to develop further new therapeutic approaches to impact on the damage that mitochondrial ROS do in pathology and also to explore how mitochondrial ROS can act as redox signals.

L23

Mechanisms of redox responses to endothelial shear stress: from peroxide to nitric oxide

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Laminar shear stress (LSS) is a protective hemodynamic regulator of endothelial function, and limits the development of inflammatory diseases related to oxidative stress. Although several endothelial proteins have been proposed as potential shear stress mechano-sensors, the signaling role of reactive oxygen species (ROS) is still unclear. We aimed to: 1) Identify LSS-dependent signaling pathways mediated by ROS signaling 2) Characterize physiologically relevant sensors to LSS-induced ROS 3) Study the role of redox-sensitive phosphatases linked to LSS. We demonstrated that LSS after 30-60 minutes promotes the formation of signaling levels of H_2O_2, which in turn activate p38 MAPK and eNOS. Peroxide generation was dependent on NOX4 presence. At early time points of LSS, mitochondrial peroxiredoxin 3 (PRX3) became specifically activated. Simultaneous changes in mitochondrial morphology, compatible with mitochondrial fission, took place. Indeed, the fission-related protein DRP1 was activated by phosphorylation enhancing mitochondrial fission. Short periods of LSS resulted in the generation of superoxide radical anion and hydrogen peroxide, which was corroborated by the increased fluorescence of the specific peroxide sensor HyPer. By using biotinylated dimedone and proteomic approaches we detected increased total protein sulfenylation which was mediated by NOX4. Mass spectrometry analysis identified SHP2 as a sulfenylated phosphatase under LSS. FAK activation and subsequent eNOS phosphorylation were promoted by LSS and both processes were dependent on NOX4, as demonstrated by their absence in NOX4-null mice. These results support that LSS elicits redox-sensitive signaling responses involving NOX4-dependent generation of H_2O_2. This can result in a) specific activation of MAPK pathways, b) mitochondrial activation of PRX3 and 3) sulfenylation of SHP2. Some of these redox-mediated responses may converge in eNOS activation, thus conferring hydrogen peroxide signaling a protective role.

L24

Vascular endothelial and smooth muscle cell redox signaling and function in health and disease

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Cells have evolved endogenous defence mechanisms to counteract oxidative stress, and the redox sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) plays a key role in the defence against oxidative stress via the induction of phase II and antioxidant enzymes.[1-3] Under low oxidative or nitrosative stress,Nrf2issequesteredby its cytosolic binding protein Kelch-like ECH Associated Protein 1 (Keap1) and targeted for proteasomal degradation.[4] GSK-3β phosphorylation may also lead to Nrf2 degradation via an adaptor proteinβ-TrCP independent of Keap1.[5]Modification of Keap1 cysteine residues leads to nuclearaccumulationofNrf2 via *de novo* synthesis, and in the nucleus Nrf2 binds to a Maf recognition/antioxidant response element in the promoter of phaseII and antioxidant genes, such as NQO1, HO-1, and glutamate cysteine ligase and cystine transporter xCT involved in glutathione synthesis. Our studies in wild type and $Nrf2^{-/-}$ murine macrophages identified Nrf2 as an important transcription factor involved in the induction of CD36 and antioxidant stress genes in atherosclerosis.[2] Notably, Nrf2 regulated redox signalling and antioxidant defences in response to hydroxynonenal are impaired in fetal umbilical vein endothelial and artery smooth muscle cells isolated from gestational diabetic and pre-eclamptic pregnancies.[6,7] More recently, we have shown that pretreatment of rodents with the Nrf2 inducer sulforaphane increases Nrf2 mediated HO-1 expression in neurovascular unit after a stroke, maintaining blood-brain barrier (BBB) integrity and neurological function.[8] Targeting the Nrf2 defence pathway using pharmacological and/or dietary inducers may provide a therapeutic approach to prevent oxidative stress induced damage in the peripheral vasculature or the BBB in stroke.

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L25

Amyloid-β disrupts calcium and redox homeostasis in brain endothelial cells

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Alzheimer's disease (AD) is a progressive and fatal brain disease which, in sporadic form, is the most prevalent form of dementia in the elderly. Amyloid β-protein (Aβ) has been shown to accumulate in the brain of AD patients, in senile plaques, but Aβ levels alone are not a reliable predictor of cognitive decline. Evidence exists showing that cerebrovascular function is altered in AD, often preceding the onset of cognitive impairment, contributing to neurodegeneration, playing a major role in AD pathogenesis. Recent data in AD mice models demonstrated a direct correlation between microvascular impairment and Aβ accumulation and results obtained in human and animal cultured cells suggest that brain capillary endothelium dysfunction is due to the deleterious effect of Aβ peptide on endothelial cells. Taking into account our previous results, highlighting the involvement of Endoplasmic reticulum (ER) stress in neuronal dysfunction triggered by Aβ, we explored the hypothesis that endothelial cells damage occurring in AD is mediated through the induction of ER stress. In a rat brain endothelial cell, exposure to $A\beta_{1-40}$, which preferentially accumulates in brain vasculature, increased the levels of several markers of ER stress-induced unfolded protein response, activated mitochondria dependent and independent apoptotic cell death pathways and disrupted Ca^2 and redox cell homeostasis. The failure of ER stress- adaptive UPR led to a decrease in proteasome activity, promoted the accumulation of ubquitinated proteins and the impairment of the autophagic flux, culminating in endothelial cells apoptosis. Prolonged ER stress was shown to induce intracellular Amyloid Precursor Protein (APP) accumulation, which co-localizes with the ER chaperon GRP78, leading to β-secretase activation, increase in intracellular Aβ levels and apoptotic cell death. In conclusion, these data support that endothelial cells dysfunction in AD arises from $A\beta_{1-40}$ induced ER stress, opening new strategies to prevent or delay the progression to AD.

L26
Possible role of oxysterols in the brain pathophysiology

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Oxysterols are a family of 27-carbon molecules originated from cholesterol oxidation by both enzymatic and non-enzymatic mechanisms, which, with respect to cholesterol, contain an additional hydroxy, epoxide or ketone group in the sterol nucleus and/or a hydroxyl group in the side chain. This report focuses on 24-hydroxycholesterol (24OH) and 27-hydroxycholesterol (27OH), two oxysterols that are normally produced in human body, by enzyme-mediated oxidation of cholesterol, and are very good ligands of LXRα and LXRβ, two physiologically important nuclear receptors. 24OH is by far the main cholesterol metabolite in the brain and, unlike cholesterol, freely diffuses over the blood-brain barrier (BBB), by this way avoiding excess accumulation of brain cholesterol. 27OH is the most represented oxysterol in the blood stream and can inversely pass into the brain. Nowadays available data achieved in vitro using neuron-like-cell lines showed both oxysterols as able to induce amyloid $\beta_{1\text{-}42}$ synthesis, but 24OH would appear to act against Tau phosphorylation while 27OH would on the contrary stimulate the latter process. Importantly, comparing autopsy samples of brain frontal cortex from early and advanced AD cases, so classified on the basis of less or more intense phospho-Tau accumulation, 24-hydroxylase (CYP46A1) expression and its product 24OH showed a significant decrease in late AD, while 27-hydroxylase (CYP27A1) expression and its product 27OH significantly increased with the severity of the disease. Is then 27OH accumulation in the brain contributing to AD progression? Could the same oxysterol have some role in brain physiology as 24OH appears to play? That of oxysterols in brain pathophysiology is definitely a yet unfinished tale.

L27

Alheimer's Disease: from oxidative stress to ApoE- mediated therapeutics

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ApoE-mediated clearing of amyloid-β from brain is a major therapeutic target to treat Alzheimer's disease. Brain ApoE is activated by the dimerization of the retinoid X receptor with peroxisome proliferator-activated receptor gamma. Bexarotene binds to retinoid X receptor, lowers amyloid-β plaque area and rapidly reverses cognitive, social and olfactory deficits in Alzheimer's disease mouse models [4]. However, it has considerable undesirable side effects [5,6,7]. Genistein, which binds to the peroxisome proliferator-activated receptor gamma moiety of the retinoid X receptor/peroxisome proliferator-activated receptor gamma dimer receptor [8,9], is extensively used in clinical practice[10] and is devoid of significant side effects [11]. We found that treatment of an Alzheimer's mouse model with genistein results in a remarkable and rapid improvement in various parameters of cognition, such as hippocampal learning, recognition memory, implicit memory and odor discrimination. This is associated with a lowering of Aβ levels in brain, in the number and the area of amyloid plaques as well as in microglial reactivity. The lowering of amyloid deposition by genistein was confirmed *in vivo* by using positron emission tomography. Finally, incubation of primary astrocytes with genistein results in an increase release of Apo E into the medium and thus the beneficial effects of genistein are likely to be mediated by ApoE. These results strongly suggest that controlled clinical trials should be performed to test the effect of genistein as treatment of human Alzheimer's disease.

L28

Neurotransmission-dependent metabolic and redox coupling between neurons and astrocytes

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Energy and redox conservation in the brain requires metabolic cooperation between distinct cell types. We have identified mechanisms and factors that maintain cell-specific programs to allow this metabolic-redox collaboration. Neurons show a high dependence on mitochondrial oxidative metabolism for survival, whereas astrocytes resist to almost complete inhibition of mitochondrial respiration. A key factor in this process is PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3), an enzyme that promotes glycolysis by activating its regulatory enzyme PFK1 (6-phosphofructo-1-kinase). We demonstrated that PFKFB3 is a substrate of the E3 ubiquitin ligase, anaphase-promoting complex/cyclosome (APC/C)-Cdh1. By regulating PFKFB3 protein stability, APC/C-Cdh1 controls the glycolysis versus pentose-phosphate pathway (PPP) switch, redox status, and survival of neurons. During glutamatergic neurotransmission, (APC/C)-Cdh1 is inhibited leading to PFKFB3 stabilization, glycolysis activation and PPP inhibition in neurons. This unavoidably leads to oxidative stress, since the intrinsic antioxidant defense of neurons is weak. However, the mechanism whereby these cells are physiologically protected against oxidative damage during glutamatergic neurotransmission is unknown. We have found that the antioxidant defense of neurons is repressed owing to continuous protein destabilization of the master antioxidant transcriptional activator, Nrf2. By contrast, Nrf2 is highly stable in neighbor astrocytes explaining their robust antioxidant defense and resistance against oxidative stress. Subtle and persistent stimulation of N-methyl-D-aspartate receptors (NMDAR) in astrocytes up-regulates a signal transduction pathway involving p35/Cdk5-mediated Nrf2 phosphorylation and activation, boosting antioxidant protection of closely spaced neurons. Thus, intercellular communication through astrocyte NMDAR couples glutamatergic neurotransmission with neuronal survival.

L29

The role and mechanisms of mitochondrial cholesterol in neurodegeneration

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Alzheimer disease (AD) is the leading cause of dementia and neurodegeneration characterized by cognitive decline and memory impairment. The Niemann Pick disease type C (NPC) is a lysosomal storage disorder that affects the cerebellum and peripheral organs that results in neurodegeneration and premature death. While AD pathology is poorly understood, NPC disease is caused by loss-of-function of the endolysosomal proteins NPC1/2 involved in cholesterol homeostasis and trafficking that leads to lysosomal cholesterol accumulation. Unlike NPC, increased NPC1 levels have been described in AD, suggesting the lack of increased lysosomal cholesterol content in AD. A common feature in both cases is the accumulation of cholesterol in mitochondria due to the overexpression of StARD1, a mitochondrial cholesterol transporting polypeptide. Using a newly generated genetic model of AD in mice overexpressing SREBP-2, APP/PS1/Tg-SREBP2, we observed the accumulation of mitochondrial cholesterol and the depletion of mitochondrial GSH that precedes the pathological hallmarks of AD, including beta amyloid accumulation, tau phosphorylation and cognitive decline. In NPC null mice, besides lysosomes cholesterol accumulates in mitochondria causing mitochondrial GSH depletion, oxidative stress and Purkinje cell death. The depletion of mitochondrial GSH by cholesterol contributes to the progression of these diseases as treatment with the GSH ethyl ester, which recovers mitochondrial GSH, improves the cognitive decline in APP/PS1/Tg-SREBP2 mice and motor deterioration in NPC null mice. Moreover, GSH ethyl ester extends the lifespan of NPC null mice to an extent similar to that caused by 2-hydroxycyclodextrin treatment, while N-acetylcysteine administration failed to exert any effect. Collectively, our findings identify the small pool of cholesterol in mitochondria as a key pathogenic factor in AD and NPC disease, pointing that strategies targeting mitochondrial cholesterol may be of relevance in the treatment of these diseases.

L30

Impaired inducibility of the mitochondrial lon protease in ageing: an example of declining adaptive homeostasis

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Mitochondria are major sites of free radical generation and mitochondrial proteins undergo significant oxidation. To minimize the accumulation of oxidative damage, mitochondria utilize reducing enzymes, repair systems, and targeted proteolysis of damaged proteins to maintain function. A key mitochondrial-matrix proteolytic enzyme is the nuclear-encoded Lon protease: an ATP-stimulated protease that preferentially degrades oxidized proteins. We have shown that Lon is a stress response protein whose increased levels and activity during stress provides significant protection for cultured mammalian cell lines, primary cells, worms (*C. elegans*), flies (*D. melanogaster*), and rodents. Now we find that the inducibility and adaptability of the Lon protease declines with age, and that this decline is linked with decreased robustness, healthspan, and lifespan. Thus, Lon appears to be very much like the Proteasome in its age-dependent loss of activity and loss of inducibility. These aging-mediated changes sensitize both mitochondria (Lon) and the cell nucleus and cytoplasm (Proteasome) to oxidative stress. Our studies indicate that dysregulation of Lon (and Proteasome) signaling is part of an overall age-dependent decline in Homeostatic redox control. The classical view of homeostasis considers a single range of biological capacities, extending above and below a mean value. Over the past two decades, however, studies from this laboratory (and several others) have demonstrated that cells, simple organisms, and even mammals, can temporarily expand the homeostatic range by undergoing transient adaptation. Such adaptive responses depend on altered gene expression, orchestrated by signal transduction pathways, such as the Nrf2-Keap1 system, and allow cells and organisms to cope with transient changes in many forms of stress. Thus, in addition to the 'normal' range of homeostatic capabilities, there is an additional range of adaptive capacity that I propose should be called, 'Adaptive Homeostasis.' Importantly, several studies from this laboratory now show that Adaptive Homeostasis declines with age in cells, worms, flies, and rodents; in other words, a decline in Adaptive Homeostasis appears to be a 'normal' age-dependent phenomenon that, unfortunately, makes older organisms (and people?) more susceptible to multiple stresses, and disease.

ORAL COMMUNICATIONS

INDEX

O1

Nitrite reductase activity of xanthine oxidase, xanthine dehydrogenase and aldehyde oxidase: evaluation of their contribution to NO formation *in vivo*

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Nitrite is presently considered a NO storage form that can be made available, through its one-electron reduction, to maintain NO formation under hypoxia/anoxia. The molybdoenzymes xanthine oxidase/dehydrogenase (XO/XD) and aldehyde oxidase (AO) were identified as "non-dedicated" mammalian NO-forming nitrite reductases. The unequivocal identification of NO as the product of enzymatic nitrite reduction was achieved with a NO-selective electrode and by EPR spectroscopy. The NO formation was shown to be dependent on the simultaneous presence of enzyme, a reducing substrate and nitrite. The molecular mechanism of XO/AO-catalysed nitrite reduction was also investigated and discussed: nitrite binding and reduction was shown by EPR spectroscopy to occur on the enzymes molybdenum centre, with NO formation being dependent on the presence of a reduced and sulfo-centre. A comprehensive kinetic characterisation of the effect of pH on XO and AO-catalysed nitrite reduction showed that the enzyme's specificity constant for nitrite increase 8-fold, while the K_m^{NO2-} decrease 6-fold, when the pH decreases from 7.4 to 6.3. These results demonstrate that the ability of XO/AO to trigger NO formation would be greatly enhanced under the acidic conditions characteristic of ischaemia. The dioxygen inhibition was quantified, and the K_i^{O2} values found (24.3-48.8μM) suggest that *in vivo* NO formation would be fine-tuned by dioxygen availability. The potential *in vivo* relative physiological relevance of XO/XD/AO-dependent pathways of NO formation was evaluated using HepG2 and HMEC cell lines subjected to hypoxia. NO formation by the cells was found to be pH, nitrite, and dioxygen-dependent, and the relative contribution of XO/XD plus AO was found to be as high as 50%. Collectively, our results supported that XO/XD and AO can contribute to NO generation under hypoxia inside a living human cell.

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O2

Peroxynitrite-dependent inactivation of human glutamine synthetase: biochemical characterization and functional consequences

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Glutamine synthetase (GS) is a key metabolic enzyme that catalyzes the ATP-dependent synthesis of glutamine from glutamate and ammonia. In the nervous system, it is mainly located in the cytosol of astrocytes, playing an important role in ammonia detoxification and prevention of glutamate-dependent excitotoxicity. Alterations in GS activity may lead to astroglial dysfunction, affecting neuronal function and survival. Several *in vitro* and *in vivo* studies have shown that GS activity is highly susceptible to biologically-relevant reactive species, in particular peroxynitrite **($ONOO^-$**). Peroxynitrite-derived radicals, promote protein tyrosine nitration yielding 3-nitrotyrosine (3-NT). Tyrosine nitration of GS has been identified as one of the main oxidative modifications associated to enzyme inactivation in a series of pathological conditions. Critical tyrosine residues participate in the enzyme active site. This work aims to establish the molecular mechanisms involved in GS inactivation by oxidative modifications mediated by $ONOO^-$. We have performed studies with the pure enzyme and in human astrocytes. Firstly, we purified recombinant human GS (rGS) expressed in *Escherichia coli*, exposed it to $ONOO^-$ under different conditions and evaluated enzyme function and oxidation/nitration. Bolus addition of $ONOO^-$ caused a dose-dependent decrease in GS activity; concomitantly, an increase in relative 3-NT levels was observed. In addition, human astrocytes were exposed to the peroxynitrite donor, SIN-1 and protein tyrosine nitration was quantitated immunochemically by an anti-3-NT antibody. Similarly to what was observed in the pure GS, SIN-1-derived $ONOO^-$ exposure lead to a decrease of cellular GS activity in parallel with a concomitant increase of cellular 3-NT levels; importantly, preliminary data obtained from immunoprecipitated GS after $ONOO^-$ challenge to astrocytes support the formation of nitrated GS. Further studies are underway to quantitatively establish the role of tyrosine nitration on GS inactivation and determine the specific nitration sites.

O3

Chronic intake of the optimized EPA:DHA 6:1 formulation protects against angiotensin II-induced hypertension, oxidative stress and endothelial dysfunction in rats

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The optimized omega-3 formulation EPA:DHA 6:1 has been shown to be a potent inducer of endothelium-dependent nitric oxide (NO)-mediated relaxations in isolated arteries. This study examined whether chronic intake of EPA:DHA 6:1 affects experimental hypertension and endothelial dysfunction induced by angiotensin II (Ang II) in rats.

Male Wistar rats daily received 500 mg/kg of either EPA:DHA 6:1 (omega 3) or corn oil (control) by gavage for 5 weeks. After 1 week, rats underwent sham surgery (sham rats) or surgery with implantation of an osmotic mini-pump infusing Ang II (0.4 mg/kg/d) for 4 weeks.

The Ang II-induced hypertension was associated with blunted relaxations of second branch mesenteric artery rings to acetylcholine (Ach) affecting the endothelium-dependent hyperpolarization (EDH)-mediated component to a greater extent than the NO-mediated component. The NADPH oxidase inhibitor (VAS-2870) improved both the NO and the EDH component in the Ang II group. Pronounced endothelium-dependent contractile responses to Ach were observed in the Ang II group compared to the control group in the presence of inhibitors of both the NO and the EDH component, which were abolished by indomethacin (cyclooxygenase inhibitor). The endothelial dysfunction was associated with increased vascular oxidative stress as assessed using dihydroethidium staining, and expression of NADPH oxidase subunits ($p47^{phox}$ and $p22^{phox}$), cyclooxygenase-2, eNOS, and Ang II type 1 receptor whereas SK_{Ca} and connexin 37 (involved in the EDH component) were down-regulated as assessed by immunofluorescence staining.

Chronic intake of EPA:DHA 6:1 prevented the Ang II-induced hypertension and endothelial dysfunction by improving the NO- and EDH-mediated relaxations and reducing endothelium-dependent contractile responses, and the expression of target molecules.

The present findings indicate that chronic intake of EPA:DHA 6:1 prevented the development of Ang II-induced hypertension and endothelial dysfunction in rats, most likely by preventing oxidative stress.

O4

Effects of high versus low dietary intake of bioactive food compounds on biomarkers of oxidative stress and metabolic and vascular health in the BIOCLAIMS cohort

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In the BIOCLAIMS cohort (n=1310, 606 M, 704 F, age 18-85 yrs) dietary intake was estimated from consumption frequencies in the preceding 12 months, using a 240-item food frequency questionnaire with picture-supported portion sizes. Subjects of the highest (Q5) and lowest quintiles (Q1) of consumption of bioactive compounds were compared with each other.

Biomarkers of nutritional exposure (vitamins A, C, E, D, B_{12}, folate, α-/β-carotene, lycopene, β-cryptoxanthin, lutein/zeaxanthin, zinc), oxidative stress (NF-κB p50, p65; human mercaptalbumin; malondialdehyde, MDA; total peroxides), metabolic and vascular health (leptin, adiponectin; HOMA-IR index; C-reactive protein, CRP; interleukin-6, IL-6; sICAM-1, sVCAM, MCP-1, e-selectin; homocysteine, HCY; asymmetric dimethylarginine, ADMA; intima-media thickness) were determined.

Plasma levels were related to intakes of carotenoids, vitamin C, folate (P<0.001) and B_{12} (P=0.007). Lower HOMA-IR index was found for higher intakes (Q1>Q5) for *carotenoids, folate* (P<0.001) and *vitamin E* (P=0.001); CRP (P=0.007) and IL-6 (P=0.009) for *carotenoids*; HCY for *carotenoids, vitamin E, folate, zinc* (P<0.001) and *vitamin K* (P=0.001); ADMA for

B_{12} and *zinc (P<0.001);* adiponectin for B_{12} (*P*<0.001); leptin: adiponectin for *retinol* (*P*=0.002); cholesterol for *fiber* (*P*<0.001). Higher adiponectin was found for higher intakes (Q5>Q1) for *carotenoids* (*P*<0.001) and *retinol* (*P*=0.002); MDA for *vitamin K* (*P*=0.002) and *fiber* (*P*<0.001). Conclusions: Biomarkers applied in this study respond to dietary intake levels of bioactive compounds and could be useful for monitoring health benefits in clinical studies. *The research leading to these results received funding from the European Union's 7th Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria.*

O5

Pancreatic beta cell response to nutrient overload is triggered by phospholipid remodelling and lipid peroxidation

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Phospholipids are a major cellular source for oxidized bioactive lipids that regulate myriad cell functions. It has been shown that oxidized polyunsaturated fatty acids or their metabolites amplify insulin secretion from pancreatic beta cells. Using the beta cell line INS1E and rat isolated islets of Langerhans we aimed at identifying these mediators and their targets following exposure to increasing levels of glucose (5-25 mM) and/or palmitic acid (PA; 50-500 μM). The cells were then taken for lipidomic analysis and glucose-stimulated insulin secretion assays (GSIS). Nontoxic combinations of glucose and PA amplified significantly GSIS. They also increased intracellular Ca_{ion} level by closure of K_{ATP} channels or activation of the free fatty acid receptor GPR40, leading to the activation of $cPLA_2$. The latter mediated massive phospholipid remodeling and released substantial amounts of arachidonic acid (AA) and linoleic acid (LA) into the cells. Both glucose and PA also intensified the production of oxygen free radicals in the cells. These radicals prompted nonenzymatic peroxidation of the free AA and LA to generate the bioactive aldehyde 4-hydroxy-2*E*-nonenal (4-HNE). Lcarnosine-mediated scavenging of cellular 4-HNE abolished both glucose- and PA-induced stimulation of GSIS. Exogenously added 4HNE mimicked these GSIS amplifying effects. We tested the idea that peroxisome-proliferator activate receptor-δ (PPARδ) was involved in the upregulation of insulin secretion. Indeed, the GSIS amplifying effects of glucose and PA were blocked by GSK0660, a selective PPARδ antagonist, or by silencing the expression of PPARδ with siRNA. Moreover, glucose, PA and 4-HNE induced luciferase expression in a PPAR-response element/PPARδ-dependent transactivation assay. The discovery that nutrient-induced phospholipid remodeling and lipid peroxidation generates lipid-derived signaling molecules, such as 4HNE, may explain the adaptive response of beta cells to nutrient overload.

O6

Redox dependent selective targeting of mutant K-Ras expressing cancer cells

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Ras is amongst the most commonly mutated oncogenes in a variety of human cancers. Despite the heightened interest in developing targeted therapeutics, the success rate of most commonly used inhibitors of signaling upstream or downstream of Ras has been disappointing. Therefore, there is a need to identify novel small molecules with the ability to target cancer cells harboring mutant Ras. To that end, we recently described the death inducing activity of a small molecule compound, C1, which triggered ROS dependent autophagy-associated apoptosis in cancer cells[1]. Here we report that the compound specifically targets human colorectal and pancreatic cancer cells harboring mutant K-RAS with minimal activity against wild type RAS expressing cells. Moreover, deletion of the mutant allele renders cells resistant to the compound by compromising intracellular ROS production. Interestingly, C1 triggers activation of K-RAS, which results in the downstream phosphorylation and activation of Akt, and gene knockdown of K-RAS or Akt resulted in the abrogation of C1-induced ROS production and rescued tumor colony forming ability. These data indicate a synthetic lethal effect against cells carrying mutant K-RAS, which could have therapeutic implications given the paucity of K-RAS-specific chemotherapeutic strategies.

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O7

Repair of peroxidized membrane phospholipids: critical role of peroxiredoxin 6

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Phospholipids are a major structural component of all cell membranes; their peroxidation represents a severe threat to cellular integrity and their repair is important to prevent cell death. Peroxiredoxin 6 (Prdx6), a protein with both GSH peroxidase and phospholipase A_2 (PLA_2) activities, plays a critical role in antioxidant defense of the lung and other organs. We investigated the role of Prdx6 in the repair of peroxidized cell membranes in pulmonary microvascular endothelial cells (PMVEC) and isolated mouse lungs treated with tert-butylhydroperoxide, and in lungs from mice exposed to hyperoxia (100% O_2). Lipid peroxidation was evaluated by measurement of thiobarbituric acid reactive substances (TBARS), oxidation of diphenyl-1-pyrenylphosphine (DPPP), and the ferrous xylenol orange (FOX) assay. The exposure dose was varied to give a similar degree of end-exposure lipid peroxidation in the different models. Values for lipid peroxidation returned to control levels within 2 h after oxidant removal in wild type (WT) PMVEC and perfused lungs but were unchanged in Pxdx6 null preparations. Prdx6 null mice showed markedly delayed recovery from lung lipid peroxidation during 20 h observation following exposure to hyperoxia. For all 3 models, an intermediate degree of repair was observed with PMVEC and lungs that express C47S or D140A mutant but not WT Prdx6; the former mutant does not express peroxidase activity of Prdx6 while the latter loses its PLA_2 activity. Thus, Prdx6 plays a critical role in the repair of peroxidized phospholipids in cell membranes and the recovery of lung cells from peroxidative stress; both the peroxidase and the PLA_2 activities of Prdx6 contribute to the recovery process.

O8

Oxidant-induced cellular senescence is caused by alteration in telomere shelterin complex

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Premature lung aging/cellular senescence is known to occur in environmental-induced airway diseases, such as Chronic Obstructive Pulmonary Disease (COPD) and Idiopathic Pulmonary Fibrosis. This may be associated with increased oxidative stress and redox changes in the cells by tobacco smoke. In fact, cigarette smoke induced oxidative and carbonyl stress by increased levels of 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA), and protein carbonylation measured by oxyblot in mice lungs, human lung fibroblasts (HLF-1) and human small airway epithelial cells (SAECs).

Senescent cells have increased senescence-associated β-galactosidase activity, senescence-associated heterochromatic foci, p16INK4a, p21CIP/WAF1, and pro-inflammatory cytokines with reduced telomere length. Telomeres are bound to a multiprotein complex that protects the chromosome, which has a role in the regulation of the telomeres length, called shelterin. This complex is composed of six core proteins: telomeric repeat binding factor 1 and factor 2 (TRF1/2), the TRF1- and TRF2-Interacting Nuclear protein 2, Protection Of Telomeres 1, TPP1 (also known as TINT1, PToP and PIP1) and the human ortholog of the yeast Repressor/Activator Protein 1. It is possible that this complex is altered by oxidative stress during stress-induced premature senescence, which may be the cause of telomere attrition.

Cellular senescence was established in HLF-1 after cigarette smoke extract (CSE) for 15 days. The markers of senescence, such as increased senescence-associated β-galactosidase activity, along with DNA damage, IL-8 release, p21 expression, and telomere attrition were observed in HFL-1. In addition to the results obtained in HFL-1, CSE-induced senescence phenotype and TPP1 reduction were also occurred in lung fibroblasts from senescence susceptible mice and in SAECs from COPD patients. We conclude that cigarette smoke as a form of oxidative/carbonyl stress disrupt shelterin complex, associated with telomere attrition, a hallmark of chronic airway diseases associated with accelerated aging.

O9

Complex I assembly into supercomplexes regulates mitochondrial ROS production in neurons and astrocytes

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Understanding the physiological roles of reactive oxygen species (ROS) in the brain requires dissecting out the contribution of different neural cell types to ROS formation. Here, the abilities of neurons and astrocytes to spontaneously generate ROS were characterized. We found that ROS production is higher (from 1.5- to 10-fold) in astrocytes than in neurons, as assessed in primary cultures prepared from Wistar rats and C57Bl/6 mice, regardless of the culture medium conditions and methods of ROS assessment. Such difference in ROS production was confirmed in neurons and astrocytes acutely dissociated from adult C57Bl/6 mice, strongly suggesting an *in vivo* phenomenon. Furthermore, higher ROS production was found to take place in mitochondria isolated from cultured astrocytes when compared with those isolated from neurons. To understand the mechanism explaining this different mitochondrial ROS production, we investigated the assembly of the mitochondrial respiratory chain. Using blue-native gel electrophoresis, proteomic analysis, and western blotting, we found that, in astrocytes, a large proportion of complex I occurs free, whereas in neurons most complex I is embedded into supercomplexes. Furthermore, the abundance of complex I subunit, NDUFS1, in free complex I, is lower in astrocytes than in neurons. Over-expression of NDUFS1 in astrocytes decreased mitochondrial ROS and increased the proportion of complex I into supercomplexes. Conversely, knockdown of NDUFS1 in neurons increased mitochondrial ROS and decreased the proportion of complex I into supercomplexes. These results are the first to demonstrate that the modulation of complex I assembly into supercomplexes regulates mitochondrial ROS production in an intact biological system. Moreover, this mechanism explains intrinsic differences in ROS production between neurons and astrocytes, likely playing different cell signalling physiological functions.

O10

Maintenance of mitochondrial function by site-specific ROS signalling extends animal lifespan

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Reactive oxygen species (ROS) have long been considered damaging agents that cause ageing and age-related diseases. However, they are also vital secondary messengers implicated in correct cellular signaling and homeostasis. Here we show that the site where ROS are originated from significantly contributes to their apparent dual nature. We show that ROS increase with age as mitochondrial functions worsens. However, we further demonstrate that specific induction of reverse electron transport (RET) through respiratory complex I produces a ROS signal that extends *Drosophila* lifespan. We show that induction of RET rescues pathogenesis induced by severe oxidative stress, confirming the importance of origin site in ROS signaling. Furthermore, preventing ubiquinone reduction through PTEN-induced putative kinase (Pink1) knockdown shortens lifespan and accelerates aging; with both phenotypes being rescued by increasing ROS through RET. Finally, we show that RET is instrumental for appropriate mitochondrial turnover and fine-tune of Target of rapamycin (Tor) signaling under stress. Our results demonstrate that origin site of any ROS signal is vital to determining what effect it has on cellular physiology and indicate that manipulation of ubiquinone redox state is a valid strategy to delay related pathogenesis.

Supported by ERC and Finnish Academy.

O11

Regulation of endothelial function and angiogenesis by PGC-1α relies on ROS control of vascular stability

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Background: Peroxisome proliferator activated receptor g co-activator 1α (PGC-1α) is a regulator of mitochondrial oxidative metabolism and reactive oxygen species (ROS) homeostasis that has been show to play a relevant role in angiogenesis. This study aims to investigate the role of ROS homeostasis on the regulation by PGC-1α of angiogenesis. ***Methods and Results:*** We found that endothelial cells (ECs) from mice deleted for PGC-1α display attenuated adhesion to the extracellular matrix, together with slower spreading, reduced formation of cellular junctions, a disorganized cytoskeleton and random motility, and a enhanced tip phenotype. Additionally, PGC-1α-deleted ECs exhibit an altered response to vascular endothelial growth factor-A (VEGF-A). *In vivo*, deletion of PGC-1α results in reduced pericyte coverage, a de-structured vascular plexus, and low perfusion. Exposure of PGC-1α$^{-/-}$ mice to hyperoxia during retinal vascular development exacerbates these vascular abnormalities. Mice show extensive retinal hemorrhaging and highly unstructured areas compared with wild-type mice. Structural analysis demonstrates a reduction of membrane bound VE-cadherin, suggesting defective inter-cellular junctions. Interestingly, PGC-1α$^{-/-}$ retinas and ECs show a constitutive activation of the VEGF-A signaling pathway and a poor response to VEGF-A stimulation. This phenotype is partially reversed both in vitro and in vivo by antioxidant administration, indicating that elevated production of mitochondrial ROS in the absence of PGC-1α is a key factor in the alteration of the VEGF-A signaling pathway. ***Conclusions:*** In summary, our findings indicate that PGC-1α control of ROS homeostasis plays an important role in the control of *de novo* angiogenesis, and is required for vascular stability.

O12

The transcription factor Nrf2 mediates UCP3 upregulation in response to 4-hydroxynonenal in mouse cardiomyocytes

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The transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) is a master regulator of the cellular defences against oxidative stress. In the presence of oxidizing agents or electrophiles, Nrf2 translocates to the nucleus, where it induces the transcription of genes involved in the defence against oxidative damage. 4-Hydroxy-2-nonenal (HNE) is a highly cytotoxic product of lipid peroxidation. Nevertheless, at low concentrations, it is able to activate protective pathways, including that of the transcription factor Nrf2. In addition, HNE activates uncoupling proteins (UCPs), mitochondrial inner membrane proteins that mediate uncoupling of oxidative phosphorylation and have been proposed to prevent excessive superoxide production and to protect against oxidative stress. It is not known, however, whether HNE might induce UCPs expression via Nrf2 to cause mitochondrial uncoupling. We investigated the effects of sublethal HNE concentrations on UCP3 expression in mouse cardiomyocytes and the involvement of Nrf2. HNE induced the nuclear accumulation of Nrf2 and enhanced UCP3 expression. After HNE treatment, Nrf2 bound to a specific antioxidant response element (ARE) conserved in the *Ucp3* promoter to activate *Ucp3* transcription in response to oxidative stress. The effects of HNE on UCP3 expression were prevented in both Nrf2-silenced cells and cells treated with the antioxidant N-acetylcysteine. Cardiomyocytes treated with Nrf2- or UCP3-specific siRNA were less tolerant to HNE as reflected by increased cell death. The treatment with HNE greatly altered cardiomyocyte bioenergetics. Thus, the leakage of protons across the inner mitochondrial membrane was increased and the maximal respiratory capacity and the respiratory reserve capacity were severely reduced. These results suggest that the upregulation of UCP3 mediated by Nrf2 in response to HNE might be important in the protection of the heart under conditions of oxidative stress such as ischemia-reperfusion.

O13

Searching for biomarkers of Charcot-Marie-Tooth, a neuromuscular rare disease

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Rare diseases are paradigms for specifically impaired molecular pathways, thus providing valuable information on the discovery of new metabolic profiles shared by different diseases and involving similar physiological events. One of the pleiotropic events that occur during aging is the gradual deterioration both of the function and mass of components of the neuromuscular system.

Charcot-Marie-Tooth disease (CMT; ORPHA166) is the most frequent hereditary neuropathy. CMT designates a heterogeneous group of disorders which, despite some variability in their clinical features, share the same general phenotype, characterized by progressive loss of muscle tissue and touch sensation across various parts of the body. Despite the advances in the clinical and molecular description of this disease during the last years, there are no effective drugs or advanced therapies available to date.

We have pursued the identification of proteins and oxidative stress parameters to be used as biomarkers, in plasmas from patients with duplication at the PMP22 gene, the most frequent mutation causing CMT, clinically characterized as CMT1A subtype. Samples were collected at the neuropathy units from 4 important Spanish hospitals. For the oxidative stress parameters analysis, we analyzed carbonylated proteins, nitrosylated proteins, total antioxidant capacity, GSSG/GSH ratio, and MDA levels. The proteic biomarker candidates search was performed using 2D-DIGE analysis. After protein identification, an interactomic map of these proteins and PMP22 was obtained with PhenUMA software. Finally, gelsolin protein levels were analyzed by enzyme-linked immunosorbent assay (ELISA).

We did not find differences in oxidative stress parameters between the different groups analyzed (mild phenotype CMT patients, severe phenotype CMT patients, and controls); however we observed significant differences in gelsolin levels between mild phenotype CMT patients and severe phenotype CMT patients. In addition, we found a correlation between the age of the patients and gelsolin protein levels.

O14

Oxidative stress and NOX4 up-regulation contribute to aortic wall injuries in Marfan syndrome aortic aneurysms

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Marfan syndrome (MFS) is characterized by ascending aortic aneurysms resulting from altered assembly of extracellular matrix microfibrills and chronic activation of TGF-ß signaling. TGF-ß is a potent regulator of reactive oxygen species (ROS) production and expression of some cardiovascular relevant NADPH oxidases, particularly the isoform 4 (NOX4). We hypothesized that vascular smooth muscle cells (VSMCs) from human Marfan aortic aneurysms overproduce ROS mediated by NOX4, in response to TGF-ß signaling, which could contribute to aneurysms development. Aortic tissue from Marfan patients showed increased levels of protein nitration, markers of oxidative stress, and NOX4 expression. VSMCs explanted from these tissues confirmed NOX4 overexpression at transcriptomic level and a higher production of H_2O_2 . To examine the functional significance of NOX4 overexpression in MFS, we generated a murine Marfan model NOX4 KO and evaluated the aortic elastic fiber integrity loss and aortic root diameters, as features of aneurysm progression. Marfan-NOX4 KO mice showed lesser elastic fibers fragmentation in comparison with Marfan littermates. In addition, aortic wall architecture revealed by elastic fibers organization was also less severe in Marfan-NOX4 KO animals. No differences were observed in elastic fiber integrity in NOX4 KO mice in comparison with control animals, but the NOX4 absence in Marfan mice seemed to cause a remodeling of elastic lamellae organization. On the other hand, the aortic root vasodilatation evaluated by echocardiography, the main aneurysm progression marker, was not prevented in Marfan-NOX4 KO mice compared with Marfan mice. Our results confirm the presence of oxidative stress in Marfan aneurysm tissue, and identify the NOX4 as an important source of the ROS produced. However, although NOX4 seems relevant to aortic wall detriment in Marfan aneurysms, its removal was not enough to arrest the aortic root dilatation progression, reinforcing the complexity of this phenotype as a result of a multitude of mechanisms.

O15

Nitrite/ascorbate redox interaction leads to nitric oxide production in the brain hippocampus, supporting neurovascular coupling. An in vivo study in real-time.

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The energetic demand of the brain requires a constant supply of oxygen (O_2) and glucose. To maximize energetic efficiency, metabolic requirements due to neuronal activation are satisfied through local and transient increase in cerebral blood flow (CBF), via the mechanism of neurovascular coupling (NVC). It has recently been shown that •NO produced by the neuronal-synthase (nNOS) mediates NVC in hippocampus under physiological conditions. However, when O_2 supply is diminished (e.g., stroke, ageing), this pathway for NVC is compromised. We hypothesized that under these hypoxic and acidic conditions nitrite becomes a source of •NO in the brain, in a process independent of enzymatic control, involving its reduction by ascorbate. For that, we used an *in vivo* approach for measurement of •NO and CBF dynamics in the hippocampus, involving the insertion of selective microelectrodes and arrays. Results show that, upon glutamate stimulus, nitrite supplementation (10 **μ**M) induces an increase of •NO and CBF signal area of 19% and 38%, respectively. The localized microinjection of nitrite (50 **μ**M) in animals submitted to short-term acidosis (in the absence of glutamate stimulus) induced a transient increase of •NO in the nM range, concomitantly with transient increased CBF. Furthermore, it was observed a decrease in extracellular levels of ascorbate coupled in time with the production of •NO. Results support that reduction of nitrite to •NO by ascorbate occurs in the brain and underlines NVC. This novel pathway for NVC is particular relevant in conditions where the synthesis of •NO from nNOS is impaired due to limited availability of O_2.

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POSTERS

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NUTRITION (P1-P20)

P1

Nitric oxide bioavailability: effect of (–)-epicatechin supplementation.

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Nitric oxide synthase (NOS) and NADPH oxidase (NOX) activities are key players in blood pressure regulation. To evaluate the effect of (–)-epicatechin (EC) on nitric oxide bioavailability in rats, we studied NOS and NOX activities in aorta, heart and kidney of fructose-fed rats.

Rats were randomly divided in 3 groups depending on beverage and diet consumed: Control (C); Fructose (F:10 % in beverage, 8 wk); Fructose (–)-epicatechin (FEC: 10 % in beverage 20 mg/ Kg wb, 8 wk).

The treatments did not affect animal's body weight. Blood pressure (BP) in fructose-fed rats was significantly higher while EC-treated rats showed BP values similar to control group.

NOS activity in the three tissues was higher in F with respect to C. EC administration increased the activity to values even higher than in F ($p< 0.05$). In this line, eNOS activation/phosphorylation was significantly higher in FEC compared to F. This result may account, in part, for the increase observed in NOS activity.

NOX activity was significantly higher in F-treated group with respect to C and FEC, in the three tissues studied. This augmented activity was accompanied by higher expression of the activating subunit of the NOX-2 isoform, $p47^{phox}$. Besides, in aorta and heart, NOX-4 was also increased in F compared to the other groups.

In conclusion, dietary EC avoid the increase in BP in fructose-fed rats and modulates NO bioavailability by increasing NOS activity and expression and/or by diminishing NOX activity and expression.

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P2

Effects of (-)-epicatechin on hepatic redox-signaling and insulin sensitivity in *in vitro* and *in vivo* obesity models

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Obesity is a major public health problem because of the associated pathologies, including the development of insulin resistance and type 2 diabetes (T2D). Our previous results showed that the flavan-3-ol (-)-epicatechin (EC), the most abundant flavonoid present in fruit and vegetables, improves inflammation and insulin sensitivity in adipocytes in culture and in high fructose-fed rats. The aim of this study was to investigate the capacity of EC and its metabolites (ECM) to modulate hepatic redox-signaling, and as a consequence improve insulin sensitivity in a mouse model of obesity-induced T2D. The underlying mechanisms were investigated in human hepatocytes (HepG2 cells) incubated with palmitate (Pal) (0.25 mM), and in the absence/presence of EC and ECM (0.25-1 μM). Mice fed a high fat diet (HFD) (60% total calories from fat) for 15 weeks developed obesity and insulin resistance. Dietary EC supplementation (20 mg EC/kg BW/d) mitigated/prevented HFD-induced alterations in glucose homeostasis (ITT, GTT, fast/fed glucose and insulin) and in the insulin pathway. EC acted inhibiting events involved in hepatic insulin resistance: oxidative stress, increased expression/activation of the superoxide anion ($O_2^{\cdot-}$) generating enzyme NADPH oxidase (NOX) (no changes in mitochondria $O_2^{\cdot-}$ production were associated to HFD), activation of redox-sensitive signals, and inflammation. In HepG2 cells Pal caused an increased oxidant production, increased NOX expression and activity, and activation of redox-sensitive signals (JNK and IKK) known to down regulate the insulin cascade. EC decreased/prevented all these events. In summary, EC and its metabolites at concentration found in plasma after consumption of EC-rich foods, improved obesity-associated insulin resistance. This occurred in part through EC-mediated inhibition of NOX expression and activity and the modulation of hepatic redox signaling. Thus, consumption of EC-rich foods may provide benefits against obesity-induced T2D.

P3

DHA diet supplementation and *in vitro* vitamin C effect on oxidative and inflammatory neutrophil response to PMA

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Acute exercise primes neutrophils to oxidative burst, and increases neutrophil ROS production after immune stimulation. Ascorbate influences some neutrophil functions, as increasing chemotaxis and particulate ingestion. Omega3 fatty acids increase phagocytic and anti-fungal neutrophil activities, increase ROS production in the presence of PMA. The aim was evaluate the effects of diet supplementation with docosahexaenoic (DHA) and in vitro vitamin C (VitC) at physiological concentrations on oxidative and inflammatory neutrophil response to PMA. 15 footballers ingested a beverage enriched with DHA or a placebo for 8 weeks in a randomized double-blind study. Neutrophils were isolated from blood samples collected in basal conditions at the end of nutritional intervention. Neutrophils were cultured for 2 hours at 37ºC in a) control media (culture media RPMI1640), b) media with PMA (5μg/ml), c) media with PMA (5 mg/ml) VitC (3 μM, physiological concentration). PMA induces neutrophil degranulation with increased extracellular myeloperoxidase and catalase activities, expression of the inflammatory genes cyclooxygenase-2, nuclear factor κB, interleukin 8 and tumor necrosis factor α, and interleukin 6 production. DHA diet supplementation boosts the exit of CAT from neutrophils but moderates the degranulation of myeloperoxidase granules induced by PMA. VitC facilitates azurophilic degranulation of neutrophils and increases gene expression of myeloperoxidase induced by PMA. VitC and DHA diet supplementation prevent PMA effects on inflammatory gene expression; although together do not produce additional effects. DHA diet supplementation enhances antioxidant defenses and anti-inflammatory neutrophil response to in vitro PMA activation. VitC facilitates neutrophil degranulation but prevents an inflammatory response to PMA. *Grants: DPS2008-07033-C0303, P 11/01791, CIBERobn CB12/03/30038. We also acknowledge the Ph.D. grant provided by the University of the Balearic Islands.*

P4

A Mediterranean diet with extra virgin olive oil may have a beneficial effect against hepatic steatosis

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Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease, therefore its prevention and treatment is highly relevant to health. Olive oil, rich in monounsaturated fatty acids, is the main source of fat in the Mediterranean diet (MedDiet) but little is known about its effect on NAFLD. Within the PREDIMED trial of nutritional intervention for the primary prevention of cardiovascular disease we evaluated its effect on NAFLD.

100 men and women aged 55-80 years at high cardiovascular risk were randomly assigned to receive MedDietEVOO, MedDietNuts, or a control low-fat diet for 3 years. Lifestyle, adherence to MedDiet, the liver fat content, the prevalence of hepatic steatosis assessed (NMR imaging), biomarkers of inflammation and oxidative stress were measured.

After follow-up there were no changes in adiposity, energy expenditure or macronutrient consumption among the groups. The liver fat content was the lowest in the MedDietEVOO. Hepatic steatosis was seen in 8.8%, 33.3% and 33.3% of participants in the MedDietEVOO, MedDietNuts and control diet groups, respectively. Urine values of 12-HETE/creatinine were lower (41%) in the MedDietEVOO than in the control diet. MedDietNuts increased nitrites plus nitrates (30%) when compared to the control. There were no changes on serum 3-nitrotyrosine, ferric reducing ability power and alanine aminotransferase (ALT) between groups. When grouping participants as non steatotic and steatotic, the systolic and diastolic blood pressures, insulin, insulin resistance and ALT were higher in participants with steatosis. Steatohepatitis involves oxidative stress. However, in steatosis, a previous step to steatohepatitis we did not observe changes in oxidative stress and inflammatory parameters.

A MedDietEVOO without energy restriction showed a lower prevalence of hepatic steatosis. This observation that could be related to the antioxidant and anti-inflammatory effect of EVOO needs further research.

We thank PREDIMED and FISS PI13/01123, both from ISCIII for their financial support.

P5

Effect of diets enriched with quercetin on oxidative stress biomarkers and hormone somatolactin expression in silver catfish (*Rhamdia quelen*)

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Quercetin has several beneficial effects and could be an important tool to be used on fish farms in order to reduce the stress linked to cultivation.This study evaluated the somatolactin hormone expression in pituitary, plasma levels of cortisol and the oxidative biomarkers in brain, liver and muscle of silver catfish fed diets containing quercetin. Three diets were tested: control; 0.15% quercetin; and 0.30 % quercetin, whose concentrations were added in the control. After 21 days of feeding, blood was collected and the animals were euthanized. The blood was utilized for analysis of plasmatic cortisol levels using an enzyme-linked immunosorbent assay. In the pituitary, the mRNA levels of somatolactin were determined by qRT-PCR. The brain, liver and muscle were removed for the follow analysis: thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) levels; enzyme activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST); and non-enzymatic antioxidants through the determination of the ascorbic acid (AA), the non-protein thiols levels (NPSH) and total reactive antioxidant potential (TRAP). The silver catfish fed with diet containing quercetin showed no differences in the cortisol concentration and the mRNA levels of somatolactin levels when compared to the control animals. All the tissues of silver catfish fed with diets containing quercetin exhibited decreased LOOH and TBARS levels than the control ones. Regarding the antioxidant enzymes, there was an increase in SOD, GPx and GST activities in all the organs of silver catfish fed with diets containing quercetin when compared to the control. There was also an increase in AA, NPSH content and TRAP in the same tissues of the silver catfish fed diets containing quercetin when compared to the control ones. These results suggest that the quercetin-supplemented diet is recommended for silver catfish feeding because it increases the tissue antioxidant responses, thus preventing the oxidative damage.

P6

17β-Estradiol enhances sulforaphane protective effects against oxidative stress in cardiomyocytes

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Female gender is associated with improved heart failure survival and estrogens seem to play a fundamental role in this protection. It has been suggested that 17 β-Estradiol (E2) reduces apoptosis in animal model of myocardial infarction through the modulation of MAPKs involved in oxidative stress. Another estrogen positive action could be the enhancement of the protective effects of some nutraceutical compounds, such as the isothiocyanatesulforaphane (SF), mainly present in Brassica vegetables. To demonstrate this hypothesis, we investigated the effects of low concentrations of SF in the presence/absence of E2 against oxidative stress in primary cultures of rat cardiomyocytes.

Cell viability was evaluated by MTT and LDH assays, ROS production by the 2',7'-dichlorofluorescein-diacetate (DCFH-DA) assay and immunofluorescence staining with anti- 8-hydroxyguanosine (8-OHdG), GSH levels by the monochlorobimane assay, Nrf2 and antioxidant enzyme expression by RT-PCR.

Cells were treated with SF (0.1-0.5 μM) and/or E2 (10-50 nM). Oxidative stress was induced by 100 μM H_2O_2 exposure. SF and E2 co-treatment protected cardiomyocytes against oxidative stress induced-damage most effectively than SF and E2 alone as measured by MTT and LDH assays. The co-treatment was also able to significantly reduce intracellular ROS production in respect to E2 or SF. As SF up-regulates phase II enzymes, we investigated if the co-treatment enhances this over-expression. As expected, SF in the presence of E2 significantly increased the expression of NAD(P)H: quinone oxidoreductase 1, thioredoxin reductase, hemeoxygenase 1, and catalase in respect to SF or E2. Moreover the co-treatment up-regulated the transcription factor Nrf2 and increased GSH intracellular level. These findings demonstrated, for the first time, that E2 enhances the protective effects of SF, suggesting that nutraceutical compounds could have different outcomes in males and females.

P7

Blood phenolics levels as biomarkers of its concentration and bioactivity on central nervous system of mice exposed to plant infusions

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Natural phenolics are phytochemicals present in plant foods, which have antioxidant properties. After intake, they are transported by blood proteins to different organs, such as metabolic organs and the central nervous system (CNS) given their capacity to cross the blood-brain barrier. Thus, it might be possible to correlate their blood concentration with those found in CNS tissues and with their redox state. The aim of our work was to assess the value of blood phenolic dosage as biomarker of organic phenolics and oxidation in BALB/C mice after 0-30 days of intake of different infusive extracts from *L. grisebachii* (LG), *I. paraguariensis* (IP) and *A. quebracho-blanco* (AQB) (100 mg/Kg/d). The following parameters were analysed: hydroperoxides (AHP), lipoperoxides (LHP) and phenolics in CNS (thelencephalon, diencephalon, mesencephalon, encephalic stem, and cerebellum), liver, kidney and blood. It was found a positive correlation between phenolics in blood and in brain ($r=0.81$; $p<0.05$). Also, blood phenolics were inversely correlated to LHP in murine diencephalon ($r= -0.57$; $p<0.01$). These results were independent of the source (extracts). Other significant associations were not found. Therefore, blood phenols could be used as a biomarker of CNS bioavailabilty of phenolics and their antioxidant capacity, with further studies being encouraged to fathom the impact of these dietary compounds on nervous tissues

P8

Effect of montmorency cherry supplementation on ischemia-reperfusion in middle aged men

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High habitual flavonoid intake, particularly anthocyanins, has been associated with reduced risk of hypertension, and acute supplementation with fruit-derived flavonoid supplementation improves brachial artery endothelial function (flow-mediated dilatation, FMD). Since repeated cycles of endothelial ischemia-reperfusion (IR) injury contribute to vascular dysfunction, we investigated whether chronic supplementation with anthocyanin rich Montmorency cherry (MC) attenuated the suppression of brachial artery FMD induced by IR. In a double-blind, placebo-controlled, crossover design, 10 sedentary middle aged men (54.0±4.6y; 28.6±4.9 $kg.m^{-2}$) consumed MC capsules (235$mg.d^{-1}$anthocyanins) and placebo (PL) for 4 weeks in pseudo-random order separated by 4 weeks washout. Pre and post supplementation, FMD responses were measured at baseline and 15, 30, and 45 min after a 20 min forearm occlusion to induce IR. Prolonged occlusion resulted in significant reduction of FMD, 15 min post occlusion in both conditions (baseline vs 15min; MC: -57.9±17.1%; PL: -48.1±23.8%, time effect: $P<0.001$) and remained depressed after 45 min (baseline vs 45 min MC: -4.8±39.3%; PL: -26.2±12.6%, time effect: $P=0.035$). Brachial artery baseline diameter increased after prolonged occlusion ($P=0.001$). The change from baseline to 15 min post-occlusion tended to be increased post vs pre MC (1.2±2.7%), but was reduced when post vs pre PL (-1.5±2.7%, condition x time interaction: $P=0.091$). Area under the curve (AUC) for shear decreased after prolonged occlusion ($P=0.036$). This reduction in shear AUC from baseline to 15 min post-occlusion tended to be attenuated post vs pre MC (-6.9±25.6%) but increased post vs pre PL (22.9±37.9%, condition x time interaction: $P=0.094$). Prolonged forearm occlusion impaired FMD for at least 45 min, and MC supplementation tended to improve FMD recovery after IR. These effects may contribute to the vascular health benefits associated with flavonoid intake.

P9

N-acetylcysteine attenuates the toxic effects of aspartame on the glutathione-related antioxidant system in rat brain regions

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This study evaluated the effects of N-acetylcysteine on the impairment in the glutathione (GSH)-related antioxidant system in the cerebral cortex, cerebellum, brainstem, hypothalamus, amygdala and hippocampus after aspartame intake. The rats were divided into four groups: control - received both aspartame and N-acetylcysteine vehicles; NAC - received aspartame vehicle and N-acetylcysteine treatment (1 mmol kg^{-1}, i.p.); ASP - received aspartame treatment (40 mg kg^{-1}, v.o.) and N-acetylcysteine vehicle; ASP-NAC - received both aspartame and N-acetylcysteine treatments. Aspartame was administrated for six weeks; whilst N-acetylcysteine was injected from the fifth to the sixth week. N-acetylcysteine reduced the aspartame-induced lipid hydroperoxides formation in the cerebral cortex, cerebellum, brainstem, hypothalamus, amygdala and hippocampus. N-acetylcysteine also led to the reestablishment of the glutathione peroxidase activity in the cerebral cortex, brainstem, hypothalamus and amygdala; glutathione reductase activity in the cerebral cortex, cerebellum, hypothalamus and amygdala; glutathione S-transferase activity in the cerebral cortex, cerebellum, brainstem, hypothalamus, amygdala and hippocampus; and total glutathione levels in the cerebral cortex, cerebellum, brainstem, hypothalamus, amygdala and hippocampus of the aspartame-treated rats to the control and NAC values. In conclusion, N-acetylcysteine protected the cerebral cortex, cerebellum, brainstem, hypothalamus, amygdala and hippocampus through GSH production; and the modulation of GSH-related enzymes, triggering different defensive responses according to each brain region.

P10

Modulation of nuclear factor-kB signalling pathway by anthocyanins in human intestinal Caco-2 cells exposed to Tnf-α.

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Inflammatory bowel diseases (IBDs), the collective name for Crohn's disease (CD) and ulcerative colitis, are characterized by persistent and unpredictable attacks of inflammation of the intestine, causing weight loss, diarrhoea, rectal bleeding, abdominal pain, fever and anemia. Previuos studies have shown that TNF-α plays a central role in the intestinal inflammation of IBDs supporting beneficial effects of flavonoids against chronic inflammatory diseases. Among these compounds, anthocyanins, which are widely distributed in mediterranean diet, have been demonstrated to inhibit inflammation as protective mechanism in many cell lines.

In this study, we employed an *in vitro* model of the acute phase of intestinal inflammation using differentiated Caco-2 cells, a continuous line of human epithelial colorectal cells, exposed to the proinflammatory cytokine TNF-α. The protective effects of Cyanidin-3-glucoside (C3G) were then evaluated. Caco-2 cells exposure to TNF-α for 6 h activated IKK/NF-kB proinflammatory pathway, and induced COX-2 and IL6 expression. Interestingly, cells pretreatment for 24h with C3G (20 and 40 μM) was effective in preventing TNF-α-induced changes. Furthermore, C3G was able to improve intracellular redox status altered by TNF-α.

Finally, our data suggest that C3G may have protective effects against TNF-α-mediated intestinal mucosal damage in intestinal epithelial cells probably by modulating intracellular redox status.

P11

Effect of microvesicles released from polyphenol-treated immune cells on stem cells

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Microvesicles are membranous elements, ranging from 30 to 1000 nm in diameter, which are released from and taken up by most types of cells. Microvesicles transport proteins, mRNA, microRNA, noncoding RNA, and DNA fragments with the potential to alter the fate of the recipient cells. They can be secreted by immune cells and play a physiological role in target cells. The nutrients we get from food contact with immune cells in the bloodstream and can promote the formation of microvesicles. Some foods contain molecules with regulatory activity, such as genistein and resveratrol, which are polyphenols found in soy and red wine, respectively.

Therefore, we aimed to study the effect of microvesicles released from polyphenol-treated immune cells on stem cells.

For that purpose, blood samples from 7 women (aged 20-30) were collected in VACUTAINER® CPT tubes with heparin, obtaining mononuclear cells by centrifugation. The cells were further cultured and treated with: 1nM resveratrol, 0.5μM genistein and 0.01% DMSO as a control. After 48h, the microvesicles were isolated by ultracentrifugation and co-incubated with dental pulp stem cells (DPSC) for 48h. Then the DPSC were treated with 130μM of H_20_2 for 24h. Cell viability was determined by Tripan Blue and mRNA expression of antioxidant genes was determined by RT-PCR.

We observed that DPSC incubated with microvesicles isolated from genistein-treated mononuclear cells were protected against oxidative stress, as demonstrated by its increased viability. Furthermore, these cells overexpressed Mn-SOD mRNA expression.

As a conclusion, microvesicles secreted by genistein-treated mononuclear cells exert a protective effect against oxidative stress in DPSC.

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P12

In vitro cholesterol assimilation and production of conjugated linoleic acid by two *Lactobacillus plantarum* strains isolated from dairy products

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Two autochthonous *Lactobacillus plantarum* strains, isolated from raw ewe's milk and artisanal cheese were studied for some functional properties relevant to their use as probiotic cultures. An *in vitro* screening study on the ability to reduce cholesterol level in the culture medium and to convert linoleic acid to conjugated linoleic acid (CLA) was applied. The ability to adhere to intestinal epithelial cells was further evaluated.

Our results showed that both the two strains were able to reduce the percentage of cholesterol in the culture medium after 24 h of incubation at 37°C. However, the two strains showed a different efficacy in removing the cholesterol added to the growth media. *Lactobacillus plantarum* 62LP39 showed a percentage of cholesterol removal ability higher (78±1.7% of cholesterol with respect to the control medium) than *Lactobacillus plantarum* 1C3M (89±5.19% of cholesterol with respect to the control medium).

The two strains also showed CLA-producing abilities from linoleic acid. The percentage of linoleic acid conversion calculated with respect to the CLA of the control medium was 4.37±1.19% for *Lactobacillus plantarum* 62LP39 and 9.73±0.27% for *Lactobacillus plantarum* 1C3M. The adhesion assay was performed in cultured Caco-2 cells, and both the two strains showed excellent adhesion capabilities.

These preliminary results suggest that the two *Lactobacillus* strains analyzed could be considered appropriate probiotic candidates for additional studies in order to be used as functional food materials.

P13

Microwave heating influence on oxidative stress parameters in vegetable oils

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Numerous studies claim that there is a correlation between the type of oils used for cooking and various health benefits or issues. Amongst the most important issues addressed in nutrition is the antioxidant effect of vegetable oils as promoters of health benefits. Also of interest are the oxidative processes that take place during cooking and the connection of these processes with health issues. For these reasons we have studied the variation of total antioxidant capacity, vitamin E and polyphenols in relation to lipid peroxides formation during microwave oils heating.

Rapeseed, sesame, olive, walnut, sea buckthorn, coconut, sunflower, corn, soybean, palm and mixed oil (sunflower, grape, flaxseed and rice oil) were purchased from the local market. Each oil was exposed to microwaves for 5, 10 and 15 minutes. For all samples and each exposure time Trolox Equivalent Antioxidant Capacity (TEAC), lipid peroxides as thiobarbituric reactive substances (TBARS), conjugated dienes (CD), vitamin E as α-tocopherol and total polyphenols were determined.

After 15 minutes heating the best retention for vitamin E was observed for the palm oil (12mM/l). The highest total antioxidant capacity after heating belonged to the corn, mixed & palm oil (4.6 eq. TROLOX/l). The best retention for polyphenols was found in olive oil (1.5 mg/ml). Highest levels of TBARS (83 uM/l) and CD (17 mM/l) were found in soybean oil.

Initial TEAC values were positively correlated with initial polyphenols concentration. After 15 min microwave treatment, final TEAC values were negatively correlated with CD.

In conclusion, after 15 min of microwave treatment the best antioxidant properties were determined for palm and olive oil while soybean oil showed the most extensive oxidation effects.

P14

Study of alcoholic fermentation effect on the total phenolics of the algerian date variety « deglet-nour »

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In this study, we tried to analyse the total phenolic content of date fruit variety «Deglet-Nour » using the method of Folin-Ciocalteu and to demonstrate the alcoholic fermentation effect on these compounds. The fermentation kinetic was followed for various parameters, pH, total sugars, biomass and total polyhenols on a period of time of 52 hours using the strain Saccharomyces cerevisiae VDH2. Samples were taken every two hours to perform different assays. The results obtained have shown a decrease in total sugar from 50g.l–1 ± 0,006 at initial time (t= 0h) to reach 20 g.l–1 ± 0,002) at the end of the fermentation process.The biomass at t=0 h was evaluated (1,6. 104 cells/ml) reaching (3,9. 105 cells/ml) at the end of fermentation. This was confirmed by the low acidity ranged from 4,6 to pH 3,4 at t=0 h and t= 52 h respectiveley. The total phenolics has increased during the fermentation from 33,3 ±0,01 EAG/100 g at t= 0h to 72,15± 0,01 EAG/100g of fresh wight at t= 52 h. Al results are means of three replicate of fermentation.

Key-words :Deglet-Nour – Fermentation – Polyphenols – Kinetic – Saccharomyces cerevisiae

P15

Anti-cancer and immune modulatory effects of palm tocotrienols

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Palm tocotrienols are known for their many health beneficial effects. In this study the anti-proliferative effects of tocotrienol-rich fraction (TRF) from palm oil, γ- and δ-tocotrienol (γ-T3 and δ-T3) and α-tocopherol (α-T) were studied on 4T1 murine mammary cancer cells. TRF, γ-T3 and δ-T3 significantly inhibited the growth of the 4T1 cells with IC50 values of 8.99, 4.79 and 3.73μg/ml respectively. Tumourigenesis was examined with TRF supplementation and compared against control in both nude and BALB/c mice models. Palm tocotrienols showed strong inhibitory effects on the growth of both MDA-MB-231 and 4T1 cells both in vitro and in vivo. In addition the immune modulatory effects of tocotrienols were also investigated and it was found that TRF enhanced production of NK cells ($P < 0.05$) as well as IFN-γ ($P < 0.05$), which in turn regulate the immune protection against cancer cells. These observations were recorded in both mice models. Several mechanisms have been postulated for the anti-cancer and immune modulatory effects of tocotrienols. In this study, the anti-cancer mechanism of tocotrienols is for the first time linked to increased expression of interleukin-24 (IL-24) mRNA, a cytokine reported to have anti-tumor effects in many cancer models. The 4T1 cells treated with TRF, δ-T3 and α-T exhibited highest levels of IL-24 mRNA in δ-T3 treated cells, followed by TRF and α-T. The IL-24 mRNA levels in tumor tissues of BALB/c mice supplemented with TRF increased two-fold as compared to control mice. Increased levels of IL-24 have been associated with inhibition of tumor growth and angiogenesis. TRF and δ-T3 treated 4T1 cells significantly decreased IL-8 and vascular endothelial growth factor (VEGF) mRNA levels. We hereby report that the anti-tumor including the anti-angiogenic effects of tocotrienols are associated with increased levels of IL-24 mRNA.

P16

Diet-derived bioactive compounds protect cells exposed to oxidative stress by: (a) penetrating cell membranes, and (b) chelating intracellular "labile iron"

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Epidemiological studies have shown that frequent consumption of fruits and vegetables influences positively human health by decreasing the probability of development of serious diseases, such as cardiovascular diseases and cancer. However, the molecular mechanism(s) underlying these effects remains unknown. Previous studies in our laboratory have shown that numerous components contained especially in the Mediterranean type of diet are capable to prevent oxidation of cell constituents in conditions of oxidative stress by chelating intracellular "labile iron". In this study, we investigated the effects of modification of simple plant-derived compounds (i.e. caffeic and rosmarinic acids) on their capacity to protect nuclear DNA in cells exposed to H_2O_2. Masking of the negative charge of these acids by ester or amide bonds facilitated their diffusion through plasma membrane and led to a manyfold increase of their protective capacities, with amide derivatives being somewhat less effective compared to esters. At higher concentrations, ester-derivatives exerted genotoxic effects, while the amides did not. The differences between ester and amide derivatives can be explained by the fact that the former are rapidly hydrolyzed inside the cells by the action of non-specific esterases leading to their accumulation, while the latter are less susceptible to hydrolysis. In conclusion, these results indicate that two main properties determine the protective capacity of numerous different compounds contained in diet: (i) their ability to penetrate through cell membranes, and (ii) their capacity to chelate intracellular "labile iron". Slight modifications in the structure of these components affect profoundly both arms of their mode of action.

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P17

Antioxidant and anti-inflammatory potential of bergamot powder against LPS-induced endothelial activation and dysfunction

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Inflammation and oxidative stress are key factors of vascular injury underlying chronic pathologies such as diabetes, obesity and cardiovascular disease.

The aim of this study was to characterize and to evaluate the antioxidant capacity of a lyophilized powder from whole-bergamot fruit. Moreover, we investigated, on human microvascular endothelial cells (HMEC-1), the nutraceutical effect of bergamot extract against LPS-induced endothelial activation and dysfunction.

Bergamot extract (BE) was analyzed for polyphenols, flavonoids, flavonols and ascorbic acid content. We investigated its antioxidant property by *in vitro* assay (ORAC, DPPH and ABTS) and on human erythrocytes (CAA-RBC and hemolysis test).

HMEC-1 were pre-treated for 1 hour with 50µg/ml BE, then exposed for 6 hours to 0.1ng/ml LPS. Specifically, we evaluated IL-6, ICAM-1, ET-1 and CHOP gene expression using Real-Time PCR. Furthermore, we assessed the cellular ROS production (DCFH-DA) and quantified the NO levels by a colorimetric assay.

Our results showed high levels of bioactive compounds and *in vitro* antioxidant activity; besides, we detected a better cellular antioxidant activity and anti-hemolytic effect following BE pre-treatment ($p<0.01$). Moreover, LPS-exposed HMEC-1 resulted in a significant up-regulation of IL-6, ICAM-1, ET-1 and CHOP expression compared to untreated-cells ($p<0.001$). Otherwise, BE pre-treatment exerted a significant inhibitory effect on LPS-induced genes, reducing IL-6, ET-1 and CHOP level ($p<0.05$). However, ICAM-1 expression was significantly reduced by BE pre-treatment compared to LPS-treated cells ($p<0.01$) but still higher than control ($p<0.05$). Furthermore, compared to control, we found a higher ROS production and a reduced NO level following LPS addition, which is completely reversed by BE pre-treatment.

Our data suggest that BE was able to protect HMEC-1 from LPS-induced endothelial activation and dysfunction. This protective effect is probably linked to the high content of bioactive compounds that exhibit radical scavenging and anti-inflammatory activities.

P18

Nutraceutical properties of a fermented bean powder on human microvascular endothelial cells exposed to oxidized-LDL

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Clinical and experimental studies support a direct role of microcirculatory dysfunction in the onset of target organ damage under oxidative stress and dyslipidemia. For instance, diabetes and hypertension are characterized by high oxidized-LDL (ox-LDL) levels and microcirculatory dysfunction.

The aim of this study was to characterize and to evaluate the effect of a fermented bean powder (Lys LJ) on human microvascular endothelial cells (HMEC-1) following ox-LDL exposure.

Compared to the unfermented powder, Lys LJ contained a higher polyphenols and flavonoids content and showed a better antioxidant activity ($p<0.01$).

HMEC-1 were pre-treated for 1 hour with 0.7mg/ml Lys LJ extract, then exposed for 24 hours to 200µg/ml ox-LDL. The oxidation degree, induced by ox-LDL treatment, was assessed as malondialdehyde production. ICAM-1, ET-1 and IL-6 medium levels were measured by ELISA, while LOX-1 and CHOP gene expression were analyzed using quantitative Real-Time PCR (qRT-PCR).

Oxidized-LDL treatment induced a significantly higher oxidative stress ($p<0.01$), which was completely reduced by Lys LJ pre-treatment. Besides, qRT-PCR analysis showed a significant raise of both LOX-1 and CHOP gene expression after ox-LDL treatment ($p<0.05$), which is completely abolished by Lys LJ pre-treatment. Moreover, compared to basal condition, a significant increment of ICAM-1, IL-6 and ET-1 ($p<0.05$) was found after ox-LDL exposure. Otherwise, Lys LJ pre-treatment exerted an inhibitory effect on ox-LDL-induced endothelial dysfunctions with ICAM-1 levels comparable to control; however, IL-6 and ET-1, although reduced, resulted still higher than control ($p<0.05$). Finally, Lys LJ alone did not induce any differences in LOX-1 and CHOP gene expression, as well as ICAM-1, IL-6 and ET-1 concentration, compared to control.

Our data suggest an improved endothelial functions in the ox-LDL-exposed cells after Lys LJ pre-treatment, which was able to inhibit the oxidation process and to decrease the endothelial adhesiveness, but poorly active against inflammation and ET-1 production.

P19

Chronic oral intake of a standardized hawthorn extract prevents DOCA-salt-induced hypertension, and alteration of cardiac, vascular and renal structures and functions in rats: Role of oxidative stress

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The hypertension-induced end target organ damage is characterized by alterations of the heart, vascular and kidney functions and structure associated with oxidative stress. The present study has examined the possibility that a standardized polyphenol-rich Crataegus extract prevents hypertension-induced end target organ damage in an experimental model of hypertension, the deoxycorticosterone acetate (DOCA)-salt non-nephrectomized rat.

Male Wistar rats were divided into 5 groups receiving a diet containing or not the Crataegus extract (100 or 300 mg/kg/day) in the presence or absence of DOCA-salt-induced hypertension. Arterial blood pressure and target organ damage (vasculature, heart and kidney) were examined.

The DOCA-salt treatment increased systolic blood pressure, and this effect was associated with the induction of pronounced endothelium-dependent contractile responses in second order mesenteric resistance artery rings to acetylcholine. The increased contractile response was prevented by indomethacin (a cyclooxygenase inhibitor) and associated with vascular oxidative stress as assessed using dihydroethidium, a reduced expression of eNOS, and an increased expression of NADPH oxidase subunits ($gp91^{phox}$, $p47^{phox}$), COX-1 and COX-2. Echocardiography and histological analyses indicated left ventricular (LV) hypertrophy, fibrosis, and impaired systolic and diastolic cardiac functions associated with oxidative stress in the DOCA-salt group. Renal dysfunction characterized by increased urea and uric acid plasma levels, glomerular and arteriolar hypertrophy, tubulo-interstitial fibrosis associated with oxidative stress were observed in the DOCA-salt group.

Chronic intake of the Crataegus extract significantly reduced in a dose-dependent manner systolic blood pressure, target end organ damage in the heart and kidney, and improved the vascular function by preventing oxidative stress and the expression of target molecules. Thus, the Crataegus extract was able to retard the hypertension-induced end organ damage in the heart, kidney and resistance arteries most likely by preventing oxidative stress.

P20

Evaluation of antioxidant capacity, furan compounds and cytoprotective/cytotoxic effect of Colombian commercial coffee on Caco-2 cells

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Antioxidant activity (ABTS and FRAP), total polyphenols (Folin-Ciocalteau), furan compounds (HMF and furfural) and cytoprotective/cytotoxic effect in Caco-2 cells [viability (MTT), cell cycle analysis and ROS measurement] were evaluated in Colombian commercial coffee (2 ground and 4 soluble). The antioxidant capacity and polyphenols were lower ($p < 0.05$) in ground vs. soluble coffees, with values ranging between 124 and 722 pmolTrolox/g, 95 and 802 pmolTrolox/g, 21 and 100 mg gallic acid/g by ABTS, FRAP and Folin-Ciocalteau, respectively. The same trend was observed for HMF where lower values ($p < 0.05$) were observed in the ground vs. soluble samples, with values between 69 and 2900 mg/kg. Furfural content varied between 25 and 108 mg / Kg. No negative effect on mitochondrial functionality with coffee extracts (1, 10, 100 and 500 mg/mL) was observed; thus, the higher concentration was selected for cytoprotection assays. Pretreatment of Caco-2 cells 24h with coffee extracts prevented ($p < 0.05$) the decrease of cell viability compared to control stress with H_2O_2 (5mM/2h), and increased the viability to values between 34-45%. Pretreatment with coffee extracts prior to oxidative stress retrieved the control values and/or samples counterparts without decrease in the G1 cell cycle phase. Induction of oxidative stress caused an increase (up to 29.3%) of ROS vs. control (5.6%). After stress, 4 extracts prevented ROS accumulation, decreasing values ($p < 0.05$) to 22.5-24.9%. In conclusion coffee samples exert a cytoprotective effect against oxidative stress induced by H_2O_2, with an improvement in cell viability, correct distribution of G1 cell cycle phase and reduction of intracellular ROS. According to the antioxidant capacity and HMF content, soluble samples with higher content of these compounds, are those which exhibit the best cytoprotective effect. Although HMF is cytotoxic there seems to be a positive correlation between this, the antioxidant capacity and the cytoprotective effect of coffee.

ANTIOXIDANTS (P21-P39)

P21

Maternal supplementation of allergic female mice with gamma-tocopherol increases the development of select dendritic cell subsets and allergic lung inflammation in neonates.

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Offspring from allergic mothers have elevated numbers of CD11b lung dendritic cells and develop allergic lung responses to suboptimal allergen challenge. We recently reported that maternal supplementation with alpha-tocopherol (αT) reduced development of these dendritic cell subsets and allergic responses in offspring of allergic female mice. It is not known whether gamma-tocopherol (γT) supplementation regulates development of allergic responses. To address this, allergic female mice were supplemented with γT during pregnancy/lactation. Then, offspring were given a suboptimal allergen challenge. γT supplementation of allergic mothers elevated pup responses to allergen challenge. There were increased numbers of pup lung eosinophils, inflammatory mediators, and CD11b expressing but not CD11b- subsets of CD11c dendritic cells. There were elevated numbers of IRF4 CD11b CD11c expressing dendritic cells, a dendritic cell subset critical for development of allergic responses. There were also fewer pups from γT supplemented allergic mothers. In conclusion, maternal αT supplementation reduced and maternal γT supplementation increased development of CD11b CD11c dendritic cells and allergic responses in offspring from allergic mothers. These results have implications for supplementation of allergic mothers with tocopherol isoforms and for development of allergies in future generations. Grant Support: NIH R01HL111624

P22

Dietary polyphenols modulate mitochondrial physiology as novel substrates in ubiquinone biosynthesis

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Plant polyphenols have attracted a strong interest as dietary antioxidants mainly due to their ability to scavenge free radicals and other reactive species *in vitro*. They have been also recognized to influence many mitochondrial processes such as mitochondrial biogenesis, electron transport, membrane potential, ATP synthesis and apoptosis. Although much progress has been indeed achieved, the mechanisms involved in these actions are far to be completely understood. Ubiquinone (coenzyme Q, Q) is a well-known lipophilic antioxidant and mitochondrial electron carrier which is synthesized endogenously and also incorporated from the diet. As reported, *p*-hydroxybenzoate (pHB) was the only confirmed precursor of Q benzoquinone ring. However, it has been recently found that *Arabidopsis* can use *p*-coumarate as another ring precursor. Moreover, p-coumarate and resveratrol can also serve as a ring precursor of Q in *E. coli*, yeast and human cells. We hypothesized that other natural polyphenols could also behave as biosynthetic precursors of Q. Mouse kidney proximal tubule (TKPTS) cells were cultured in the presence of four polyphenols: resveratrol, kaempferol, quercetin and piceatannol. Our results showed increased Q levels in TKPTS cells treated with resveratrol, kaempferol and quercetin, with kaempferol having the strongest effect. The action of kaempferol was related directly with Q biosynthesis because it was totally inhibited by pABA, a well-characterized inhibitor of Coq2 activity, but it was not due to the stimulation of the mitochondrial sirtuin Sirt3. A competitive assay with radiolabelled ^{14}C-pHB showed that kaempferol acted as a substrate in Q biosynthesis. Kaempferol treatment also produced a significant decrease of cellular peroxides and mitochondrial generation of superoxide. The role of polyphenols, and specially kaempferol, as novel substrates in Q biosynthesis must be considered to understand their action in modulating ROS levels and mitochondrial function.

P23

Lemon verbena polyphenols activate AMPK, improve lipid metabolism and decrease ROS generation in hypertrophic adipocytes.

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Dietary energy excess causes lipid accumulation in adipocytes and other cells resulting in obesity, metabolic stress and low-grade chronic inflammation, which establishes altered homeostasis between metabolic and immune cells. It is evident that metabolism of adipose tissue itself is important for the control of body fat content. In fact, cellular energy and adenosine monophosphate-activated protein kinase (AMPK) are involved in the control of lipid metabolism and mitochondrial function in adipocytes. It has been demonstrated that AMPK in adipocytes can be activated by adipocyte-derived hormones such as adiponectin, and also by thiazolidinediones or polyphenols. We hypothesized that *Lippia citriodora* (LC) polyphenols may prevent energy excess-induced metabolic stress through AMPK-dependent mechanism. The potential of LC and its major compound, verbascoside (VB), to protect against inflammatory metabolic disturbances was studied.

A well-established insulin-resistant hypertrophic 3T3-L1 adipocyte model was used to examine the capacity of LC polyphenols on AMPK activation and mitochondrial function, and a murine model of diet-induced obesity was used to assess the *in vivo* metabolic response.

Cellular studies showed that LC and VB restored mitochondrial membrane potential. The activation of AMPK, the mRNA expression upregulation of PPAR-α and fatty acid synthase (FASN) mRNA expression downregulation were also involved. Experiments in mice suggested a significant improvement in fat metabolism. Decreased lipogenesis, enhanced fatty acid oxidation and the activation of the energy sensor AMPK, probably through activating transcriptional factors, are involved in the observed beneficial effects. VB effects were less potent than those observed with the extract, so a potential synergistic, multi-targeted action is proposed.

The polypharmacological effects of plant-derived polyphenols from lemon verbena may have the potential for clinical applications in obesity.

P24

Flavonoids in silica nanoparticles: adsorption, stability and reactivity against singlet oxygen

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The main concern for the study and the application of flavonoids is their poor water solubility and stability. Strategies proposed to overcome these difficulties consist of inclusion in vesicles, in cyclodextrins and on nanoparticles. In this work, the incorporation of flavonoids on silica nanoparticles, the kinetics and thermodynamics of adsorption and finally the evaluation of antioxidant efficiency were studied. Besides, the behavior of nanoparticle-polyphenol system in different dispersion media formed by compounds frequently used in pharmaceutical and cosmetic formulations for topical use was evaluated. The first step was to develop a preparation process optimized in order to obtain nanoparticles of sizes of 100 nm, spherical geometry and monodisperse. NPs obtained were characterized in terms of size, zeta potential, FTIR and image by scanning electron microscopy. After generating mesoporosity and surface modification with NH_2 groups, six flavonoids (morin, quercetin, rutin, diosmin, hesperidin and hesperetin) were incorporated.

Adsorption efficiency values of flavonoids on NPs were less than 3 % for all compounds and the maximum monolayer capacity of Langmuir isotherm adsorption was 20.8 mg g^{-1} for morin, which showed the best fit. Our results suggest that the pseudo-second-order model is the nest model to predict the kinetic mechanism for the adsorption of morin on silica nanoparticles. The thermodynamic data revealing a spontaneous process, exothermic and the negative value of entropy indicates that the organization of the adsorbate at the solid/solution interface becomes less random. Moreover, among the different constituents studied, benzalkonium chloride showed the most effect on the release of morin. On the other hand, carbomer was the only viscosant agent studied that presented incompatibilities due to the nature of charge.

Antioxidant efficiency of flavonoids adsorbed on nanoparticles, specifically against singlet oxygen attack follows the next order: morin> quercetin> rutin> diosmin> hesperidin> hesperetin

P25

Role of Nrf2 in the antioxidant response of esculetin-induced apoptosis in human leukemia NB4 cells.

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Esculetin (6,7-dihidrocoumarin) is an antioxidant compound with antitumour properties. We have previously demonstrated an apoptotic action of esculetin on human acute promyelocytic leukaemia NB4 cells [1] that could be related with imbalance of redox equilibrium. Nrf2 is a transcription factor that regulates expression of many detoxification or antioxidant enzymes and, consequently, can activate antioxidant-defense genes and promote survival. In addition, phosphorylation of Nrf2 by stress kinases such as c-Jun NH2-terminal kinase (JNK) or p-38 MAPK is assumed to facilitate its translocation to the nucleus. In order to know the involvement of Nrf2 transcription factor in NB4 cells antioxidant response presumibly elicited by esculetin, we have analyzed the possible correlation between superoxide dismutase activity and factor Nrf2 levels. Apoptosis induction by esculetin on these cells correlated well with a reduction of metabolic activity in a concentration- and time-dependent manner. Moreover, a significant increase of Nrf2 in the nucleus of NB4 cells treated with 100 μM esculetin for 19 hours correlates with the observed increased superoxide dismutase (SOD) enzymatic activity. In addition, higher levels of SOD were observed as studied by Western-blot analysis. Since we observed increased activation of stress kinases such as JNK and p-38 MAP kinases, an induction of antioxidant cellular response can be also inferred. The phosphorylated forms of these kinases appeared significantly increased after treatments with either 100 or 500 μM esculetin for 19 hours. All these data could support a relevant role for Nrf2 as a factor involved in the response to changes in the redox state in the apoptosis induced by esculetin in human leukemia NB4 cells.

[1] V. Rubio et al. Chemico-Biological Interactions 220 (2014) 129-139.

P26

Effect of dietary polyphenols on metal homeostasis and lipid oxidation of murine liver.

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Phenolic compounds from plants present well-studied beneficial effects on human health, which are mediated by different mechanisms, such as anti-oxidation, control of stress-related pathways, and metal chelation. In this regard, there is scarce information about effects of phenolic intake on organic levels of biometals, which are important physiological regulators. Thus, the aim o our work was to assess hepatic bioaccumulation of Cu, Fe and Zn (by XRF) after oral consumption (0-30 days) of phenolic-containing infusions (100 mg/kg/day) from South American plants, and lipoperoxidation (LHP) in BALB/C mice. Also, hepatic phenolic level was measured by the Folin-Ciocalteau method, with infusions of *L. grisebachii (LG)*, *A. quebracho-blanco* (AQB) and *I. paraguariensis* (IP) which were compared ($p<0.05$). Zn was decreased in males by IP and AQB, whereas Cu was increased by IP, AQB and LG. Fe was not affected. In females, Zn was increased by the three treatments. Male liver showed elevated phenolics and LHP after IP treatment. Therefore, there was a sex-dependent response, with females showing a better profile, whereas males were negatively affected by phenolic overload, leading to Zn depletion, Cu accumulation and subsequent oxidation. Further studies are encouraged given the high intake of IP by South American people and its potential deleterious effects.

P27

Effect of *XDH* (xanthine oxidoreducatse) and *SLC2A9* (uric acid and glucose transporter) gene polymorphisms on the concentration of serum uric acid is probably sex specific.

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Background: Uric acid is an antioxidant, which scavenges free radicals in the body. Uric acid is formed from hypoxanthine by the enzyme XDH. The SLC2A9 is a transport protein for uric acid and glucose. We tested whether polymorphisms in *XDH* and *SLC2A9* affect the concentration of uric acid in healthy subjects.

Subjects and methods: We recruited 165 subjects in this study. Fasting blood samples were collected in serum vials from all subjects. The concentration of serum uric acid was measured with COBAS, and genotyping of *XDH* (rs206812) and *SLC2A9* (rs1014290) was done with PCR methods.

Results: The concentration of serum uric acid was 297±70 µmol/L (mean±SD) in all subjects. The subjects with *AA* genotype in *XDH* had serum uric acid concentration 301±70*, GA 298±79* and *GG 288±53* µmol/L (mean±SD) (p=0.28). The polymorphisms in *SLC2A9*, *AA* 299±65, *AG* 295±80 and *GG* had serum uric acid 267±73 µmol/L respectively (mean±SD), (p=0.32). Nevertheless, there was a significant difference in the concentration of uric acid between men and women, *SLC2A9* polymorphisms: in women with *CT CC* the concentration of uric acid was 257±52 and in men 350±88, and in women with *TT,* the concentration of uric acid was 283±60 vs men 347±58 (p=0.039). Similarly, in *XDH*, women with *AA* had uric acid 270±53 vs men 369±53, and women with *GA* 277 vs men 349 and women with *GG* 282±60 vs men 308±25 µmol/L (mean±SD), (p=0.025).

Conclusion: Our preliminary findings suggest that effect of *XDH* and *SLC2A9* gene polymorphisms on concentration of serum uric acid is due to sex differences.

P28

Chemometric techniques for predicting the antioxidant activity of molecular entities

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INTRODUCTION: Nowadays there is a growing interest in oxidative stress, which is associated with a variety of pathologies, and the search for new antioxidant molecules.

AIM: To design a model based on Molecular Topology using molecular descriptors to predict antioxidant activity of target molecules.

MATERIALS AND METHODS: A group of 91 and 98 molecules with and without known antioxidant activity respectively were selected. 326 molecular descriptors were chosen to characterize antioxidant capacity using PaDEL-Descriptor software. Data was processed by artificial neural networks of the statistical software SPSS. 70% of the molecules were used to create the model and 30% to validate it. Results were compared with the calculations done before with R, an open statistical access software. The data base used was the 2013 Spanish Catalogue of Drugs. Lipid peroxidation inhibition of target molecules was determined by measuring MDA concentration with an HPLC system and total antioxidant capacity (TAC) was measured by the ELISA kit from Cayman-chemical™. Antioxidant capacity was expressed in TEAC (Trolox Equivalent Antioxidant Capacity) units.

RESULTS: The best computational neural network model was a fully-connected, feed-forward method with 326−10−2 architecture. The training group and the test group obtained 97% and 93% of successful results respectively with the use of SPSS. These groups obtained 96% and 91% of successful results respectively with the use of R. 54 potential antioxidant drugs were detected by both methods. We observed a significant decrease in MDA production of 15.2% by nystatin, 15.8% by captopril and 1.9% by atenolol. Furthermore, nystatin showed a TAC of 0.72 TEAC, captopril 0.64 TEAC, atenolol 0.10 TEAC, hydroquinone 0.96 TEAC, doxycycline 0.93 and azelaic acid 0.51.

CONCLUSIONS: The use of chemometric methods such as lineal discriminant analysis and artificial neural networks are good tools to predict the antioxidant activity of new molecular entities.

P29

Prevention of UVB-induced Oxidative Stress and DNA damage in human keratinocytes by a combination of citrus and rosemary extract

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UV radiation is the major cause of a variety of cutaneous disorders including photoageing and skin cancers. In recent years, an increase in the use of botanicals with antioxidant and anti-inflammatory properties as skin photoprotective agents is emerging. In the present study, a combination of rosemary extract and citrus bioflavonoid extract has been challenged to inhibit UV harmful effects using human keratinocytes and human volunteers.

The protection study was performed determining the viability of HaCaT cells exposed to UVB and their level of DNA damage through MTT and Comet assays. In addition, we evaluated the antioxidant activity and attenuation of oxidative stress on UVB-irradiated cells by the combined extract. Finally, we carried out a human trial, inducing erythema in volunteers, and assessed the skin protective effect against UV radiation.

The combination of the extracts exhibited higher protective effects on cell survival upon UV radiation than the individual extracts. The combined extract also showed the capacity of scavenging intracellular radical oxygen species at non-cytotoxic concentrations. The extract also decreased UV-induced DNA damage as shown by the comet assay. In a pilot trial, the oral daily consumption of 250 mg of the combination in human volunteers revealed a significant UV protection after 57 days, through the increase of the minimal erythema dose (MED) upon UV radiation. A second human study on 90 volunteers revealed a better performance on MED increase, skin elasticity and wrinkle decrease as soon as 15 days consumption of only 100 mg of the supplement.

These results indicate that the combination of citrus flavanones and caffeoyl derivatives from rosemary may be considered as an ingredient for oral photoprotection and skin antiaging. The implication of the antioxidant capacity in its molecular mechanism may deserve further attention.

P30

Prevention of UVB-induced Oxidative Stress and DNA damage in human keratinocytes by lemon balm polyphenols

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Solar ultraviolet (UV) radiation, especially UVB (290 nm-320 nm) component, causes DNA damage, pyrimidine dimmers, 8-hydroxy-2´-deoxyguanosine (8-OHdG), p53 induction, protein oxidation and generation of reactive oxygen species (ROS). UVB radiation, absorbed by the epidermis, is the major cause of a variety of cutaneous disorders including photoageing and skin cancers. In recent years, an increase in the use of botanicals with antioxidant and anti-inflammatory properties as skin photoprotective agents is emerging. Some polyphenols show the ability to protect the skin from the adverse effects of UVB radiation, including the risk of skin cancers.

This study evaluated the protective effects of lemon balm extract (LBE) (*Melissa Officinalis*, L) and its main phenolic compound, rosmarinic acid (RA), against UVB-induced damage in human keratinocytes.

The composition of LB was determined by HPLC analysis coupled to photodiode array detector and electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS/MS). Rosmarinic acid derivatives were the major compounds and some flavones, isoflavones, bisresorcinol, naphthodianthrone and anthocyanin structures were also found. LB and RA significantly inhibited the decrease of UVB-induced cell viability on irradiated cells, but LB showed stronger effect. In addition, treatment with LB suppressed UVB-induced ROS production and also reduced DNA damage by Comet assay. However, the putative molecular mechanism deserves further attention.

In conclusion, these results suggest that LBE may have better potential for photoprotective effects of human skin against UVB-induced damage than purified rosmarinic acid. This suggests that other components of LBE, probably flavones, might act synergistically with rosmarinic acid in their protective action.

P31

Extract from both orange and bergamot juices protect human lung epithelial A549 cells from hydrogen peroxide-induced oxidative stress

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The oxidant/antioxidant imbalance has been proposed to trigger cell damage that in turn causes several lung diseases. Flavonoids are known for their health benefits, and the *Citrus* fruits juices are one of the main food sources of these secondary plant metabolites.

The present study was designed to evaluate the flavonoid fraction's effect of both bergamot and orange juices, against the H_2O_2-induced oxidative stress in human lung epithelial A549 cells.

First we tested the antioxidant properties of both extracts in cell-free experimental models and then we assayed their capability to prevent the cytotoxic effects induced by H_2O_2.

The results demonstrated that both *Citrus* juice extracts reduce reactive oxygen species generation and membrane lipid peroxidation, improve mitochondrial functionality and prevent DNA-oxidative damage in A549 cells incubated with H_2O_2.

Our data indicate that the flavonoids mixtures present in both bergamot and orange juices may be useful to prevent oxidative cell injury, opening the way for further study on a novel healthy approach to avoid lung disorders.

P32

Flavonoids in microheterogeneous media, relationship between their relative location and their reactivity towards singlet oxygen

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There are several studies determining reactivity of flavonoids with singlet oxygen in heterogeneous media, but there are no reports in biological systems as membranes. In this work, the relationship between the molecular structure of three flavonoids (kaempferol, quercetin and morin), their relative location, their relative localization in liposomes (POPC, DPPC and mixtures) and in erythrocyte membranes and their reactivity against singlet oxygen was studied.

Results for log D determination show that quercetin and kaempferol have similar hydrophobicity, due to their structural similarities. However, morin, sharing these similarities in structure, has a lower value. Differences of quercetin and morin could be due to the OH group increased interaction of n-octanol with the neighboring hydroxyl in the B ring of quercetin, which results in increased solubility of this flavonoid in n-octanol.

The changes observed in membrane fluidity induced by the presence of flavonoids and the influence of their lipophilicity/hydrophilicity on the antioxidant activity were evaluated by means of fluorescent probes such as Laurdan and diphenylhexatriene (DPH). The small differences observed for the value of generalized polarization of Laurdan (GP) curves in function of the concentration of flavonoids, indicate that these three compounds promote similar alterations in liposomes and erythrocyte membranes. In addition, these compounds do not produce changes in fluorescence anisotropy of DPH, discarding their location in deeper regions of the lipid bilayer.

The determined chemical reactivity is similar in all the studied media (kaempferol < quercetin < morin). The hydroxyl moiety in R_2' makes morin reactivity to be approximately 10 times higher than quercetin and 20 to 30 times greater than kaempferol, depending on the medium. The flavonoids studied show a similar magnitude of their total rate constants, having kaempferol lower value ($2.1x10^7$ $M^{-1}s^{-1}$), followed by quercetin ($4.3x10^7$ $M^{-1}s^{-1}$), and finally morin shows the highest value ($14.4x10^7$ $M^{-1}s^{-1}$).

P33

Effect of gene polymorphisms on the concentration of total glutathione in plasma from healthy subjects.

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Background: Glutathione (GSH) is an antioxidant, which is utilized by GSH peroxidase (GPX) and GSH transferase (GST) to neutralize hydrogen peroxide and lipid peroxides in the body. During these reactions GSH is oxidized (GSSG), which is reduced back to GSH by the enzyme GSH reductase (GSR). Data on the effect of gene polymorphisms on the concentration of total GSH in plasma is limited therefore we investigated the effect of gene polymorphisms on the concentration of GSH in plasma from healthy subjects.

Subjects and methods: We recruited 126 subjects in this study. Fasting blood samples were collected in heparin vials from all the subjects. The concentration of plasma GSH was measured with an HPLC method. The genotyping of GSH enzyme genes was done with PCR methods.

Results: The concentration of GSH in plasma in healthy subjects was 4.1±1.3 µmol/L (mean±SD). Subjects with *GSRint3 (A>G) (rs2978663)* polymorphisms, AA, AG and GG had plasma GSH concentration 4.2±1.3 (n=68), 4.2±1.2 (n=56) and 3.5±1.2 (n=22) µmol/L (mean±SD) respectively (p=0.06). Similarly, the concentrations of plasma GSH were not significantly different in individuals with gene polymorphisms *GPX1(C>T) (rs1050450)*, CC, CT and TT, 3.9±1.1 (n=63), 4.4±1.5 (n=44), 3.7±1.2 (n=8) (p=0.3). Subjects with polymorphisms in GSH transferase, *GSTM1* (n=80) and *GSTM1-* (n=46) had plasma GSH concentrations 4.1±1.2 vs 4.0±1.3 µmol/L (mean±SD), (p=0.4) and the subjects with *GSTT1-* (n=17) had 3.9±1.0 vs *GSTT1 (n=109)* plasma GSH concentration 4.1±1.3 µmol/L (mean±SD) (p=0.9).

Conclusion: Our preliminary findings suggest that the studied GSH gene polymorphisms do not affect the concentration of GSH in plasma from healthy subjects

P34

Cytoprotective effect of the polyphenol quercetin against the cytotoxicity of *Alternaria* mycotoxins in Caco-2 cells.

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Molds of the genus *Alternaria* have been reported as contaminants of a variety of food and feed such as cereals, vegetables and fruits. Thereby *Alternaria ssp*. is able to produce mycotoxins under a wide range of environmental conditions. Alternaria toxins such as alternariol (AOH) and its naturally occurring monomethylether (AME) produce oxidative stress in cell culture and possess genotoxic and mutagenic properties. On the other hand, it has been proved that natural polyphenols have antioxidant effect. Quercetin is a polyphenol present in berries and other commodities. It exhibits antioxidant, antitumoral, anti-inflammatory, antiplatelet, and vasoprotector activities. The objectives of this study were to evaluate the cytotoxicity of AOH and AME, and the cytoprotective effect of quercetin exposed simultaneously with AOH, AME and the mixture AOH plus AME in human adenocarcinoma (Caco-2) cells. Concentrations ranged from 0.3125 to 100 μM for individual and binary (quercetin plus AOH and quercetin plus AME) mixtures. For the tertiary (AOH, AME and quercetin) mixture, the concentration ranged from 0.3125 to 50 μM. The cytotoxicity was determined by the MTT test after 24 and 48 h of exposure. Individual cytotoxicity assays decreased cell viability after 48 h of AOH and AME exposures. Quercetin showed cytoprotective effect when cells were exposed to AOH and AME. The protective effect of cells against quercetin depends on a balance between antioxidants and pro-oxidant components. Increased levels of antioxidants in food commodities may help to regulate oxidative damage.

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P35

Five-membered nitroxyl derivatives and anticancer drugs synergize in human breast cancer cells

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Nitroxides is a group of stable free radicals possessing one or more nitroxyl groups. Currently under extensive research is the utility of nitroxides in supporting radiotherapy and chemotherapy of cancer.

The aim of our study was to determine the type of interaction between the two nitroxyl derivatives (3-carbamoyl-dehydroPROXYL, 3-carbamoyl-PROXYL) and three anticancer drugs commonly used in the treatment of breast cancer (doxorubicin, paclitaxel, docetaxel). We also evaluated the impact of nitroxide on the inhibition of depolymerization of microtubules, which is the basal activity of the taxane drugs. The study was conducted on two breast cancer cell lines, MCF-7 and MDA-MB-231.

Analysis of drug drug interaction was performed by the Chou-Talalay method using Campusyn software. Cells were treated with drug at IC50 concentrations in combination with 50 μmol/l 3-carbamoyl-dehydroPROXYL/3-carbamoyl-PROXYL. Cells were pre-incubated time with nitroxides for 1 h and treated with drugs in presence of nitroxides for 2 h. After incubation the medium was removed and the cells were cultured for 72 h. Then he MTT assay was performed and data were subjected to combination analysis. Condition of microtubules was assessed by fluorescence microscopy using fluorescent monoclonal antibodies directed against the β-tubulin.

Both nitroxides have synergistic activity with doxorubicin and docetaxel in MCF-7. In the case of MDA-MB-231 cells nitroxides had synergistic activity only in cells treated with docetaxel. Paclitaxel had synergistic activity only with cells preincubated with 3-carbamoyl-dehydroPROXYL. Microscopic observations showed no influence of the nitroxides on the activity of taxanes in inhibiting depolymerization of microtubules.

In conclusion, 3-carbamoyl-dehydroPROXYL and 3-carbamoyl-PROXYL seem to be promising for chemotherapy, exhibiting no cytotoxic properties by themselves but enhancing the antitumor effect of doxorubicin, paclitaxel and docetaxel.

P36
Protein glycation by ascorbic acid

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The aim of the study was to compare the effects of ascorbic acid (AA) *in vitro* in the absence and in the presence of cell-dependent recycling. In a cell-free system, AA enhanced glycoxidation of bovine serum albumin (BSA) by glucose and induced BSA glycation in the absence of sugars. On the other hand, AA did not affect erythrocyte hemolysis, glycation of hemoglobin and erythrocyte membranes and inactivation of catalase, protected against inactivation of erythrocyte membrane acetylcholinesterase and enhanced the loss of glutathione in erythrocytes incubated with high glucose. These results can be explained by assumption that AA acts as a proglycating agent in the absence of recycling while is an antiglycating agent when metabolic recycling occurs. AA-induced glycation of BSA in vitro was prevented by antioxidants (glutathione, captopril, polyphenols) but not by nitroxides which are known to oxidize ascorbic acid. These findings are in agreement with the view that protein glycation is induced but product of AA oxidation but not by AA itself.

P37

Efficacy of topical formulations containing *Azadirachta indica* (neem) extract against UVB-induced oxidative stress in hairless mice

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The skin is continuously exposed to a combination of environmental insults including UV radiation and high oxygen concentration, which constantly jeopardizes the integrity of cellular oxidizable structures of the skin. In this regard, antioxidants from natural sources present novel possibilities for the treatment and prevention of oxidative stress-mediated skin diseases. *Azadirachta indica*, popularly known as neem, presents various biological activities and pharmacological actions such as antiviral, antibacterial, antifungal, anti-inflammatory, antipyretic, antiseptic and antioxidant. Therefore, in the present study it was evaluated *in vivo* the photochemioprotective effect of two topical formulations (non-ionic emulsion with high lipid content and anionic emulsion with low lipid content) containing neem extract (NE). The skin oxidative stress was induced in hairless mice by UVB irradiation and the protective effect of NE was determined by skin edema and the cutaneous antioxidant capacity using ABTS and FRAP methods. Only the formulation with high lipid content containing NE showed ability to inhibit edema and decreased antioxidant capacity of the skin caused by UV-B irradiation. The present results suggest that formulations containing NE may be a topical source of antioxidant compounds to decrease oxidative damages of the skin and the type of emulsion used can influence the antioxidant effectiveness of NE.

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P38

Free radical scavenging capacity, polyphenolic content and bioactivity of extracts derived from *Cistus salviifolius* and *Thymus moroderi.*

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Dietary polyphenols are a source for antioxidant compounds which may prevent the impact of stress oxidative-related pathologies. Previous research has reported the polyphenolic composition and the antioxidant capacity of extracts derived from species well adapted to the Mediterranean area such as *Cistus salviifolius* (*Cistus* genus) and *Thymus moroderi* (*Thymus* genus).

In the present study, the extraction procedure of the aerial part of these two plants has been optimized to maximize their polyphenolic content and antioxidant capacity through a guided multistep protocol. The extactions were performed using solvents with different polarities (water, acetone, ethyl acetate and methanol) and extraction time and plant/solvent ratio was varied in order to maximize polyphenolic content and antioxidant capacity.

The composition of the extracts was also characterized by using high performance liquid chromatography coupled to electrospray ionization and ion trap mass spectrometry (HPLC-ESI-MS/MS). Main compounds of the extracts bearing the highest polyphenolic content and antioxidant activity were identified. A principal component analysis was performed on the polyphenolic composition and the antioxidant capacity in relation to the different extraction procedures. *Cistus salviifolius* extract was assayed in a murine 3T3-L1 preadipocytes differentiation model in high glucose conditions.

The results show that *Cistus salviifolius* polyphenolic extract inhibits triglyceride accumulation and glucose-induced metabolic stress which may lead to the development of food ingredients with potential applications in the management of obesity.

P39
5-FU and new fluorinated uracil derivates which inhibit lipid peroxidation

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INTRODUCTION: There is a current controversy about the beneficial use of antioxidants combined with antineoplastic therapy because of the possible interference with its mechanism of action (Papaioannou D. et al, 2011; Fu Y. et al, 2014). A widely antineoplastic drug used against colorectal cancer is the fluorinated uracil molecule 5-Fluorouracile (5-FU).

AIM: The purpose of our research is to study de antioxidant capacity observed by the inhibition of lipid peroxidation by 5-FU and other fluorinated uracil derivates.

MATERIALS AND METHODS: Target molecules studied were 5-FU and 6-[Difluoro(phenyl) methyl]-3-(2,4,6-trifluorophenyl)pyrimidine-2,4(1H,3H)-dione (S-81), 3-[2-Chloroethyl]-6-[difluoro(phenyl)methyl]pyrimidine-2,4(1H,3H)-dione (S-86),6-[Difluoro(phenyl)methyl]-3-(4-trifluoromethoxyphenyl)pyrimidine-2,4(1H,3H)-dione (S-123).

Lipid peroxidation inhibition was determined by measuring MDA concentration with an HPLC system: 5 ml of liver homogenate, obtained from C57BL/6 mouse liver and adjusted to a final protein concentration of 12 mg/mL, were placed in bottles. A range of concentrations between 0 and 10 μM of fluorinated uracil derivates and peroxidation inducers were added to the homogenates and incubates at 37ºC during 2 h. After incubation homogenates were stored at -80ºC until MDA analysis. Total antioxidant capacity (TAC) of 5-FU was measured by the ELISA kit from Cayman-chemical™. Antioxidant capacity was expressed in TEAC (Trolox Equivalent Antioxidant Capacity) units.

RESULTS: We have observed a significant decrease in MDA production of 58.12% in S-86, 44.61% in S-123, 24.11% in 5-FU and 10.83% in S-81 in the sample with highest concentration (10uM). 5-FU also showed a TAC of 0.68 TEAC.

CONCLUSIONS: 5-FU and other fluorinated uracil derivates have a significant antioxidant capacity. Specifically, the antineoplastic 5-FU has both biological and antioxidant activity *per se.*

INFLAMMATION (P40-P57)

P40

Lipid-Induced oxidative stress and inflammation in brain microvascular endothelial cells

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Elevation of blood triglycerides, primarily triglyceride-rich lipoproteins (TGRL), is an independent risk factor for atherosclerotic cardiovascular disease. The accumulating evidence indicates that the development of atherosclerosis and vascular dementia are linked to vascular inflammation. Our previous investigation has shown that TGRL lipolysis products have both pro- and anti-inflammatory effects on endothelium.

Brain microvascular endothelial cells critically contribute to brain development and homeostasis besides their role in maintaining the anatomical and functional integrity of the blood-brain barrier (BBB) and hemodynamic of blood flow. The injuries to the brain microvasculature can have deleterious consequences on the neurovascular unit and play an important role in the pathogenesis of several neurological disorders. The mechanisms and impact of lipid-induced injury on neurovascular inflammation remain unknown.

To address this question, we treated human brain microvascular endothelial cells with TGRL lipolysis products derived from human postprandial TGRL by lipoprotein lipase (LpL). Superoxide radical generation was analyzed in supernatant by ESR and in mitochondria by MitoSOX red and MitoTracker green at 15 mins. Lipolysis-iinduced oxidative stress (SOD-2, HOX-1, COX-2), inflammatory (E-selectin, ICAM-1, CXCL3, KLF4) and stress response (ATF3 and ATF4) genes expressions at 3 h. Additionally, TEM analysis also showed that elongated tubules shape to donut/Blob shape of mitochondrial after treatment for 3h. Moreover, increased expression of mitochondria apoptosis protein Caspase-9 and Caspase-3 activity was also observed by treatment with TGRL lipolysis products. Our data demonstrated that the mechanism of TGRL lipolysis products activates the production of mitochondrial ROS that possibly results in induction of pro-inflammatory signaling in brain. In summary, reactive oxygen species and pro-inflammatory lipid mediators released from endothelial cells are implicated in endothelial cell physiological responses.

P41

Protective effects of conditioned media from adipose stem cells on human monocytes

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INTRODUCTION: In the inflammation process, reactive oxygen species are early released by monocytes. Adipose-derived mesenchymal stem cells (ASC) act as a cellular source of soluble factors exerting immunomodulatory effects on other cells. The purpose of this study was to investigate whether the conditioned medium from human adipose-derived mesenchymal stem cells (ASC-CM) improves the oxidative and inflammatory response in stimulated human monocytes.

METHODS: Mononuclear cells were isolated by density gradient from human peripheral blood and monocytes were isolated by adherence. Monocyte phenotype was analyzed by flow cytometry and confocal microscopy with specific antibodies: anti-CD45-FITC, anti-CD14-PE and anti-CD105-PE. Cellular viability was assesed with propidium iodide. Adipose tissue from patients subjected to abdominal lipectomy was used for ASC isolation. Cells were incubated in DMEM/F12 containing 15% human serum to confluence and ASC-CM was collected after 48h of culture. Isolated monocytes were stimulated with TPA (tetradecanoilforbol acetate) or LPS (lipopolisaccharide). Mitochondrial membrane potential was monitored using JC-1. Myeloperoxidase activity was determined by spectrophotometric procedures and prostaglandin E_2 (PGE_2) by RIA.

RESULTS: Our results indicate that cell population had a phenotype of monocyte cells with ≥ 95% CD45 , CD14 and CD105. After TPA treatment, we observed an increased myeloperoxidase release. We have evaluated the effects of ASC-CM on monocyte functions at 3 and 24h. ASC-CM significantly reduced myeloperoxidase levels and normalized mitochondrial membrane potential. The production of inflammatory mediators was induced by LPS stimulation of monocytes for 24h. In these experiments, ASC-CM significantly reduced the levels of PGE_2.

CONCLUSIONS: The findings of the present study show that ADSC-CM treatment results in early down-regulation of oxidative and inflammatory responses, and preserve human monocyte cells viability and function.

P42

Novel inflammatory biomarkers revealed from proteomic analysis of TGRL lipolysis product-induced exosomes from human brain microvascular endothelial cells

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Exosomes are 40-100 nm diameter endocyticnanovesicles, present in many body fluids, and are secreted by mammalian cells under normal and pathological conditions. Exosomes are involved in cell-to-cell communication and protein, and RNA delivery, which regulate biological processes of target cells.

Proteins contained within exosomes hold great promise as novel biomarkers for clinical diagnostics. The traditional methodology for clinical diagnostics is the measurement of soluble markers in plasma/serum by ELISA techniques. Importantly, many target proteins in clinical diagnostics may not be truly soluble, but may instead be released on exosomes. Therefore, we employed LC-MS/MS quantitative proteomics to identify the novel biomarkers of neurovascular inflammation from isolated exosomes.

Conditioned media was collected from human brain microvascular endothelial cells (HBMVEC) treated with TGRL lypolysis products (TL) or non-treated cells. Exosomes were isolated by ultracentrifugation from conditioned media of HBMVEC treated or media and proteins were identified by LC-MS/MS in a bottom-up proteomic workflow.

LC-MS/MS analysis identified kinesin-like motor proteins, adhesive glycoproteins, zinc finger transcriptional repressor protein, and blood zymogens in exosomes harvested from non-treated HBMVEC. The proteomic composition reflected a normal homeostasis state of HBMVEC. However, the generation of intracellular exosomes increased in HBMVEC after 3 hours of TL treatment. The accumulation of exosomes was confirmed by EM and immunofluorescence. MS data of exosomes from HBMVEC treated with TL revealed several potential inflammation markers, including: inflammatory protein, endothelial cell polarity protein, inflammatory monocyte trafficking, extracellular matrix adhesion proteins, pathologic enzyme for creating fibrosis, and clearance of peptides from the brain.

Thus, exosomal proteomes may represent novel diagnostic tools to determine the state of neurovascular diseases.

P43

Peroxiredoxin II negatively regulates lipopolysaccharide-induced osteoclast formation and bone Loss *via* JNK and STAT3

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Aims: Lipopolysaccharide (LPS) is considered a prominent pathogenic factor in inflammatory bone diseases. LPS challenge contributes to the production of reactive oxygen species (ROS) in diverse inflammatory diseases. However, its mechanism remains to be clarified in bone. Thus, we investigated the critical mechanism of ROS in LPS-induced osteoclastogenesis and bone loss. ***Results:*** Antioxidants prevented LPS-induced osteoclast formation *via* inhibition of nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) and c-Fos expression in preosteoclasts. Moreover, LPS-induced osteoclast formation *via* ROS was attenuated by treatment with c-Jun N-terminal protein kinase (JNK) inhibitor. Interestingly, LPS also activated signal transducer and activator of transcription 3 (STAT3), which is suppressed by antioxidants. We found that knockdown of STAT3 or use of a STAT3 inhibitor resulted in a significant reduction in interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and nitric oxide (NO) production, followed by decreased osteoclast formation by LPS. Peroxiredoxin II (PrxII) is a member of the antioxidant enzyme family, and it plays a protective role against oxidative damage caused by ROS. In our study, ROS production and osteoclast formation by LPS was significantly enhanced in $PrxII^{-/-}$ cells. Moreover, JNK-mediated c-Fos and NFATc1 expression was promoted in $PrxII^{-/-}$ cells. Furthermore, STAT3 activation and accompanying IL-1β, IL-6, and NO production was also increased in $PrxII^{-/-}$ cells. Consistent with the *in vitro* result, PrxII-deficient mice showed increased osteoclast formation and bone loss by LPS challenge compared with wild-type mice. ***Innovation:*** For the first time, we showed that LPS-induced ROS signaling is dependent on the coordinated mechanism of JNK and STAT3 during osteoclastogenesis, which is negatively regulated by PrxII. ***Conclusion:*** We suggest that PrxII could be useful in the development of a novel target for inflammatory bone loss.

P44

Mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglia cells by regulating MAPK and NF-κB pathways

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Activation of microglia cells in the brain contributes to neurodegenerative processes promoted by many neurotoxic factors such as pro-inflammatory cytokines and nitric oxide (NO). Reactive oxygen species (ROS) actively affect microglia-associated neurodegenerative diseases through their role as pro-inflammatory molecules and modulators of pro-inflammatory processes. Although the ROS involved in microglia activation are thought to be generated primarily by NADPH oxidase (NOX) and are involved in the immune response, mitochondrial ROS have also been proposed as important regulators of the inflammatory response in the innate immune system. However, the role of mitochondrial ROS in microglial activation has yet to be fully elucidated. In this study, we demonstrate that inhibition of mitochondrial ROS by treatment with Mito-TEMPO effectively suppressed the level of mitochondrial and intracellular ROS. Mito-TEMPO treatment also significantly prevented LPS-induced increase in the TNF-α, IL-1β, IL-6, iNOSand Cox-2 in BV-2 and primary microglia cells. Furthermore, suppression of LPS-induced mitochondrial ROS generation not only affected LPS-stimulated activation of MAPKs, including ERK, JNK, and p38, but also regulated IκB activation and NF-κB nuclear localization. These results indicate that mitochondria constitute a major source of ROS generation in LPS-mediated activated microglia cells. Additionally, suppression of LPS-induced mitochondrial ROS plays a role in modulating the production of pro-inflammatory mediators by preventing MAPK and NF-κB activation in microglia cells. Our findings suggest that a potential strategy in the development of therapy for inflammation-associated degenerative neurological diseases involves targeting the regulation of mitochondrial ROS in microglial cells.

P45

Effect of selected milk thistle polyphenols on the oxidant response of neutrophils and myeloperoxidase (MPO) activity

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Silymarin, a standardised extract of *S. marianum*, and its constituent polyphenols, are known hepatoprotectants. Recent studies highlight their anti-cancer and anti-inflammatory effects. We investigated the effects of some molecules found in silymarin: silybin (S), taxifolin (T), quercetin (Q), and dehydrosilybin (DHS) on ROS production and MPO release by stimulated neutrophils and on MPO activity through the lens of structure-activity relationships.

Each compound was tested at a range of concentrations between 100nM and 100**µ**M on the inhibition of i) superoxide anion production monitored by chemiluminescence (CL) and MPO release by PMA activated neutrophils, ii) purified MPO activity by classic and SIEFED assays, and iii) OH radical formation by Fenton reaction as monitored by spin-trap EPR.

All compounds inhibited the superoxide anion production by stimulated neutrophils in a dose depend manner. DHS and Q inhibited CL response and MPO release from 10 **µ**M. MPO activity as measured by the classical assay showed Q as the most potent inhibitor, followed by T, DHSB and S. The SIEFED assay highlighting a link between a tested molecule and MPO showed that only Q and T maintained an efficient inhibition above 90% at 10 µM. EPR experiments showed that Q is the most potent radical scavenger of the compounds tested.

Our results showed a strong inhibition on PMN activation by the planar compounds Q and DHS and a strong inhibition of MPO activity by the smallest molecules (Q and T). Only Q reduced levels of OH radicals as shown by EPR. The planar compounds could favour a better interaction with membrane proteins involved in PMN activation, while the smaller flavonoids have less steric hindrance, allowing a tighter interaction with MPO. In conclusion, the compounds from silymarin, may be useful for modulating the excessive oxidative response of neutrophils, but further *in vivo* studies are needed.

P46

Trans-chalcone, a flavonoid precursor, inhibits UVB-induced skin inflammation and oxidative stress in mice by targeting NADPH oxidase and cytokine production

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The anti-inflammatory and antioxidant activities of a wide variety of plant polyphenols have been reported, and supplementation with flavonoids has been shown to prevent UVB irradiation-induced skin damage. In the present study, the *in vitro* antioxidant activity and anti-inflammatory and antioxidant mechanisms of systemic administration with trans-chalcone (TC), a flavonoid precursor, on UVB-induced skin inflammation and oxidative in mice were investigated. Hairless mice were divided in groups (n=5): non-irradiated control, irradiated control (4.14 J/cm^2) and three group of irradiated and treated with TC (10-300 mg/kg, ip). This study was approved by the Ethics Committee on Animal Research of UniversidadeEstadual de Londrina (Process no. 3344.2012.08). Data were statistically analyzed by one-way ANOVA followed by Tukey's*t* test, $p<0.05$. Systemic treatment with TC prevented skin inflammation by reducing neutrophil recruitment (25-75%) and skin edema (47-100%), and also inhibited matrix metalloproteinase-9 activity (18-71%). TC also inhibited gp91phox (95%) mRNA expression, oxidative stress (GSH: 37-60%; catalase: 68-100%; lipid peroxidation: 59-77% and superoxide anion production: 61-100%), and a wide range of cytokines production (TNF-α: 85%; IL-1β: 54%; IL-6: 66% and IL-17: 55.5%). However, TC did not presented antioxidant activity by ABTS (electron transfers), DPPH (H transfer), iron chelation and superoxide anion (free radical biological) assays *in vitro*. These results can be explained because TC does not posses classical antioxidants groups in its structure such as hydroxyl group. In conclusion, treatment with TC inhibited UVB-induced skin inflammation that resulted in minor oxidative stress *in vivo*, which suggests that systemic supplementation with this compound may represent an important therapeutic approach in inflammatory skin diseases induced by UVB irradiation.

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P47

Quercetin microencapsulated to improve the anti-inflammatory and analgesic effects in rats

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Quercetin presents limited oral bioavailability reducing its per oral therapeutic use. In the present study, quercetin-loaded microcapsules were developed in an attempt to improve its anti-inflammatory and analgesic effects. Morphology of quercetin-loaded microcapsules was evaluated by Scanning Electron Microscopy and size distribution by light scattering. Structural and polymorphic changes were determined by X-ray diffraction and by differential scanning calorimetry, respectively. In carrageenan-induced inflammation, an electronic pressure meter was used to evaluate mechanical hyperalgesia, neutrophil recruitment was evaluated in Neubauer chamber and stained slices, TNF-α and IL-1β levels by ELISA, and total antioxidant capacity by colorimetric assays. Non-microencapsulated quercetin diffractogram showed high crystallinity while microencapsulated quercetin showed mixed crystalline quercetin and amorphous polymer phase. Crystallinity of pure quercetin was 25% higher than microencapsulated quercetin. In contrast to non-microencapsulated quercetin and inert microcapsules, pretreatment with quercetin-loaded microcapsules significantly decrease carrageenan-induced mechanical hyperalgesia, neutrophil recruitment, and TNF-α and IL-1β production. Microencapsulated and non-microencapsulated quercetin prevented carrageenan-induced decrease of total antioxidant capacity in the paw tissue. Therefore, microencapsulation enhanced quercetin anti-hyperalgesic and anti-inflammatory effects due to increased inhibition of cytokine production and oxidative stress. This better therapeutic profile was related to increased amorphous phase of quercetin-loaded microcapsules compared to quercetin.

P48

Antinflammatory and antioxidative effect of quercetin on rat adjuvant arthritis

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Beyond the classical folic acid analogue methotrexate, novel therapies for rheumatoid arthritis have been studied during the last years in the attempt to reduce the side effects of traditional drugs. Natural compounds with antioxidant and anti-inflammatory properties have been largely tested both *invivo* and *invitro*. This study aimed to determine the effects of oral administration of quercetin (QUE) in a rat model of adjuvant arthritis. Arthritis was induced by a single intradermal injection of heat-inactivated *Mycobacterium butyricum* in incomplete Freund's adjuvant. The experimental groups were treated with an oral daily dose of 150 mg/kg b.w. of QUE for 28 days. Markers of oxidative stress and inflammation were assessed in plasma and in selected tissues, such as joint, lung and liver. Results indicated that QUE lowered levels of interleukin-1β, C-reactive protein, and monocyte chemotactic protein-1 and restored plasma antioxidant capacity. In addition, QUE inhibited the enzymatic activity of pro-inflammatory 12/15-lipoxygenase in lung and liver and increased the expression of heme oxygenase-1 in joint and lung of arthritic rats. To better investigate the mechanism of action of QUE, we measured the level of activation of ERK in joint and lung. Results showed that ERK activity is inhibited by QUE. Finally, QUE reduced the 2-fold increase of NF-κB activity observed in lung, liver and joint after induction of arthritis. This study demonstrates that orally administrated QUE ameliorated all markers of inflammation and oxidative stress measured in a rat model of adjuvant arthritis not only in joint tissue and plasma but also in extra-articular tissues such as lung and liver (*Acknowledgement: project VEGA 2/0044/15, SAS-CNR bilateral project coordinated by Dr. Bauerova (Slovakia) and Dr. Russo (Italy) "Phytochemicals in ameliorating rheumatoid arthritis therapy: from preclinical studies to clinical applications."*).

P49

Could an antiinflammatory response be correlated with NFκB changes in esculetin-induced apoptosis of human leukemia NB4 cells?.

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Esculetin (6,7-dihydroxycumarin) is an antioxidant compound that can change redox equilibrium in different cell types. It has also been claimed for esculetin to produce antiinflamatory effects. The NFκB transcription factor is critical in the inflammatory response by controlling expression of genes enconding pro-inflammatory effectors such as enzymes like 5-LOX. The antiinflamatory properties of esculetin levels have been studied in human acute promyelocytic leukemia NB4 cells by measuring 5-lipoxygenase levels in relation to changes in NFκB levels and its localization. These cells were treated with increasing concentrations of esculetin for different times (0.5, 3, 6 and 9 hours). Esculetin decreased metabolic activity of NB4 cells at longer times and as esculetin concentration increased. NFκB p105 levels in the cytosol decreased as time treatment increased and NFκB p105 was not observed in the cell nucleus at any of the used treatment times. The levels of pro-inflammatory NFκB p65 decreased in the nucleus at higher esculetin concentration treatments for longer times. Concomitantly, the levels of anti-inflammatory NFκB p50 increased in the nucleus suggesting a possible formation of inhibitory p50 homodimers that could be related with an antiinflamatory response. Furthermore, lipoxygenase expression was not induced by esculetin treatment as tested by Western-blot analyses. These results could suggest a possible role for NFκB as modulator of antiinflamatory response in apoptosis induced by esculetin in human leukemia NB4 cells.

[1] V. Rubio et al. Chemico-Biological Interactions 220 (2014) 129-139.

P50

Glutathione deficiency attenuates endotoxic fever in rats

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Fever is a common response to infection and inflammation that is beneficial for healing process. Despite the well documented ubiquity of fever, there are clinical reports suggesting a decreased frequency of fever within cancer patients. This finding places the patients in an unfavourable position. The reason of fever inhibition is not known. We hypothesized that it can be an effect of deficiency in glutathione, what is a frequent consequence of oxidative stress observed in many pathological processes.

Purpose: The aim of the present study was to investigate the time-course of LPS-induced fever in rats with glutathione deficiency.

Material and methods: Intraperitoneal injection of endotoxin (LPS) from E. coli was used to provoke fever in Wistar rats. The level of liver glutathione was decreased by administration of phorone – an α,β-unsaturated carbonyl compound. Body temperature (Tb) in free running rats was recorded using biotelemetry system. Moreover, the concentrations of liver glutathione and plasma tumor necrosis factor-α (TNF-α) were estimated.

Results: Animals with decreased glutathione level responded with lower fever after LPS injection (average Tb in Pho/LPS-treated and oil/LPS-treated animals were 36.9 ± 0.11 and 37.8 ± 0.16 degrees Celsius, respectively). This response was accompanied by a significant decrease in LPS-induced TNF-α level (TNF- α in Pho/LPS-treated group was 10.68 pg/ml ± 2.24, versus 113.35 pg/ml ±13.93 in oil/LPS-treated rats). Pretreatment of rats with TNF-α partially restored fever.

Conclusion: Based on our data one may conclude that, glutathione deficiency may be responsible for the inhibition of fever via TNF-α dependent pathway.

P51

Resveratrol activates Nrf2 pathway in cytokine-stimulated human intestinal cells: comparison with 5-aminosalicylic acid

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Introduction: Inflammatory Bowel Disease (IBD) is a chronic and intermittent inflammatory disorder of the gastrointestinal tract, for which no cure has been found so far. Since current treatment options for IBD patients often bring marginal results with significant long-term side effects, it is imperative to find preventive strategies or better targeted therapies effective in preventing IBD progression. Resveratrol is a polyphenolic compound widespread in the human diet, particularly in fruits and red-wine, with recognized beneficial health effects. Our work aimed to compare the antioxidant efficiency of Resveratrol (Resv) with 5-aminosalicylic acid (5-ASA), a first-line therapy of IBD patients, using the HT-29 cell line as a cellular model.

Materials and Methods: HT-29 cells were pretreated with 25 **μ**MResv and/or 500 **μ**M 5-ASA and then exposed to a combination of cytokines (IL-1**α**, TNF-**α**, IFN-**γ**) for a period of time. The GSH/GSSG ratio and the generation of reactive species were evaluated by fluorimetric assays and the mRNA expressions of heme oxygenase-1 and of both subunits of glutamate cysteine ligase were evaluated by RT-PCR. The activity of the transcription factor Nrf2 was assessed by an Elisa-based method.

Results: Our data showed, for the first time, the ability of Resv to induce Nrf2 activation, to increase the mRNA expression of heme oxygenase-1 and of both subunits of glutamate cysteine ligase, to enhance the GSH/GSSG ratio and to decrease the production of reactive species, in cytokine-challenged cells, at a much lower concentration than 5-ASA.

Conclusion: Resveratrol displays superior efficiency as antioxidant than the drug 5-ASA, in our experimental conditions, being able to give complementary benefits in the context of Inflammatory Bowel Disease.

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P52
Liver glutathione and endotoxic fever in aged Wistar rats

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According to published data, the aging of the immune system is probably the major determinant for susceptibility to endotoxin (LPS). Furthermore, ageing is associated with glutathione decline, thereby putting rats at increased risk of succumbing to oxidative stress.

Purpose: The aim of the present study was to investigate the time-course of LPS-induced fever and the level of liver glutathione in aged rats.

Material and methods: Intraperitoneal injection of endotoxin (LPS) from E. coli was used to provoke fever in aged Wistar rats. Body temperature (Tb) in free running rats was recorded using biotelemetry system. Moreover, the concentration of liver glutathione was estimated.

Results: Aged rats revealed natural rhythm of body temperature with low day-time and high night-time temperatures, however these temperatures were significantly lower than those observed in young animals.Total glutathione level was similar in both groups of animals. LPS injection provoked endotoxic fever, which was prolonged compared to fever observed in young rats.

Conclusion: Based on our data one may conclude that, aged rats are still able to react with fever in response to LPS, although natural rhythm of Tb is lower than in young rats. In our experimental conditions, total liver glutathione content did not differ significantly between young and old rats.

P53

γ-Glutamyl cysteine attenuates the inflammatory response in acute pancreatitis *via* protein phosphatases

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Acute pancreatitis (AP) is an acute inflammatory process of the pancreatic gland that may lead to local and systemic complications. Cytokines and oxidative stress play a role in the early pathophysiological events of the disease. Previous studies have shown the antioxidant properties of gamma-glutamyl cysteine (γ–GC), a metabolic precursor for the synthesis of glutathione.

Acute pancreatitis was induced in C57BL/6 mice by seven subcutaneous injections of cerulein (50 μg/kg bw). To evaluate the effects of γ-GC, a group of mice with AP was treated with γ-GC administered in two doses (37.5 mg/kg bw each) at 3 and 6 hours after the first cerulein injection. The induction of AP was confirmed by measuring plasma lipase activity and performing histological studies.

The aim of this work was to evaluate the role of γ–GC in the modulation of the inflammatory response and oxidative stress in PA.

The histopathological study showed that the inflammatory process and tissue edema were reduced with the γ-GC treatment. The increase in pancreatic lipase activity was lower with γ-GC treatment. Moreover, the increase in pancreatic myeloperoxydase activity and the expression of the pro-inflammatory cytokine TNF-α were abrogated in mice treated with γ-GC after AP induction. The c-Jun N-terminal kinase (JNK) pathway activation was blocked upon γ-GC treatment. Protein tyrosine phosphatases SHP1, SHP2 and protein serine threonine phosphatase PP2A were reduced upon AP induction but they were recovered after γ-GC administration. Redox pairs, such as reduced glutathione/ oxidized glutathione, and cysteine/cystine, were not affected by the γ-GC treatment.

In conclusion, our results show the anti-inflammatory properties of γ-GC in acute pancreatitis by avoiding the activation of the JNK pathway and by recovering protein phosphatase levels, independently of the redox thiol status.

P54

The effects of HMG-Co a reductase inhibition on trace elements in endotoxemic rats

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Introduction: It has been known that increased cytokine levels cause metabolic and cytosolic changes in tissues during Lipopolysaccharide (LPS) induced sepsis. Systemic inflammation causes alterations the levels of trace elements in tissues and organs that are involved in immune defense. Trace elements are essential for direct antioxidant activity as well as functioning as cofactors for a variety of antioxidant enzymes. Simvastatin is known as a HMG-CoA reductase enzyme inhibitor that has anti-inflammatory, antioxidant, anti-apoptotic effects. Previous studies have demonstrated changes of trace element levels during sepsis, which were found to increase tissue damage. We aimed to study the effect of simvastatin on trace elements levels in liver tissue in Wistar albino rats with LPS inducing sepsis.

Material/Method: In our study, adult rats (Wistar albino) were divided in control, LPS, Simvastatin (20mg/kg, o.p., 5 days), Simvastatin LPS. LPS was given in a single dose by intraperitoneal injection (20mg/kg) to create early phase of sepsis. Liver tissue samples were taken for measuring selenium, zinc, iron, manganese, magnesium, calcium, copper and potassium element levels and also for investigating histological changes. Element levels inductive coupled plasma/Optical Emission spectroscopy was measured by optical emission spectroscopy (Parkin Elmer- Optima 7000 DV) Results were evaluated by one way analysis of variance.

Results: In liver tissue, Selenium and Copper elements levels were found decreased but iron level increased in LPS group compared to those in control ($p < 0.01$). As a result of the light microscopic examination with H&E stained sections, in LPS group, hepatocyte cell membrane and sinusoid structure were damaged. In Simvastatin LPS group, hepatocytes and sinusoidal cord structure partially were improved.

Discussion and Conclusion: In conclusion, we suggest that the Simvastatin treatment may improve tissue damage through the oxidant/antioxidant system in septic rats in spite of increased levels of magnesium and iron

P55

A novel phagocytosis and oxidate burst assay in whole blood by Conventional - and Imaging Flow Cytometry with GFP-expressing bacteria

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Phagocytosis is essential for the clearance of pathogens, apoptotic bodies and necrotic debris. Defects in the phagocytic process are diagnostic and prognostic biomarkers in deficiencies of the innate immune system. Also, novel cell-based assays may complement current approaches for in vitro immunotoxicology. We have developed a flow cytometric method based on the use of green fluorescence protein labeled Escherichia coli (GFP-E. coli) and dihydroethidine (DHE) to assess phagocytosis and the subsequent oxidative burst in human whole blood samples and in the human monocytic cell line U937.

For the whole blood assays, GFP-E.coli was added at different bacterium:leukocyte ratios (1, 0.5 and 0.25) to 50 µL of heparinized whole blood incubated with CD45-APC antibody. After incubation at 37ºC for 30 min, 1 mL of BD FACS lysing solution was added. For the U937 study, GFP-E.coli was added to U937 cells at bacterium:leukocyte ratio of 30. After incubation at 37ºC for 90 min, 400 µL of RPMI were added. For oxidative burst quantitation in both assays, DHE was added prior incubation at 37ºC. Positive controls were samples stimulated with PMA. Negative controls included Cytochalasin A and 0ºC incubation. Samples were analyzed by using three different flow cytometers: FACSVerse, Accuri C6 and AmnisImageStream.

A bacterium:leukocyte ratio as low as 0.25 is enough to trigger phagocytosis and oxidative burst by neutrophils in whole blood samples. The capability of GFP –E. coli to elicit radical oxygen species is similar to PMA. Contrarily, cytochalasin A decreased markedly the phagocytic potential of blood and U937 cells. Analysis by imaging flow cytometry of U937 cells and whole blood samples allowed us to discriminate between surface-adherent and internalized bacteria.

This assay has been applied successfully to estimate the phagocytic potential of leukocytes in whole blood samples of normal subjects and it may provide a fast, simple and accurate protocol for diagnosing phagocytic disorders and toxic effects on phagocytic cells.

P56

Peroxisome proliferator-activated receptor β/δ activation prevents lipopolysaccharide-induced endothelial dysfunction

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Objective: Bacterial endotoxin lipopolysaccharide (LPS) activates inflammatory pathways, induces cytokine expression in the endothelium, augments reactive oxygen species (ROS) production in the vascular wall, and induces endothelial dysfunction. The aim of the present study was to analyse the protective effects of peroxisome proliferator-activated receptor (PPAR)-β/δ activation on LPS-induced inflammation, oxidative stress and endothelial dysfunction and determined whether or not uncoupling protein-2 (UCP2) could contribute to the endothelial benefit of PPARβ/δ activation.

Methods and results: A23187-stimulated nitric oxide (NO) production and reactive oxygen species (ROS) production were studied in mouse aortic endothelial cells (MAECs) incubated with LPS. Acetylcholine-stimulated relaxations and gene and protein expression in aorta from LPS-treated mice were also analyzed. We show that in MAECs, the PPAR-β/δ agonist GW0742 prevented the decreased A23187-stimulated NO production, and the increased intracellular ROS levels caused by exposure to LPS *in vitro*. *In vivo,* GW0742 treatment prevented the reduced aortic relaxation, the increased vascular ROS production, NADPH oxidase activity, NOX1, NOX2, p47phox, p22phox, toll-like receptor (TLR)4, and tumor necrosis factor (TNF)α mRNA levels in mice treated with LPS. The PPAR-β/δ antagonist GSK0660 abolished all these protective effects induced by GW0742. This agonist also restored the reduced expression of UCP-2 induced by LPS. The effects of GW0742 on NO and ROS production in MAEC exposed to LPS were abolished by the UCP-2 inhibitor genipin or by siRNA targeting UCP-2.

Conclusion: PPAR-β/δ activation restored the LPS-induced endothelial dysfunction by upregulation of UCP2, thus reducing intracellular ROS production and increasing NO bioavailability.

P57

Superoxide anion induces pain and inflammation in mice dependent on endothelin: Effect of bosentan, a mixed endothelin receptor antagonist

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Superoxide anion stimulates the production of cytokines and other molecules contributing to inflammation and pain. Bosentan is a mixed endothelin receptor antagonist under clinical use in the treatment of pulmonary arterial hypertension. Emerging literature suggests bosentan as a potent anti-inflammatory drug due to the pro-inflammatory effects of endothelin. However, it remains to be determined whether endothelin contributes to superoxide anion-induced pain and inflammation. The effects of bosentan in a mouse model of inflammation and pain induced by potassium superoxide, a superoxide anion donor, were investigated. Male Swiss mice (approved by University Animal Ethics Committee under process number 71.2012.68) were treated with bosentan (10–100 mg/kg, 1h) by oral gavage before potassium superoxide injection. The inflammatory response was evaluated locally and at spinal cord (L4–L6) levels. Bosentan inhibited superoxide anion-induced paw hyperalgesia (pain) to mechanical and thermal stimulus, overt pain-like behavior (abdominal contortions, paw flinching and paw licking), paw edema, myeloperoxidase activity (neutrophil marker) in the paw skin, and leukocyte recruitment in the peritoneal cavity. Bosentan treatment inhibited superoxide anion-induced IL-1beta and TNF-alfa production while enhanced IL-10 production in the paw skin and spinal cord. Bosentan inhibited the reduction of antioxidant capacity (reduced glutathione, ferric reducing antioxidant power, and ABTS radical scavenging ability) induced by superoxide anion. Furthermore, potassium superoxide induced the increase of prepro-endothelin-1 mRNA expression in the paw skin and spinal cord. In conclusion, our results demonstrated that superoxide anion-induced inflammation, pain, cytokine production and oxidative stress depend on endothelin, therefore, these responses are amenable by bosentan treatment. Financial support: CNPq, FAPESP, CAPES, MCTI, SETI, Fundação Araucária and Parana State Government, Brazil

AGING (P58-P76)

P58

Methylation of ribosomal RNA by NSUN5 is a conserved mechanism modulating organismal life span and oxidative stress resistance

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Aging, one of the best known but still not well understood biological phenomena, is among the big challenges to our society. The aging process in all organisms is characterized by a reduction in the cellular oxidative stress response, as well as a decline in overall protein synthesis. While experimental evidence is accumulating that several pathways modulating longevity and stress resistance converge on translation by targeting ribosomal proteins or translation initiation factors, modifications of ribosomal RNA were so far not studied in this context. Recently, we identified a putative RNA methyltransferase, NSUN5, as differentially regulated during aging in large scale transcriptional profiling of human and yeast cells.

Here we confirm that Rcm1, the yeast homologue of NSUN5, is a conserved RNA methyltransferase and specifically methylates a single nucleotide in the peptidyl transferase centre of the ribosome. Low levels of NSUN5 increase lifespan and tolerance to oxidative- and heat stress in yeast, worms and flies. Using yeast as model system we demonstrate that bulk translation is not altered in Rcm1 compromised cells, but that translational fidelity and the mRNA recruitment profiles into polysomes are different. Interestingly, Rcm1 deficiency favours recruitment of a distinct subset of mRNAs, all linked to the oxidative stress response.

Thus, our results suggest that the ribosome, rather than merely being a static molecular machine executing translation, exhibits functional diversity by modification of just a single rRNA nucleotide. Our findings support that there is a functional link between translational regulation and modulation of life span, mediated by a differential stress response of the ribosome.

P59

S-nitrosoglutathione reductase (GSNOR) is a new molecular determinant of aging

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S-nitrosylated proteins (PSNO) extent depends on NO production versus denitrosylation rate, with the latter reaction largely ascribed to *S*-nitrosoglutathione reductase (GSNOR). Interestingly, some phenotypes of GSNOR ablated (GSNOR-KO) mice resemble those commonly related to ageing.

Here we show that brain cortex of young GSNOR-KO mice showed an unusual increase of protein aggregates, which correlated with a severe neuromuscular phenotype. Moreover GSNOR-KO mouse embryonic fibroblasts (MEFs) showed clear signs of senescence when maintained in culture. On the basis of these pieces of evidence, we focused only on WT strains and observed that GSNOR decreased during age in brain, peripheral blood mononuclear cells, MEFs and primary cortical neurons (PCN) maintained in culture. Data obtained in WT brain and MEFs indicated that GSNOR promoter undergoes methylation as it showed a gradual increase of 5-methylcytosine (5meC) and a concomitant decrease of 5-hydroxymethylcytosine (5hmeC) levels during age or passages in culture. We found out that this phenomenon was due to a general decreased expression of ten-eleven translocation protein 1 (TET1), as TET1 silencing decreased, while overexpression of TET1 catalytic domain increased GSNOR expression.

GSNOR-KO MEFs and PCN showed a severe drop of mitochondrial transmembrane potential associated with a high *S*-nitrosylation rate of the Dynamin-related protein 1 (Drp1) and Parkin, both leading to a damaged and fragmented mitochondrial network, this matching the mitochondrial theory of aging. In line, TET1 downregulation phenocopied GSNOR-KO molecular phenotypes (e.g., impaired mitochondrial homeostasis, PSNOs increase and early senescence). Interestingly, we finally observed that GSNOR and TET1 expression decreased in old human individuals, while they were kept sustained in centenarians, reinforcing the hypothesis that GSNOR could be a new longevity gene

P60

J147 attenuates the metabolic profile of aging and Alzheimer's disease-related pathology in senescence-accelerated SAMP8 mice

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Alzheimer's disease (AD) drug discovery has rarely been addressed in the context of aging even though sporadic AD accounts for 99% of the cases. Phenotypic screens based upon old age-associated brain toxicities were used to develop the potent AD drug candidate J147. Here, we hypothesized that J147 would be effective against both brain aging and AD-associated pathology in rapidly aging SAMP8 mice, a model for early sporadic AD. An inclusive and integrative multi-omics approach was used to investigate protein expression, RNA expression, metabolite levels as well as cognition in old and young SAMP8 mice. J147 not only reduced the cognitive deficits and associated metabolic changes observed in old SAMP8 mice, it restored the levels of multiple markers of AD, vascular pathology, synaptic function, and inflammation to those approaching the young phenotype. Our data show that a drug candidate selected upon the basis of preventing old age-related brain toxicities also reduces AD-associated pathology.

P61

Oxidative stress or not in healthy older subjects ?

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The normal ageing process is thought to be associated with increased oxidative stress (OS). However, we have to admit that there is a large number of discrepancies about this matter. In order to better understand the presence or not of OS in the ageing process, we propose therefore to check if mean (or individual) blood values of fifteen OS biomarkers in older but robust subjects (65 to 85 years, n = 38, 19 men and 19 women) are or not in the normal reference values (used in a clinical routine*) established on populations of being healthy subjects (n = 60 to 120) aged between 18 and 60 years.

Data indicated that the mean blood value of antioxidants in older subjects (vitamin C, alpha and gamma - tocopherol, beta - carotene, thiol proteins, ubiquinone, total glutathione, SOD and GPx) ranged within the normal reference values. With respect to trace elements, selenium was at the limit of the inferior normal value while zinc and copper were inside the reference values. However, the ratio Cu/Zn was a little bit higher than the upper value. The mean concentration of lipid peroxides detected by their - OOH function was widely above the upper value (608 µM vs 432 µM) while oxidized LDL and isoprostanes ranged, by contrast, within the normal values. Statistical analysis revealed a strong correlation between the Cu/Zn ratio and circulating lipid peroxides ($r = 0.72$, $p < 0.0001$). Women exhibited a significant higher mean value in lipid peroxides than men (808 /-340 µM vs 407 µM /174 µM, $p < 0.0001$), consequently to a higher Cu/Zn ratio (1,45 /-0.40 vs 1.12 /-0.24, $p = 0.0039$).

In conclusion, our study raises the question to define which marker or group of associated OS markers must be investigated in order to evidence of not the presence of OS in healthy older subjects.

* Pincemail et al. On the potential increase of the oxidative stress status in patients with abdominal aortic aneurysm. Redox Report 17:139-144, 2012. This work was supported by FIRS grant, CHU of Liège.

P62

The hawthorn extract WS1442® retards replicative senescence in endothelial cells by preventing the redox-sensitive expression of p53/p21 and p16, and eNOS down-regulation

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Endothelial cell senescence has been suggested to contribute to the development of age-related vascular disorders. Endothelial senescence is characterized by an irreversible cell cycle arrest, oxidative stress and down-regulation of endothelial nitric oxide synthase (eNOS). The present study has evaluated whether the Crataegus special extract WS1442®, a rich source of polyphenols and a potent activator of eNOS, prevents replicative senescence in cultured porcine coronary artery endothelial cells, and, if so, to elucidate the underlying mechanism.

Replicative senescence was induced by sequential passaging of primary cultures of endothelial cells up to the fourth passage (P4). Senescence was assessed using senescence-associated β-galactosidase (SA-β-gal) activity, the formation of reactive oxygen species (ROS) using dihydroethidium (DHE) by flow cytometry, and the level of protein expression by Western blot analysis.

Passaging of endothelial cells was associated with a gradual increase in the SA-β-gal activity, DHE signal and expression level of p53, p21 and p16, and the down-regulation of eNOS from P1 to P4. The increased DHE signal was associated with increased expression of NADPH oxidase subunits ($gp91^{phox}$, $p47^{phox}$, $p22^{phox}$), COX-1 and COX-2. The Crataegus extract, apocynin and indomethacin prevented the increase in SA-β-gal activity, oxidative stress, and the up-regulation of $gp91^{phox}$, $p47^{phox}$, $p22^{phox}$, COX-1, COX-2, p53, p21 and p16, and the down-regulation of eNOS in P3 cells.

In conclusion, the Crataegus extract WS1442® has been able to retard endothelial cell replicative senescence most likely by preventing oxidative stress, which leads to down-regulation of eNOS and cell cycle arrest subsequent to the up-regulation of the p53/p21 and p16 pathways.

P63

Critical dynamics of gene networks is behind ageing and Gompertz law

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Several animal species are considered to exhibit what is called negligible senescence, i.e. they do not show signs of functional decline or any increase of mortality with age. Recent studies in naked mole rat and long-lived sea urchins showed that these species do not alter their gene-expression profiles with age as much as other organisms do. This correlates well with exceptional endurance of naked mole rat tissues to various genotoxic stresses. We quantitatively analyzed the relation between stability of gene regulatory networks (GRNs), mortality and the process of aging, constructed stochastic models of ageing in age-dependent microarray datasets and found that gene networks of most species are inherently unstable. Over a time the instability causes an exponential accumulation of gene-regulation deviations leading to death. However, should the repair systems be sufficiently effective, the gene network can stabilize so that gene damage remains constrained along with mortality of the organism. We applied the suggested model to analyze age-dependent gene expression datasets of model animals and derived a form of the Gompertz law, relating ageing and mortality with the stochastic genetic network instability. At the same time, this model accounts for the apparently age-independent mortality observed in some exceptionally long-lived animals. The presented analysis provides a new way to analyze effects of aging encoded in the modern -omic data. We suggest a systematic approach to identify biomarkers of aging and develop anti-aging therapeutics.

P64
Systems biology of human aging - network model 2015

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This network diagram is presented to aid in conceptualizing the many processes of aging, the causal chains of events, and the interactions among them. Contemplation of this network suggests promising intervention points for therapy development. This diagram is maintained on the Web as a reference for researchers and students. Content is updated as new information comes to light.
[www.LegendaryPharma.com/chartbg.html]
At first glance the network looks like a complicated web. However, as a conceptual summary, in one view, we can see how most biogerontological processes relate to each other. Importantly, examination of these relationships allows us to pick out reasonably plausible causal chains of events. Within these chains, we can see age-related changes or accumulations that appear to be promising targets for future therapy development. The many observable signs of human senescence have been hypothesized by various researchers to result from several primary causes. Inspection of the biochemical and physiological pathways associated with age-related changes and with the hypothesized causes reveals several parallel cascades of events that involve several important interactions and feedback loops. This network model includes both intracellular and extracellular processes. It ranges in scale from the molecular to the whole-body level. Effects due to externalities, lifestyle, environment, and proposed interventions are highlighted around the margins of the network.

P65

Caloric restriction exerts lipidomic effects in liver of male mice.

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Changes in membrane lipid composition, particularly membrane unsaturation, have been associated both with mammalian aging and in the determination of inter-species animal longevity. However it is currently unknown whether caloric restriction (CR), an accepted life-extending intervention, affects cellular lipid profiles. In this study we demonstrate, by using a liquid chromatography quadrupole time of flight-based methodology, that CR in male C57BL/6 mice: a) induces marked changes in the liver lipidome, b) specifically reduces hepatic levels of a phospholipid peroxidation product, 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphatidylcholine, c) leads to changes in phosphoethanolamine and triglyceride distributional profiles, d) causes hepatic changes in the fatty acid compositional profile, and e) affects the liver lipoxidative damage. These data demonstrate that CR induces a specific lipidome and metabolome reprogramming event in mouse liver along with an adaptation in mitochondrial function. Overall, these pathways could lead to decreased lipid oxidation vulnerability, assessed by mass spectrometry-based measurements, and contribute to the age-sparing and healthspan observed following CR.

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P66

A lipidomics comparative scan for longevity-related peroxidation susceptibility in different mammalian species

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Available evidences suggest that aerobic life and long-lived species evolved by reducing the relative abundance of those structural components that are highly susceptible to oxidative damage, thus conferring to the cellular constituents a higher structural stability and lower susceptibility to oxidative damage. In this context, lipid composition of cellular membranes is an important correlate of the rate of aging of animals and, therefore, the determination of their longevity. In the present work, the use of high-throughput technologies allowed us to determine the plasma lipidomic profile of 11 mammalian species, including humans, ranging in maximum longevity from 3.5 to 120 years. Using a non-targeted approach we revealed a specie-specific lipidomic profile that accurately predicts the animal longevity. Because the only significant site of FFA liberation into plasma is adipose tissue, the results of the present study seem to suggest an evolutionary adaptation in the sense that: i) adipose tissue regulates the fatty acid type stored and released to plasma in a species-specific fashion maintaining a lower degree of unsaturation in long-lived species, and ii) adipose tissue regulates LC-FFA concentration in a species-specific fashion as expression of the different metabolic needs. In conclusion, these results indicate that the lipidomic signature is an optimized feature associated with animal longevity, emerging LC-FFAs as a potential biomarker of longevity.

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P67

The levels of basal Hsp70 in peritoneal leukocytes from mice are related to their longevity as well as their survival after an endotoxic shock

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Heat shock proteins (Hsp70) act as a cellular defence mechanism and their expression being induced under stressful conditions. An impaired induction has been related to aging whereas an increased one has been related to longevity. It has been shown, in mice, that the oxidative and inflammatory stress of the immune cells, through the exacerbated activation of NF-kB is involved in the rate of aging and longevity of each animal. Since Hsp70 modulates lipopolysaccharide (LPS)-induced NF-kB activation in immune cells and could block the establishment of a chronic pro-inflammatory state, the aim of the study was to investigate the age-related changes in basal Hsp70 levels in leukocytes from mice and if these levels are modified after LPS injection, facts that are still not known. Adult, mature, old and long-lived (6, 12, 18 and 30 months of age, respectively) ICR-CD1 female mice were used and the basal levels of Hsp70 were assessed in peritoneal leukocytes using an ELISA method. In addition, in mature mice, Hsp70 levels were quantified at 0, 2 and 24 hours after *E.coli* LPS administration (intra-peritoneally), using a dose of 25 mg/kg. The results show that the basal Hsp70 levels are decreased in peritoneal leukocytes from old mice ($p < 0,01$) in comparison to adults, whereas levels from long-lived mice are comparable to those in adults. The group of mice subjected to endotoxic shock showed decreased Hsp70 levels at 2 and at 24 hours of LPS administration in comparison to the initial levels (at 0 hours). Interestingly these Hsp70 levels were shown to be positively correlated to the survival time of each animal ($p < 0,01$). The fact that the Hsp70 levels are preserved in long-lived animals demonstrates the important role of Hsp70 in achieving the healthy aging characteristic of longevity. Moreover, the positive correlation between initial Hsp70 levels and survival time after LPS administration indicates their importance as a cellular defence mechanism.

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P68

Oxidant and antioxidant ratios are proposed as useful markers for the measurement of redox state in aging and longevity

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An optimum balance between antioxidant enzymes is more important than their absolute activities for the efficient decrease of ROS. Superoxide dismutase (SOD) and catalase (CAT) are the main defense enzymes against the ROS generated by xanthine oxidase (XO). The imbalance between XO, SOD and CAT may be an important indicator of the oxidative stress state associated with aging. This work studied the changes in both SOD/CAT and XO/(SOD CAT) ratios in tissues and immune cells of mice during aging. The XO, SOD and CAT activities were analyzed in the liver, kidney, thymus, spleen, heart, brain and peritoneal leukocytes of adult, old and long-lived (6, 18 and 30 months-old, respectively) ICR-CD1 female mice, as well as of adult mice classified as prematurely (PAM) and non-prematurely (NPAM) aging mice. The results showed age-related changes and organ-specific profiles in both SOD/CAT and XO/(SOD CAT) ratios. A significant increase in SOD/CAT (associated with peroxide accumulation) was observed in cerebral cortex and peritoneal leukocytes of old mice in comparison to adult mice, and in PAM in relation to NPAM. However, the SOD/CAT was lowered (associated with superoxide increase) in liver, spleen and brainstem of old mice and PAM in comparison to adults and NPAM, respectively. An increased XO/(SOD CAT) ratio was observed in all the tissues and peritoneal leukocytes of old mice in relation to adults. Moreover, PAM also showed altered XO/(SOD CAT) in their tissues, these being similar to those observed in old mice. Long-lived mice showed lower or similar SOD/CAT and XO/(SOD CAT) than those observed in adult mice. Our study demonstrates that SOD/CAT and XO/(SOD CAT) ratios reflect the higher oxidative redox state that PAM and old mice suffer in their tissues and immune cells, as well as the preservation of the cellular redox balance in long-lived mice. Therefore, we propose both ratios as useful oxidative stress markers for the measurement of the cellular redox state and biological age. *MINECO(BFU2011-30336), RETICEF(RD12/0043/0018)-ISCIII-FEDER-(UE),UCM-Research-Group(910379)*

P69

Antioxidant adaptation of peripheral blood mononucleated cells to regular physical activity in elderly people

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The expected increase of the elderly population is an important health challenge in our society. A correct assessment of the elderly people´ health status is essential in order to reduce socio-economic costs and improve health services. Regular physical activity prescription is also a key point for chronic disease management and prevention. The aim of the present study was to evaluate the antioxidant system in peripheral blood mononucleated cells (PBMCs) in elderly people depending on their level of physical activity. Within a broader study, a sample of 120 participants (60 men and 60 women) >60 years old was recruited using the level of physical activity performed as a criterion of selection (low, medium and high). Blood samples were collected under basal conditions and PBMC fraction and plasma were purified. The results reported a progressive increase in PBMCs antioxidant protein levels -antioxidant enzymes: catalase, Mn-superoxide dismutase, glutathione peroxidase, glutathione reductase and thioredoxin reductase, and UCP3- with the increased physical activity levels in both genders. Carbonylated proteins, nitrotyrosine in PBMCs as well as plasma malondialdehyde also progressively increased but the differences were only significant ($p<0.05$) for carbonylated proteins in the medium and high levels in women. In conclusion, elderly people practicing regular physical activity had increased antioxidant machinery. Early management of body composition with an adequate nutrition and a training exercise programme could improve and maintain physical function in older adults and promote healthy aging. This study was supported by Instituto de Salud Carlos III (PI11/01791 & CB12/03/30038).

P70

A multicomponent exercise intervention improves function, cognition, emotional and social networking in a controlled population of community-dwelling frail older people.

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Introduction: Frailty is an age-associated, biological syndrome characterized by decreased biological reserves which puts an individual at risk when facing minor stressors, and is associated with poor outcomes. One of the most important features of frailty, from the medical point of view, is that a frail individual can become nonfrail if frailty is detected and treated at the onset.

Aim: To ascertain if a multicomponent exercise programme (including aerobic, anaerobic, proprioceptive, and stretching exercises) when performed by frail old persons can improve frailty, functionality, activities of daily living, social, affective and cognitive functions, as well as anthropometric characteristics and biological biomarkers of frailty, when compared with a controlled population that received no training.

Methods: Intervention old subjects (65 years) performed one hour of daily activity, five days per week for twenty-four weeks; while the control group (65 years) received no training and were asked to continue with their routine daily activities. The physical activity sessions were individualized.

Conclusion: We report here a unique multicomponent exercise training intervention for reversing frailty and improving physical function, cognitive, emotional, and social network determinations in a controlled population of community-dwelling frail older adults. We have also found improvements in anthropometric and analytical variables some of them considered good biomarkers of frailty. These results in a significant decrease in the number of visits to the primary care physician after the training programme. This is of the utmost importance to lower health care costs as primary medicine significantly contributes to whole health-care social effort.

Acknowledgments: This work was supported by grants SAF2010-19498 and SAF2013-44663-R, ISCIII2012-RED-43-029 from (RETICEF); RS2012-609 INCLIVA Intramural Grant and EU Funded CM1001 and FRAILOMIC-HEALTH.2012.2.1.1-2. The study has been co-financed by FEDER funds from the European Union.

P71

Identification of single nucleotide polymorphisms in centenarians

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The study of human populations with extreme longevity (centenarians) is one of the major challenges facing scientists. Single nucleotide polymorphisms (SNPs), are nucleotide changes in the DNA sequence, which may affect both the structure and the regulation of protein. This change may be in the extragenic region, the coding region (exon) or the non-coding region (intron) of genome

The aim of this study was the identification of single SNPs and SNPs grouped by signaling pathways as a possible cause of extreme longevity.

We recruited 28 centenarians and 60 young and elderly individuals from Hospital de la Ribera, Alzira, Valencia, collecting whole blood for DNA isolation. Sequencing was performed using the AxiomTM Genotyping of Affymetrix®, analyzing 295.988 SNPs. After applying a series of filters and genetic quality controls, only 37.564 SNPs remained, for which the association study was performed using logistic regression analysis. Moreover the SNPs genotyped, which belonged to genes located in 117 signaling pathways, selected from three databases, were grouped in the different pathways.

The results show a group of 108 SNPs with a *p* value less than 0.05, with twelve SNPs with a *p* value less than 0.001. Seventeen of the 108 SNPs are located in fourteen different genes among ten signaling pathways.

The study of SNPs characteristic of centenarians could be a new tool to find genes and signaling pathways involved in healthy aging.

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P72

Oxidative stress mediates premature senescence in human dental pulp stem cells cultured under 21% oxygen

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In vitro culture is routinely carried out under ambient oxygen tension. Dental pulp tissue physiological oxygen tension ranges between 3-6% O_2. *In vitro* cellular senescence refers to both replicative and premature senescence. Replicative senescence is associated with a progressive increase in $p16^{INK4a}$ expression, which is downregulated by Bmi-1. Premature or accelerated senescence can be induced by ROS accumulation. It has been demonstrated that pluripotency can be induced by introducing 2 factors: Sox2 and Oct4.

The aim of this study was to investigate the involvement of oxidative stress induced by culture under 21% O_2, in the maintenance of pluripotency and in the mechanisms of chronological and premature aging of human dental pulp stem cells (hDPSC).

hDPSC cultured under 21% O_2 show DHR123 levels significantly higher, and a lower mitocondrial membrane potential in comparison to hDPSC cultured under 3% O_2. Bmi-1 expression increases with passages, but this expression is higher in hDPSC cultured under 21% O_2 in comparison to 3% O_2 since the beginning. At early passages, Sox2 and Oct4 are overexpressed when culture under 3% O_2 compared to 21% O_2, and its expression decreases with passages. $p14^{ARF}$, $p16^{INK4a}$ expression and β-Galactosidase activity remain very low in hDPSC cultured under 3% O_2, whereas under 21% O_2 they are significatively augmented. p21 expression increases with passages under both 3% or 21% O_2 culture conditions.

Because hDPSC expressed $p14^{ARF}$ and $p16^{INK4a}$ but not Bmi-1 or p21, we conclude that *in vitro* premature senescence under 21% O_2 may be associated with oxidative stress and not with Bmi-1 overexpression. hDPSC culture under 3% O_2 mantains its stemness and reduces premature senescence.

This work was supported by grants SAF2010-19498 and SAF2013-44663-R, ISCIII2012-RED-43-029 from (RETICEF); RS2012-609 INCLIVA Intramural Grant and EU Funded CM1001 and FRAILOMIC-HEALTH.2012.2.1.1-2. The study was co-financed by FEDER funds from the European Union.

P73

Effects of a resistance training program on functional performance, oxidative stress and cardiovascular risk factors in healthy older adults

Ronda Serrat, Mar[1]; Colado Sánchez, Juan Carlos[2]; Hernandez Espinilla, Amaya[3]; Gargalló Bayo, Pedro[2]; Iradi Casal, Antonio[4]; Muñoz Cutillas, Victor[2]; Estany Capell, Nuria[3]; Juesas Torres, Álvaro[2]; Tormo Muñoz, M.Carmen[1]; Monzó Beltrán, Lidia[1]; Rivera Ballesteros, Sergio[1]; and Sáez Tormo, Guillermo[5].

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Introduction

Aging is known to develop parallel to different cardiovascular and metabolic complications. Vast amount of literature supports the theory of the Free Radicals as the explanation for the aging process. Hypothesis of how physical activity can improve this aging progression are made. However, contradiction is obvious based on the concept that physical exercise increases oxidative stress.

Incongruity can come from the fact that there are different types of physical exercise, each one producing a distinct action on many body macromolecules. We therefore try to establish an exercising protocol on older adults for being trained and to assess its effects on their clinical and oxidative stress status.

Material and methods

96 healthy adults (60 – 88 years old) were randomly distributed in the control group or the exercise groups with different resistance intensities (15RS or 6RS) twice a week during a regular 4-months exercise. Functional performance was recorded and representative markers of lipid metabolism, oxidative stress and related cardiovascular risk factors were analyzed in blood and urine of all participants following standard laboratory methods.

Results and conclusions

We observed an on-going and significant decrease of the oxidative footprints on lipids (LDLox and 8-isoprostanes) together with an improvement of the lipid metabolic profile in the older adult group after exercise. Functional performance and cardiovascular risk was improved without significant differences between exercises groups.

The obtained results may emphasize the important role of a controlled exercise program for the prevention of oxidative stress and cardiovascular related alterations in the older adult subjects.

Supported by PI13/01848, Plan Estatal de I D I 2013-2016; ISCIII-Subdirección General de Evaluación y el Fondo Europeo de Desarrollo Regional (FEDER). Department of Social Welfare of the City of Valencia (Spain). Fundación Mapfre.

P74

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Introduction: Aging is known to develop parallel to different cardiovascular and metabolic complications. Vast amount literature supports the theory of the Free Radicals as the explanation for the aging process. Hypothesis of how physical activity can improve this aging progression are made. However, contradiction is obvious based on the concept that physical exercise increases oxidative stress.

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P75

Changes over life-time in morphometric and metabolic variables in the long-lived freshwater mussel *Diplodon chilensis*

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Diplodon chilensis is a long-lived freshwater mussel, the most abundant in the Patagonian region, Argentina, with a MLSP of 90. Biomarkers of oxidative stress and antioxidant defense system were investigated in digestive gland (DG) and gonad tissues, along with morphometric variables, in relation to chronological age and sex. The von Bertalanffy growth model describes the individual growth (L_∞ = 72.01 mm; k = 0.14 year^{-1}; Age_{max} = 73 years old). Several antioxidant and oxidative stress parameters (superoxide dismutase (SOD) activity, glutathione (GSH) level and oxidative damage to proteins) remain constant through life in both tissues, while catalase (CAT) and GST activities, and lipid peroxidation decrease until 24 - 27 years, to remain fairly stable (mostly in gonads) or increase slowly mostly in DG afterwards. The timing of these age-related changes is coincident with the age estimated (28 years) from the lower Confidence limit (Cl) for L_∞ (69.97 mm), at which the bivalves would reach their minimum growth rate. A continuously increase in DG mass with age was recorded.The biochemical composition analysis showed that DG protein and lipid concentrations remained unchanged, while glycogen concentration increased until 30 years and then decreased. In gonad tissue, Gonad Mass (GM) increased also with age, following different patterns in females and males and was significantly higher in males. Total lipid content increased with age in gonads while proteins decreased. Besides GM and gonad glutathione-S-transferase (GST) activity (higher in females), no differences were found between sexes for any of the variables analyzed in this study.

The role of oxidative stress in this species could contribute to understand its longevity. Considering its wide distribution in Argentina and Chile, with abundant populations in a great variety of lake and river environments becomes a promising model for aging.

P76

Lipofuscin -mediated protein oxidation in model systems and in cells in vitro.

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Retinal pigment epithelial cells (RPE) provide key metabolic support for the photoreceptor cells and entire retina. These cells are at elevated risk of oxidative stress due to high oxygen concentration, intense visible light and accumulation of the age pigment lipofuscin (LF). It has been postulated that chronic oxidative stress mediates in the human RPE by LF, and can contribute to the development of age-related macular degeneration (AMD). To assess the photosensitizing ability of LF, photoperoxidation of proteins in ARPE-19 cells, containing phagocytized LF, was determined by the use of coumarin boronic acid (CBA) probe.

Purified LF isolated from RPEs from human donors of different age, with and without enrichment with a combination of zeaxanthin and alpha tocopherol (An), were introduced into ARPE-19 cells by phagocytosis. Control cells and An-treated cells or cells with LF and An-LF were irradiated with blue light, and analyzed for the presence of protein hydroperoxides using the CBA assay. Photoreactivity of LF was tested in a model system containing albumin.

Irradiation with blue light of ARPE- 19 cells, containing phagocytized LF, reduced survival of the cells, with the effect being stronger for LF from older donors. More pronounced photooxidizing properties of LF granules from older donors was also confirmed in a model system with albumin. Zeaxanthin and alpha tocopherol substantially reduced the phototoxic effect of LF and photooxidizing efficiency of the age pigment. Photooxidation of proteins in cells mediated by LF granules can be reproducibly analyzed by CBA assay. Photosensitizing abilities of the LF and its phototoxic potential can be substantially modulated by combination of antioxidants.

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MUSCLE & EXERCISE (P77-P80)

P77

Effects of training and acute exercise on mitochondrial biogenesis and oxidant and antioxidant capabilities in football players' blood mononuclear cells

Busquets, Carla[1]; Capó , Xavier[1]; Martorell, Miquel[2]; Sureda, Antoni[1]; Drobnic, Franchek [3]; Tur, Josep Antoni[1]; and Pons, Antoni[1].

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Acute exercise is associated with oxygen overconsumption resulting in high rate of ROS production. Regular physical activity induces oxidative stress but also causes adaptations in antioxidant defences including the NF-κB pathway, which activates target genes related to antioxidant defences like UCPs, and the mitochondrial biogenesis mediated by PGC1α.

The aim was to determine the effect of a long-term training and an acute exercise bout on the oxidant/antioxidant status and on the expression of mitochondrial biogenesis genes in peripheral blood mononucleated cells (PBMCs) from professional football players.

Twelve male football players performed a program of exercise for 8 weeks consisting of a 2-h/day football training session. One blood sample was obtained in basal conditions before the training period. After 8 weeks, another two samples were taken, before and after an acute and intense exercise. PBMCs fraction was isolated and the gene expression was assessed by Real Time-PCR and western-blot for the specific protein.

The results reported a significant increase in antioxidant protein levels - UCP2 and UCP3 – and in COXIV after the 8-week training period in basal conditions. PGC1α and UCP2 protein levels were also increased after acute exercise respect to pre-exercise value. After the training, expression of PGC1α, COXIV and mitND5 mRNA significantly augmented after the acute physical activity respect to pre-exercise value, while no changes occurred in these mRNAs in basal conditions. NF-κB activation reported a significant increase after acute exercise

In conclusion, training increases the levels of proteins related to mitochondrial biogenesis and improves antioxidant capabilities of mitochondria in PBMCs of well-trained football players. Acute exercise can act as an inducer of the mitochondrial biogenesis through NF-κB activation and PGC1α gene expression.

This study was supported by Instituto de Salud Carlos III (PI11/01791 & CB12/03/30038).

P78

Effects of docosahexanoic diet supplementation, acute exercise and aging on plasma and erythrocyte oxidative damage markers

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Omega 3 diet supplementation increases polyunsaturated fatty acids in cell membranes and the susceptibility to induce lipid oxidation. Omega 3 diet supplementation has healthy effects against diseases in which oxidative stress is in the origin or is instituted. Ageing is also associated with accumulation of oxidative damage. The aim was to determine the effects of DHA diet supplementation, acute exercise and aging on oxidative and nitrosative damage markers in plasma and erythrocytes. Five young athletes and five master athletes participated in a nutritional intervention with a DHA-enriched beverage. Each athlete performed an exercise test at the beginning and at the end of nutritional intervention. Blood samples were taken before and 1 hour after the exercise test. Exercise significantly increased body temperature, lactate blood levels and fatigue perception (Borg index). Aging significantly reduced the values of Borg index during exercise test. DHA diet supplementation significantly increased the levels of this fatty acid in erythrocyte membranes. DHA diet supplementation reduced plasma MDA levels, but not affected erythrocyte MDA. Exercise and age significantly increased plasma carbonyl index, but unaffected erythrocyte carbonyl index. Exercise increased plasma nitrate levels mainly in young sportsmen; while increased nitrate levels in master sportsmen only after DHA diet supplementation. In conclusion, aging reduces the fatigue perception during exercise. Oxidative damage markers in plasma are influenced by DHA diet supplementation, acute exercise and age. DHA diet supplementation promotes a reduction of oxidative damage; acute exercise and aging increases oxidative damage in proteins. Exercise also increases nitrate plasma levels which are modulated by DHA diet supplementation in master but not in young sportsmen.

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P79

Oxidative stress and inflammation are involved in the activation of E3 ubiquitin ligases during skeletal muscle atrophy

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INTRODUCTION: Muscle atrophy plays a relevant role in the most prevalent illnesses and conditions. Generation of reactive oxygen species (mainly by the xanthine oxidase) and inflammation are two of the main triggers of muscle atrophy (1).

Aim: The major aim of our study was to determine the mechanism by which reactive oxygen species cause muscle atrophy and its possible prevention by allopurinol, a well-known xanthine oxidase inhibitor widely used in clinical practice; and indomethacin, a non-steroidal antiinflamatory drug.

Methods: Male C57BL/6J mice (3 months old) conditioned by 14 days of hindlimb unloading with or without the treatments or its combination (n=48) were compared with freely ambulating controls (n=48). After the experimental intervention, we found that hindlimb unloading induced a significant increase in xanthine oxidase activity and prostaglandins in plasma (209%, $p<0.001$ and 114%, $p<0.05$; respectively).

Results: The most relevant new fact reported is that the combination of allopurinol and indomethacin prevents soleus muscle atrophy (from -41% to -16%, $p<0.001$). This is mediated by the inhibition of the E3 ubiquitin ligases MAFbx, MuRF-1 and Cbl-b, related to the inhibition of p38 MAPK and NF-κB and the stimulation of Akt pathways respectively.

Conclusion: Our data point out the potential benefit of allopurinol and indomethacin administration for bedridden, astronauts or muscle disuse; as well as a potential benefit in other atrophy models such as pathology-related cachexia or sarcopenia.

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P80

De novo ceramides are not necessary for induction of disuse-induced muscle atrophy

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Patients admitted to the intensive care unit commonly develop skeletal muscle weakness that can exacerbate illness and complicate their recovery. Beyond the primary disease or aging, weakness is promoted by a variety of prolonged hospitalization-associated conditions. These include altered nutritional status, physical inactivity, and prolonged bed rest. The two latter conditions are the most ubiquitous, affecting all patients during a prolonged hospitalization. In both cases, skeletal muscle utilization is decreased with a concomitant reduction in fatty acid oxidation. Subsequent fatty acid accumulation converted to ceramides could be a cellular mechanism leading to muscle wasting. Indeed these sphingolipids act as second messengers in several of molecular signaling pathways involved in muscle atrophy. Consequently, the aim of this work was to determine the effects of immobilization on muscle ceramide accumulation, and identify the role of these ectopic lipids in molecular mechanisms involved in skeletal muscle atrophy. For this purpose, male Wistar rats were treated with an inhibitor of de novo synthesis of ceramides (i.e. myriocin) and subjected to hindlimb unloading for 7 days. We found that hindlimb unloading increases total muscle ceramide content and decreases soleus muscle weight and fiber diameter. Immobilization increased the level of polyubiquitinated proteins and induced muscle apoptosis. Despite a reduction in total muscle ceramide content, myriocin treatment did not prevent skeletal muscle atrophy and concomitant induction of apoptosis and proteolysis. In conclusion, these results show that *de novo* synthesis of ceramides is not involved in muscle atrophy induced by a short period of immobilization.

NITROSATIVE STRESS (P81-P87)

P81

Exploit the *S*-nitrosylation of the mitochondrial chaperone TRAP1 to selectively kill GSNOR-deficient hepatocellular carcinoma cells.

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S-nitrosoglutathione reductase (GSNOR) is the enzyme controlling protein *S*-nitrosylation and the intracellular signal transduction depending on this redox post translational modification. GSNOR depletion has been recently demonstrated to contribute to the development of hepatocellular carcinoma (HCC) and is downregulated in almost the half of HCC cases. In the present work, we demonstrate that GSNOR-downregulating HepG2 HCC cell line (siGSNOR HepG2) exhibits an unusual upregulation of succinate dehydrogenase (SDH). This upregulation is associated with the proteasomal degradation of its inhibitory partner, the mitochondrial chaperone TNF receptor-associated protein 1 (TRAP1) which, upon *S*-nitrosylation at Cys501, undergoes proteosomal degradation. Such a molecular feature represents the *Achilles' heel* of GSNOR-deficient HCC cells, as they are highly susceptible to cytotoxicity induced by SDH-directed drugs, mostly, α-tocopheryl succinate (α-TOS). We provide evidence that the cell death induced is caspase-independent and ROS-dependent, and proceeds *via* the PARP1/RIP1-mediated necroptotic pathway. Mice experiments confirmed that stably-downregulating GSNOR HepG2 xenografts are susceptible to α-TOS with both growth rate and size of tumors reduced after 2-weeks of systemic treatment. Since, to date, chemotherapy has failed in providing possible cures for HCC, our results provide the molecular basis for paving the way to the development of new therapeutic approaches.

P82

Regulation of cell death receptor S-Nitrosylation and apoptotic signaling by Sorafenib in hepatoblastoma cells

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Nitric oxide (NO) plays a relevant role during cell death regulation that limits the survival of tumor cells. We have recently shown that the overexpression of nitric oxide synthase type III (NOS-3) induces oxidative and nitrosative stress, p53 and cell death receptor expression and apoptosis in hepatoma cells. Sorafenib is the unique recommended molecular-targeted drug for the treatment of patients with advanced hepatocellular carcinoma. The present study was addressed to elucidate the potential role of NO during Sorafenib-induced cell death in hepatoblastoma cells. We determined the intra- and extracellular NO concentration, cell death receptor expression and their S-nitrosylation modifications, and apoptotic markers in Sorafenib-treated HepG2 cells. The effect of NO donors on above parameters has also been determined. Sorafenib induced cell death in HepG2 cells. However, low concentration of the drug (10 nM) increased the expression of cell death receptors and extrinsic apoptotic pathway (caspase-8) that both diminished at higher concentrations of the drug (10 μM). High doses of Sorafenib correlated to a rise of caspase-9 and caspase-3 activities, as well as DNA fragmentation in HepG2 cells. The shift of cell death signaling pathway was associated with a reduction of S-nitrosylation of cell death receptors in cells treated with Sorafenib. The administration of NO donors increased S-nitrosylation of cell death receptors and overall induction of cell death markers in control and Sorafenib-treated cells. In conclusion, Sorafenib induced alteration of cell death receptor S-nitrosylation status which may have a relevant repercussion on cell death shift from the extrinsic to intrinsic apoptotic signaling in hepatoblastoma cells.

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Mechanism of the Reaction of Peroxynitrite with Mn-Superoxide Dismutase: Nitration of Critical Tyrosine-34

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MnSOD is a mitochondrial enzyme that acts as a superoxide detoxifier. Superoxide can react with nitric oxide to yield peroxynitrite ($ONOO^-$), reaction that can outcompete MnSOD for superoxide. One of the principal biomarkers for $ONOO^-$ formation *in vivo* is tyrosine (Y) nitration, which can alter both protein structure and function. One of the most widely reported nitrated proteins is MnSOD, which, when nitrated at Y34 becomes inactivated. The reaction between MnSOD and $ONOO^-$ was reported to occur with a rate constant of ~$1.0x10^5 M^{-1}s^{-1}$ and to cause the oxidation of the Mn^{III}, according to Quijano *et al* (1); generating an oxo-metal complex (Mn^{IV}=O). Recently, Surmeli *et al* (2) reported a rate constant value significantly smaller than the previous one and proposed a different mechanism of reaction, in which the Mn^{III} was *reduced* by $ONOO^-$. Thus, the aim of this study was to reassess the second order reaction constant value and the mechanism of reaction between MnSOD and $ONOO^-$ by a combination of experimental and computational methods. Competition stopped-flow spectrophotometry experiments were performed in order to determine the rate constant value using the novel probe coumarin boronic acid (CBA), which reacts directly with $ONOO^-$ at ~$1x10^6$ M^{-1} s^{-1} to yield 7-hydroxycoumarin. The time course and yields of fluorescence emission of the reaction of $ONOO^-$ with CBA were followed in the presence of increasing concentrations of MnSOD obtaining a rate constant of 1.9 x 10^5 M^{-1} s^{-1} for human MnSOD, almost identical to the one reported in (1), that followed direct $ONOO^-$ decomposition. Theoretical methods (QM/MM) were used to study the reaction between MnSOD and $ONOO^-$ that indicated the thermodynamically feasible reaction steps in the reaction and illustrated the microscopic events that lead to nitration of Y34. Overall, the results obtained by both experimental and theoretical methods are consistent with a metal-catalyzed process that involves the formation of Mn^{IV}=O followed by site-specific nitration of Y34.

References

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P84

Characterization of peroxynitrite interaction in the metabolism of nitric oxide and prostaglandins and its impact on vascular response.

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Reactive oxygen species and reactive nitrogen species maintain a delicate balance under physiological conditions. An alteration of this balance in endothelial cells leads the overproduction of nitric oxide (NO) and superoxide (O^{-2}) that in turn triggers peroxynitrite (ONOO-) production. In endothelial cells O^{-2} can be generated through NO and prostaglandins (PGs) pathways. The aim of this work was to investigate whether ONOO- could modulate vasorelaxation mediated by NO and PGs. Since acetylcholine (ACh) activates two vasorelaxation mechanisms endothelial nitric oxide synthase and PGs production, ACh was used in this work to test our aim. Thoracic aortic rings were obtained from male Wistar rats. Rings were stripped from perivascular connective tissue and placed into an organ chamber for isometric tension. After 1 hour of stabilization, aortic rings were contracted with phenylephrine (Phe, 10^{-7}M) and relaxed with ACh in a dose-response curve (10^{-10}-10^{-4}M). Rings were incubated for 30 minutes with L-NAME (10^{-6}M), Indomethacin (Indo, 10^{-5}M), in combination of both inhibitors, in the presence of SIN-1 (10^{-6}M), SIN-1 (10^{-6}M) with L-NAME (10^{-6}M) and SIN-1 (10^{-6}M) with Indo (10^{-5}M). Then aortic rings were contracted and relaxed as described. In the second set, aortic rings were incubated for 30 min with SIN-1 (10^{-6}M), contracted with Phe (10^{-7} M), and relaxed with a dose-response curve of sodium nitroprusside (SNP,10^{-10}-10^{-4}M). Relaxation was expressed as percentage. ACh relaxation was inhibited by 70% in the presence of L-NAME, 30 % with Indo, in combination of both inhibitors the relaxation was totally inhibited. In the presence of SIN-1, NO dependent relaxation was inhibited by 50 % and did not affect PGs dependent relaxation. Rings incubated with SIN-1 and relaxed with SNP induced relaxation by 50 %. These data suggest that peroxynitrite interacts with NO bioavailability decreasing NO levels, thereby prevents NO dependent relaxation.

P85

The inhibition of mitochondrial complex III by NO involves ubisemiquinone formation

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The effects of NO on complex III were studied using bovine heart submitochondrial particles (SMP). Complex II-III activity (222 ± 4 nmol/min.mg protein) was inhibited by 50% in the presence of 1.25 μM NO, released from 500 μM GSNO or 30 μM SPER-NO. Neither GSNO nor SPER-NO were able to inhibit complex II (220 ± 9 nmol/min.mg protein) suggesting that NO affects complex III area. Complex II-III activity was also decreased (36%) when SMP were incubated with L-arginine and mtNOS cofactors, indicating that the inhibition was produced by endogenous NO. GSNO (500 μM) reduced cytochrome b_{562} by 71% in an $[O_2]$ independent manner. Hyperbolic increases in $O_2^{\cdot-}$ (up to 1.3 ± 0.1 nmol/min.mg protein) and H_2O_2 (up to 0.64 ± 0.05 nmol/min.mg protein) productions were observed with a maximal effect at 500 μM GSNO. SMP incubated in the presence of succinate showed an EPR signal (g=1.99) compatible with a stable semiquinone ($UQH^{\cdot}$), which was increased (42%) by antimycin and abolished by antimycin plus myxothiazol supplementations. In the presence of 1.25 μM NO (500 μM GSNO or 30 μM SPER-NO) the $UQH^{\cdot}$ signal was increased by 35%. When GSNO and myxothiazol were simultaneously added to the medium, the $UQH^{\cdot}$ signal was not observed, similarly to the effect of antimycin plus myxothiazol. EPR spectra obtained under N_2 atmosphere were comparable to the ones obtained in air saturated conditions, suggesting that $UQH^{\cdot}$ signal is not caused by NOx species on complex III. To conclude, NO interacts with complex III in an $[O_2]$ independent manner producing antimycin-like effects. This behaviour comprises the inhibition of electron transfer, the interruption of the oxidation of cytochromes *b,* and the enhancement of $[UQH^{\cdot}]_{ss}$ which, in turn, leads to an increase in $O_2^{\cdot-}$ and H_2O_2 production rates

P86

Nitric oxide is produced through mitochondrial reverse electron transfer

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Heart phosphorylating electron transfer particles (ETPH) showed a NAD reductase activity of 63.9 ± 3.3 nmol/min.mg protein, sustained by reverse electron transfer (RET) at expenses of ATP and succinate. This activity was inhibited by rotenone (99%), oligomycin (98%) and m-CCCP (93%). ETPH produced NO at 1.1 ± 0.1 nmol NO/min.mg protein by the mtNOS reaction. In conditions of reverse electron flow, ETPH produced 0.96 ± 0.07 nmol NO/min.mg protein, suggesting that NO production can be supported by electrons derived from the low isopotential components of the respiratory chain. Rotenone completely inhibited NO production supported by reverse electron transfer measured in ETPH, but that inhibitor did not reduce the activity of isolated nNOS, indicating that the inhibitory effect of rotenone on NO production by ETPH is due to an electron flow blockage and not to a direct action on NOS structure. A mitochondrial fraction enriched in complex I produced 1.72 ± 0.18 nmol NO/min.mg protein and reacted with anti-nNOS antibodies, indicating that complex I physically and functionally interacts with mtNOS enzyme. These data show that mitochondrial NO production can be supported by reverse electron flow of the respiratory chain, a phenomenon that could occur in physiological and pathological conditions.

P87

A no-wash, no lyse kinetic assay of the interaction between nitric oxide and superoxide anion in leukocytes

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Reactive nitrogen- and oxygen- species are crucial in leukocytes responses against pathogens, but also in inflammatory conditions. Central to both processes is the generation of peroxynitrite (ONOO) by a fast reaction between NO and superoxide anion. The strong oxidant peroxynitrite causes protein nitrosylation. Circulating by-products of protein nitrosylation are early biomarkers of inflammation-based conditions, including minimal hepatic encephalopathy in cirrhotic patients. In this context, we have designed a novel no-wash, no-lyse real-time flow cytometry assay to detect and follow up the NO- and superoxide-driven generation of ONOO in leukocyte subpopulations of normal donors and cirrhotic patients.

Whole blood samples were stained with CD45 and CD14 antibodies plus each of a series of fluorescent probes sensitive to NOS, ROS or glutathione (GSH), namely 4-Amino-5-Methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA), dihydrorhodamine123 (DHR), MitoSOX Red, Dihydroethidium (DHE) and 5-chloromethylfluorescein diacetate (CMFDA) plus a viability marker. Samples were exposed sequentially to a NO donor, and three different superoxide donors, while analyzed in real time by flow cytometry. Relevant kinetic descriptors, such as the rate of fluorescence change were calculated from the kinetic plot.

The real-time generation of ONOO, which consumes both NO and superoxide, led to a decrease in the intensity of the cellular fluorescence of the probes sensitive to these molecules in all leucocytes from samples preincubated with superoxide donors and then treated with NOR-1. In addition, the NO generation in samples treated with NOR-1 was rapidly reversed by the addition of superoxide donors. Further there are striking differences among lymphocytes, monocytes and granulocytes regarding the response to NO and superoxide, indicating a different regulation of oxidative pathways in those cells.

This is a fast and simple assay that may be used to explore ROS and NOS metabolism, especially for monitoring the intracellular generation of ONOO in blood cells.

OXIDATIVES STRESS MARKERS (P88-100)

P88

Two dimensional blue native/SDS-PAGE to identify mitochondrial complex I subunits modified by 4-hydroxynonenal (HNE)

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The lipid peroxidation product 4-hydroxynonenal (HNE) can form protein-linked HNE adducts, thereby impacting protein structure and function. Mitochondrial complex I (NADH-ubiquinone oxidoreductase), containing at least 45 subunits in mammalian cells, sits in a lipid-rich environment and is thus very susceptible to HNE modifications. In this study, a procedure for the identification of HNE-modified complex I subunits is described. Complex I was isolated by first dimensional nongradient blue native polyacrylamide gel electrophoresis (BN-PAGE). The isolated complex I band, visualized by either Coomassie blue staining or silver staining, was further analyzed by second dimensional SDS-PAGE. HNE-modified proteins were visualized by Western blotting probed with anti-HNE antibodies. HNE-positive bands were then excised and the proteins contained in them were identified by mass spectrometric peptide sequencing. The method was successfully applied for the identification of two complex I subunits that showed enhanced HNE-modifications in kidney mitochondria isolated from diabetic rats in which diabetes was induced by streptozotocin

P89

Between and within subject variability of biomarkers of antioxidant status in the BIOCLAIMS cohort

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The *BIOCLAIMS cohort* (1310 study subjects, 606 M, 704 F, 18-85, 51.3±16.1 yrs) underwent comprehensive dietary (240-item food-frequency questionnaires), anthropometric (BMI, subcutaneous adipose tissue distribution, bioelectrical impedance), clinical (blood pressure, intima-media thickness, flow-mediated dilatation) and biochemical characterization, with >500 variables, including pro-inflammatory genotype variants. Subsets participated also in *Menstrual Cycle Study* (28 F, not using hormonal contraceptives, 4 investigations: early/late follicular, early/late luteal phase), *Seasonal Variability Study* (52 subjects, M=F, 12 investigations, monthly intervals), and *Day-to-Day Variability Study* (12 subjects, 5 investigations, Monday-Friday).

Plasma concentrations (µmol/L) were (mean±SD; min-max) for ascorbate 68.2±19.0 (8.90-170); α-tocopherol 29.5±7.51 (7.87-92.5), γ-tocopherol 1.24±0.699 (0.218-7.85), β-carotene (0.85±0.72, 0.034-10.03), and lycopene 0.59±0.30 (0.001-2.38); erythrocyte GSH-Px (U/g Hb) was 6.21±1.56 (2.01-12.13). Changes across *menstrual cycle* were significant for plasma ascorbate (P<0.001) and α-tocopherol (P=0.019), both low in luteal phase, but not for α-tocopherol:cholesterol. *Seasonal variability* was significant for ascorbate (low: 04 [April], high: 06-09, 11, 12), lycopene (high: 08, low: 04), β-carotene (high: 08-09), and GSH-Px (high: 01, low: 12). *Day-to-day variability* was generally low (intraclass correlation coefficients >0.8 for vitamin E, >0.9 for other antioxidants).

Conclusions: In contrast to high between-subject variability in the cross-sectional study, longitudinal within-subject variability was low, allowing for reliably studying associations of antioxidant status with functional biomarkers.*Funded by the European Union's 7th Framework Programme FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project) and the Federal Ministry of Science, Research and Economy of Austria*

P90

Combined effect of carbamylation and oxidative stress on the antioxidant capacity of lymphocytes

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The accumulation of urea and other waste products of metabolism in blood is one of many consequences of renal failure. In human tissues, urea is in spontaneous equilibrium with cyanate. Cyanate is rapidly converted into its reactive form isocyanic acid which can react with free functional groups of proteins, especially free amino groups resulting in their carbamylation. Carbamylation is a non-enzymatic, post-translational modification that alters protein structural and functional properties and thus contributes to cell ageing.

For determination of the effect of carbamylation on lymphocytes induced by different concentrations of cyanate, cells were treated with this compound and survival MTT test was carried out. Lymphocytes were incubated with sodium cyanate for 24 h at 37ºC. Two non-toxic concentrations of cyanate were selected for further investigation. At the end of incubation hydrogen peroxide was added for induction of oxidative stress. The assays of oxidative stress parameters: concentration of thiols and antioxidant capacity (using TPTZ and DPPH) were applied to examine the changes in isolated lymphocytes.

The survival of cells did not change up to the 2 mM concentration of cyanate in comparison to the control. The significant decrease of antioxidant potential after hydrogen peroxide and combined treatment with cyanate and hydrogen peroxide, respectively, for both methods was detected. The significant decrease in total thiolsconcentration in cells after hydrogen peroxide and combination of cyanate and hydrogen peroxide, respectively vs. control, was also detected.

The 1 and 2 mM concentrations of cyanate did not change cell survival but had negative influence on oxidative stress parameters. The combined effect of the cyanate and hydrogen peroxide significantly deepened changes in the lymphocytes in comparison to cyanate alone. Carbamylation of proteins displays a weaker effect on oxidative stress parameters than carbamylation together with oxidative stress.

P91

Detection of S-(2-succinyl)cystein (2SC) by LC-MS/MS as a marker for mitochondrial metabolic abnormalities

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Background: S-(2-Succinyl)cysteine (2SC) is formed by reaction between the thiol group of proteins and fumarate of the Krebs cycle intermediate. We previously demonstrated that 2SC significantly increased during maturation of 3T3-L1 fibroblasts to adipocytes. Fumarate concentration increased > 5-fold during adipogenesis in medium containing 30 mm glucose, producing a > 10-fold increase in 2SC-proteins in adipocytes compared with undifferentiated fibroblasts grown in the same high glucose medium. Furthermore, several proteins such as adiponectin and heat shock were identified by matrix-assisted laser desorption ionization time-of-flight/time-of-flight mass spectrometry. However, little is known about the presence of 2SC in serum. In the present study, we measured 2SC level by liquid chromatography tandem mass spectrometry (LC-MS/MS) in serum.

Methods: Human serum was hydrolyzed in 6 M hydrochloric acid at 100 °C for 24 h. The samples were dried *in vacuo*, and brown materials were removed by application to a C-18 Sep Pak column, followed by elution of polar amino acids with H_2O and then analyzed by LC-MS/MS on a TSQ Vantage (ThermoFisher Scientific).

Results: 2SC was detected in human serum as well as in matured adipocytes by LC-MS/MS.Serum 2SC level was significantly higher in non-diabetic nephropathy as compared to normal subjects. However, its levels in diabetic patients were not higher than normal subjects, possibly because the blood glucose level was normalized due to medication.

Conclusions: The metabolic abnormalities of mitochondria could be estimated by measuring 2SC in serum samples.

P92

Kinetics assays shows biochemical interactions between fluorescent probes in the analysis of oxidative stress

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Background: Analysis of reactive oxygen species (ROS) is a relevant application of flow cytometry. The specificity of fluorogenic substrates for ROS analysis, and their possible interferences with ROS metabolism are still unresolved issues. We were interested in developing multicolor real-time flow cytometry (RT-FCM) assays for ROS by combining spectrally-compatible fluorogenic substrates for ROS. While setting up such assays we found discrepancies between the responses of fluorogenic substrates alone and in combination.

Methods: Suspensions of N13 rat hepatoma cells were incubated with several fluorogenic substrates used in oxidative stress studies: MitoSOX Red Dye, dihydroethidium, dihydrorhodamine 123, dihydro-dichlorofluorescein and Mitochondria Peroxy Yellow 1 (MitoPY1). For setting up RT-FCM experiments, appropriately stained cells were run on an Accuri C6 cytometer and live cells gated based on their FSC vs SSC features. After defining baseline fluorescence for about 30 sec, ROS generation was triggered by cumenehydroperoxide, and fluorescence emission was continuously measured for 10 min. Under these conditions, similar RT-FCM experiments were performed with hydrogen peroxide, NOR-1, FCCP, propranolol and epinephrine.

Results: Our results showed that kinetics of individual probes responded as expected to the addition of prooxidant compounds. However, the same probes, when paired to other spectrally-compatible ROS probes, showed important increases or reductions in the rate of fluorescence generation, induced by the same pro-oxidant. Variations were complex, and dependent on fluorochrome pairs and prooxidants, but not on fluorescence compensation.

Conclusions: These results suggest biochemical interactions among ROS and fluorogenic substrates used in oxidative stress studies and recommend caution when several probes are to be combined in multiparametric analysis of ROS.

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P93

Simultaneous detection of oxidative stress and inflammation in amniotic fluid for early assessment of chorioamnionitis

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Introduction: Intraamniotic infection/inflammation (IAI) is associated with preterm birth, adverse short and long-term clinical outcomes, oxidative stress and inflammation. The diagnosis of IAI is based on histological and clinical findings; however, often these results are unspecific. Therefore, efforts have been directed towards validating reliable methods for patients lacking overt clinical symptoms.

Inflammation and oxidative stress are interconnected. Consequently, markers of oxidative stress (8-hydroxy-2'-deoxyguanosine (8OHdG)) or interaction of oxidative stress and inflammation (3-Chloro-Tyrosine (3Cl-Tyr)) and glutathione sulfonamide (GSA) can be used as highly reliable biomarkers of inflammatory processes in the newborn period.

The aim of this work was to develop a simultaneous analysis method for 8OHdG, 3Cl-Tyr and GSA in amniotic fluid (AF) samples employing ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) and evaluate the diagnostic power of these biomarkers for chorioamnionitis.

Methods and patients: AF samples were prospectively collected from 23 women grouped into two categories with or without IAI following clinical and histological criteria. UPLC-MS/MS parameters were optimized for the detection of 8OHdG, 3Cl-Tyr and GSA in AF. Standards of 8OHdG and 3Cl-Tyr were obtained from Sigma-Aldrich and GSA was synthesized by treatment with hypochlorite followed by purification using liquid chromatography.

Results: Inflammatory markers such as GSA ($p<0.002$) and 3Cl-Tyr ($p<0.049$), and the oxidative stress biomarker 8OHdG ($p<0.021$), showed significant differences between AF with or without IAI.

Conclusions: Biomarkers of inflammation and oxidative stress determined in AF samples could represent a new approach towards an early diagnosis of IAI in the clinical setting.

P94

Novel biomarkers of oxidative stress in newborn with hyperoxia and metabolic acidosis in delivery room

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Perinatal asphyxia is the consequence of hypoxic-ischemic episodes during the labor and delivery, which cause hypoxemia, hypercapnia, and acidosis, and even brain damage[1]. The association between low umbilical cord pH and long-term outcome has been previously established[2]. However, biomarkers capable of promptly diagnosing asphyxia-derived damage are scarce and unreliable[3]. Brain has a high content of complex lipids that undergo oxidation during hypoxia-reoxygenation which leads to the production of a variety of prostaglandins-derived metabolites. We evaluated these analytes in cord serum samples from newborns using liquid chromatography coupled to mass spectrometry (LC-MS/MS).

Eligible infants were consecutively born in the Feto-maternal Medical Center (Helsinki). Systematic clinical variables (weight, gestational age, Apgar scores...) were retrieved and cord serum blood gases were determined. Babies were classified as control (n= 17; pH >7.05) and case groups with acidosis (n= 20; pH<7.05). Samples were shipped to the Neonatal Research Center where lipid peroxidation analytes were determined by LC-MS/MS. Thereafter, a univariable statistical analysis was carried out. The concentrations of 4 analytes showed significant differences between the case and control categories studied (Table, P<0.05).

Compound	Median (inter-quartilerange)		U Mann Whitney test
	Control (n= 17)	Case (n=20)	P value
PGF2α	3 (2)	6 (5)	0.004
8-iso-15(R)-PGF2α	0.0 (0.7)	2 (3)	0.004
Isoprostanes	0.0 (0.5)	0.5 (1.1)	0.03
Isofurans	0.3 (1.0)	1 (2)	0.011

Conclusions: In newborns with a cord serum pH <7.05, the determination of PGF2α, 8-iso-15(R)-PGF2α, isoprostanes and isofurans was significantly elevated. A correlation between analytes' concentration in cord blood and follow up results at a later age to assess their utility needs to be performed.

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2-BJOG 119 (2012) 824-831
3-BMC Nephr 136 (2012) 1-7

P95

Effect of irradiation on the antioxidant response and oxidative stress in cultured human blood from medical workers occupationally exposed to ionizing radiation

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The interaction of ionizing radiation with water molecules in humans cause an increase of free radicals which can lead to oxidative stress. When exposition to low doses occurs, the increase of the oxidative stress is one of the major concerns. However, some authors suggest that an adaptive response can though arise.

The objective of this study was to evaluate the effect of *in vitro* irradiation on the antioxidant response and oxidative stress in cultured blood from medical workers exposed to chronic low doses of ionizing radiation. Medical workers no occupationally exposed to ionizing radiation were used as controls. Blood samples were collected and cultured for 72 hours. Then, half of the samples were irradiated at 1 Gy. Total antioxidant capacity (TAC), nitrites (NOx), reduced and oxidized glutathione (GSH and GSSG) and free nitrotyrosine were measured in non-irradiated and irradiated samples from both groups.

TAC was statistically reduced after irradiation in both groups of medical workers, however, the effect seemed to be more pronounced in the control group. The ratio of GSH/GSSG was also lower in irradiated samples of medical workers exposed to chronic low doses than in non-irradiated samples. No signficant differences were found for the control group. Irradiation increased free nitrotyrosine in both groups. Finally, NOx were not able to be measured because culture medium interfered with their determination.

Our preliminary findings indicate that 1 Gy irradiation decreases antioxidant response in cultured human blood. However, crhonic exposition to low doses of ionizing radiation does not seem to induce an adaptive response.

P96

Dichlorofluorescein derivatives do not measure intracellular ROS in apigenin treated cells

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Apigenin, a natural polyphenol, has become a promising molecule for treatment of many diseases, such as neurodegenerative, cardiovascular and cancer diseases. Although flavonoids have been described as antioxidant molecules, apigenin has been distinguished by its pro-oxidant activity, which has been frequently demonstrated by the oxidation of reduced dichlorofluorescein derivatives, in many cell types. However, little is known about the actual mechanism by which apigenin generates radical oxygen species (ROS). Therefore, the purpose of this work is to study the oxidation of apigenin and its effects in the oxidation of 5-(and-6)-chloromethyl-2',7'dichlorodihydrofluorescein (CM-H_2DCF), a probe employed to measure intracellular ROS. Results showed that apigenin increases significantly the oxidation of CM-H_2DCF in two different immortalized cell lines derived from human and rat hepatic stellate cells (HSC), even if apigenin did not generate ROS, as judged by dihydroethidium oxidation and extracellular hydrogen peroxide production. Moreover, cell-free assays confirmed that apigenin enhances CM-H_2DCF direct oxidation without a net increase in the concentration of ROS. In conclusion, HSCs treated with apigenin increase significantly CM-H_2DCF oxidation without generation of ROS, so pro- and antioxidant effect of flavonoids must be confirmed with different techniques and not only with dichlorofluorescein derivatives, as it is usually done.

P97

Peroxidation of lipids photosensitized by a functionalized fullerene in cells and liposomes

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Fulerenes are a unique class of carbon molecules with interesting photophysical and photochemical properties. In this study, we analyzed photo-peroxidation of unsaturated lipids induced by a cationic C_{60} fullerene substituted with three quartenarypyrrolidinium groups (BB6). BB6-photosensitized peroxidation of lipids was analyzed in liposomes made of POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and cholesterol and in mause melanoma (B16) cell culture employing iodometric assay for total lipid hydroperoxidemesurement and HPLC-Ec(Hg) for sensitive and selective detection singlet oxygen specific (5α-ChOOH) and free radical dependent (7α-ChOOH, 7β-ChOOH) cholesterol hydroperoxides. Photoinduced peroxidation of lipids was also tested in the presence of iron ions biological relevant electron donor such as NADH. Moreover we analyzed the modulatory effect of endogenous and exogenous melanin on the photoinduced peroxidation of lipids. Synthetic DOPA-melanin in liposomal systems, and two mouse melanoma cells (B16) lines – F10 and F0 were employed. Melanin content of the cell was obtained using EPR spectroscopy. Irradiation of B16 mouse melanoma cells with blue light in the presence of the fulerene induced significant killing of the cells. BB6 mediated photoperoxidation of lipids in POPC-cholesterol liposomes and in membranes of cultured mouse melanoma (B16) cells. This nanoparticle generated both: singlet oxygen and free radicals; however, in the presence of reducing agents such as NADH the efficiency of photogeneration of superoxide anion was significantly higher than that of singlet oxygen.

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P98

An integrated approach to isolate, optimize and characterize LDL and its conversion to oxLDL from healthy subjects

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One of the most relevant events in atherogenesis is the chronic oxidative process of low-density lipoprotein (LDL) to oxidized (oxLDL). This bioactive modified lipoprotein is an important factor that induces oxidative stress, lipid metabolism alteration, and inflammation into the arterial wall. Currently, there is no consensus about ideal oxLDL's physicochemical characteristics to be used in models related to immunogenic activity in atherosclerotic pathology; therefore it is so important to better understand the process underlying oxLDL generation and its characterization. The aim of this study was to optimize the oxidation conditions of isolated LDL from 75 apparently healthy subjects using parameters such as lipid peroxidation, apolipoproteinB alteration, particle size and eicosanoids levels. Plasmatic basal amounts of oxLDL evaluated by ELISA were compared with lipid profile, body mass index, blood pressure, nitric oxide, plasma antioxidant activity and eicosanoids (prostaglandins-(PGs), isoprostanes-(IsoPs)) quantified by targeted lipidomic. From the mentioned group, 15 samples were selected for extracting LDL and a subsequent oxidation with different concentration of copper and AAPH. TBARS, relative electrophoretic mobility and dynamic light scattering were used to evaluate the oxidative modification. It was observed a positive *Pearson's correlation*only between LDL and total cholesterol. For the isolated LDL we could determine that copper is more efficient than AAPH (5μM; 6h) to modify oxidatively the lipidic and proteic fraction without a significant change in the particle size. Finally, eight PGs and nine IsoPs were quantified into the dialyzed LDL and oxLDL and the comparative analysis shows that both has similar content of PGs, but oxLDL exhibits a significant increase in 6 IsoPs related to *in vivo* oxidative stress. This procedure developed can be used to obtain oxLDL under controlled conditions. A better comprehension of this important process is useful to understand the effects of oxLDL on cardiovascular pathophysiology experimental models.

P99

Protective role of fetal-to-neonatal transition under hypoxic atmosphere in newborn mice

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Background. Newborn asphyxia is an acute and severe condition during the fetal to neonatal transition. The use of pure oxygen for resuscitation has been traditionally standard of care. However, recently it has been shown that oxygen overexposure induces an oxidative stress (OS) and increases mortality. **Hypothesis.** We speculate that delaying postnatal oxygenation during postnatal stabilization would preserve reducing equivalents, enhance redox adaptation, and protect oxy-regulator tissues (e.g.:brain). **Objective**. To assess oxidative stress and inflammation status, in brain of mice pups submitted to different initial inspiratory fractions of oxygen (FiO_2) during fetal to neonatal transition (FNT). **Material and Methods**. Pregnant mice were kept in 21% (Nx21) or reduced to 14% (Hx14) the night before of delivery (G18). 8 hours after birth both groups were switched to room air (Hx14/21 and Nx21/21 groups) or subjected to hyperoxia (FiO_2=100%)(Hx14/100 and Nx21/100 groups) and reset to 21% after 1 hour. At day 1 of life (P1) the pups were sacrificed and the brain tissues were snap frozen and kept at -80ºC until the analysis. We set up a Mass Spectrometry (MS) method of four selected biomarkers: i) meta-tyrosine (m-tyr)/Phenylalanine (Phe), ii) orto-tyrosine (o-tyr)/Phe, iii) $3NO_2$-tyrosine ($3NO_2$-tyr)/para-tyrosine (tyr) and iv) 3Chloro-tyrosine (3Cl-tyr)/tyr ratios, to study cerebral OS and inflammation. **Results.** Hypoxia does not promote significant changes in none of biomarkers. By contrast, hyperoxia induces a significant increase in m-tyr/phe and 3Cl-tyr/p-tyr. Remarkably, pre-conditioning hypoxia abrogates the rise of m-tyr/phe and 3Cl-tyr/p-tyr induced by hyperoxia as well as a decrease in the o-tyr/phe. **Conclusions**. Our results support the idea that fetal to neonatal transition performed under hypoxic conditions could be neuroprotective improving postnatal adaptation.

P100

Roux-y gastric bypass surgery after 6 years: focus on inflammatory and oxidative stress markers

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Objectives and methods: Considering that the Roux-Y gastric bypass surgery (RYGB) is associated with changes in inflammatory and antioxidants responses, this prospective and controlled study followed in different intervals (1, 2 and 6 years), beside biometric and lipid profile parameters, several inflammatory biomarkers such as serum concentrations of nitric oxide (NO_X), myeloperoxidase (MPO), and C-reactive protein (CRP), oxidative stress biomarkers such as catalase (CAT) activity, contents of ferric reducing antioxidant potential (FRAP), reduced glutathione (GSH) and thiobarbituric-acid reactive substances (TBARS), as well as the contents of nutritional antioxidants such as the contents of vitamins E, C and β-carotene, measured in the blood of 20 patients submitted to the Roux-Y gastric bypass (RYGB) just before and after those different post-surgical periods. Results: RYGB was effective in decreasing body weight after the first year, but revealed a body weight regain of 12% between 1 and 2 years and further 7.5% between 2 and 6 years. Serum contents of NO_X and CRP, which were decreased after 1-2 years showed enhanced levels compared to the first year and even when compared to baseline levels. Serum levels of vitamins C and E as well as total cholesterol contents were increased, while β-carotene contents were continuously decreased during 6 years. CAT activity and TBARS and GSH contents, which were decreased until the first 2 years, returned to basal levels after 6 years of postoperative period. Conclusions: The persistent lowering of serum concentrations of β-carotene, vitamin C, GSH, FRAP and CAT activity, concomitant to the enhancement of inflammatory markers and the marker of lipoperoxidation (TBARS contents) found after 2 and 6 years of the postoperative period, probably contribute to the persistent cell damage and inflammation, therefore to the maintenance of a chronic systemic oxidative stress in post-surgical RYGB patients.

REDOX SIGNALING (P101-P130)

P101

4-Hydroxynonenal activates Src through a non-canonical pathway that involves EGFR/PTP1B and direct cysteine modification

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Src, a non-receptor protein tyrosine kinase involved in many biological processes, can be activated through both redox-dependent and independent mechanisms. 4-Hydroxy-2-nonenal (HNE) is a lipid peroxidation product that is increased in pathophysiological conditions associated with Src activation. This study examined how HNE activates human c-Src. In the canonical pathway Src activation is initiated by dephosphorylation of pTyr530 followed by conformational change that causes Src auto-phosphorylation at Tyr419 and its activation. HNE increased Src activation in both dose- and time-dependent manner, meanwhile it also increased Src phosphorylation at Tyr530 (pTyr530 Src), suggesting that HNE activated Src via a non-canonical mechanism. Protein tyrosine phosphatase 1B inhibitor (539741), at concentrations that increased basal pTyr530 Src, increased basal Src activity while reduced HNE-mediated Src activation by about 60%. The EGFR inhibitor, AG1478, abrogated HNE-mediated EGFR activation and partially inhibited basal and HNE-induced Src activity. In addition, AG1478 also eliminated the increase of basal Src activation by PTP1B. Taken together current data suggest that HNE activates Src through both canonical and non-canonical pathways involving activation of EGFR. Supported by NIH grant R21-ES020942

P102

Role of cysteine 328 and interaction with zinc in the dynamics of the vimentin network

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Vimentin is a Type III intermediate filament protein that plays both architectural and functional regulatory roles. The vimentin filament network undergoes a drastic reorganization in response to electrophilic or oxidative stress. We have recently shown that the single cysteine residue of vimentin, C328, is required for this reorganization. Indeed, treatment of cells with oxidative compounds induces juxtanuclear condensation or disassembly of vimentin, which is attenuated in the C328S mutant. In addition, we have observed that this cysteine residue is important for the primary assembly of the vimentin network as well as for filament stability. Since vimentin interaction with divalent cations may influence filament mechanical properties, we have explored the effect of zinc. Our results indicate that zinc reversibly binds to vimentin *in vitro* and that its cellular availability regulates vimentin dynamics and susceptibility to oxidants in a C328-dependent manner. Zinc chelation disrupts the vimentin network and this effect is prevented or reversed by zinc supplementation. The presence of zinc protects vimentin from modification by electrophiles targeting C328. Moreover, the zinc-specific fluorophore Zinquincolocalizes with vimentin bundles in cells, thus pointing to a direct interaction between zinc and vimentin. We are currently exploring the occurrence of this interaction in several cell types in which it could be pathophysiologically relevant, and addressing the mechanisms underlying vimentin regulation by zinc.

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P103

MC1R controls mitocondrial function and oxidative stress in melanoma.

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Melanoma has recently gained attention due to the important increase in its incidence in the last 20 years. Melanocortin 1 receptor (MC1R) is a well-known key gene for melanoma development. It is involved in growth, differentiation and pigmentation of melanocytes. Recent studies point out that it also has a role in the regulation of reactive oxygen species (ROS) and the protection against ultraviolet light. PGC-1α is a key regulator of these processes, inducing melanogenesis through the activation of the microphthalmia transcription factor (MITF) and stimulating mitochondrial biogenesis mediated by TFAM.

Our aim was to analyse whether the stimulation of MC1R leads to changes in mitochondrial function and oxidative stress. For this purpose, two melanoma cell lines were studied: HBL (wild-type for MC1R) and A375 (mutant for MC1R). Cells were treated with an agonist of the MC1R, the NDP-MSH, and parameters such as ROS levels, enzymatic activities of COX, ATPase and SOD and protein levels of PGC-1α were studied.

Results showed that ROS production was significantly higher in A375 cell line compared to HBL cell line under basal conditions. The addition of NDP-MSH to stimulate the MC1R resulted in a lower ROS production in HBL cells, but not in A375 since they have a non-functional receptor. Furthermore, enzymatic activities were significantly increased in HBL cells and were enhanced when MC1R was activated. There was also a rise in PGC-1α levels in HBL-treated cells, supporting the central role of this protein in MC1R signalling.

In conclusion, these results suggest that MC1R regulates mitochondrial function and improves the antioxidant response in order to reduce ROS levels. Mutations in this receptor altering its function could be a risk factor for developing melanoma.

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P104

Acute hypoxia produces a superoxide signal through mitochondrial complex I-dependent ion exchange via NCLX

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Oxygen is a key molecule necessary for aerobic life and as a consequence of its metabolism cells produce reactive oxygen species (ROS). Acute hypoxia has been shown to produce a superoxide burst that can be a signal promoting cell adaptation. We show that hypoxia triggers the acute activation of the mitochondrial Na/Ca^2 exchanger (NCLX),promoting mitochondrial depolarization and superoxide production. NCLX inhibition is associated with suppression of HIF-1α stabilization and reduced expression of its target genes. Mitochondrial complex I is also involved in the activation of the Na/Ca^2 exchange and ROS production, probably due to the Na/Hantiporter activity of its deactive form. These results show that complex I and NCLX take part in the acute oxygen sensing in several cell types, which is also linked to hypoxia adaptation through the HIF pathway. NCLX inhibition may also have clinical relevance in diseases where hypoxia, mitochondrial ROS signaling or oxidative stress are involved.

P105

Acute hypoxia signals mediated by reactive oxygen species: from superoxide production to thiol modification

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The adaptation to decreased oxygen availability (hypoxia) is crucial for proper cell function and survival. In metazoans, this is partly achieved through gene transcriptional responses mediated by the hypoxia-inducible factors (HIF). There is still a debate on whether the production of reactive oxygen species (ROS) increases in hypoxia, which in turn may contribute to the activation of the HIF pathway. In addition to altering the cellular redox balance, leading to oxidative stress, ROS are capable of transducing signals by reversibly modifying the redox state of cysteine residues of proteins.

We have used different techniques for measuring superoxide production kinetics, and we have observed that acute hypoxia produces a superoxide burst in different types of cells. We have uncovered a mechanism by which oxidative phosphorylation complex I and mitochondrial sodium-calcium exchanger (NCLX) cooperate in oxygen sensing and ROS signal production in different cell types.

By using diverse thiol redox proteomics techniques, we have observed an increase in cysteine reversible oxidation in endothelial cells in acute hypoxia. Indeed, we have been able to identify a number of proteins that are specifically oxidised in these conditions. These cysteine oxidation signals may mediate different adaptations to hypoxia, before the HIF pathway is fully activated.

We hypothesize that complex I deactivation acts as an oxygen sensor, driving mitochondrial sodium/calcium exchange and inducing a superoxide signal in mitochondria from cells subjected to acute hypoxia. This signal, which is common to a variety of cell types, can be translated into different responses in specific cell types by the oxidation of specific sensitive cysteine residues in different proteins.

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P106

MicroRNA-9 mediates fibrogenic transformation of dermal fibroblasts

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Excessive accumulation of extracellular matrix (ECM) proteins is the hallmark of fibrotic diseases. In the skin it is induced by stimuli including persistent infections, autoimmune reactions or tissue injury that lead to a process of improper wound healing by an "excessive" degree of repair. This process is dependent on the activation of ECM synthesis in interstitial fibroblasts that evolve into α-smooth muscle actin (α-SMA)-positive myofibroblasts and is mainly mediated by TGF-β signaling. MicroRNAs are small non-coding RNAs acting as regulators of gene expression at the post-transcriptional level controlling multiple biological processes, including fibrogenesis. We found upregulation of miR-9 expression in TGF-β1-treated dermal myofibroblasts. *In silico* identification of two target sites in the 3´UTR of the TGFβRII mRNAspotted this protein as a potential TGF-β signaling related target. Decreased luciferase reporter activity of the TGFβRII 3´UTR after transfection with miR-9 confirmed its specific binding to its target sites. Consistently, this microRNA downregulated TGFBRII expression at both the mRNA and protein levels. This microRNA also targets NOX4, which is involved in the generation of reactive oxygen species that contribute to the activation of TGF-β. In keeping, over-expression of this microRNA repressed TGF-β1-induced fibrogenesis in human dermal fibroblasts, decreasing the phosphorylation and nuclear translocation of Smad2/3, the abundance of α-SMA-positive fibroblasts, as well as the levels of α-SMA, collagen1α1 and fibronectin. The expression of miR-9 was also enhanced in the *in vivo* model of bleomycin-induced skin fibrosis while its levels in the sera from these mice were also increased. Taken together, these results support *in vitro* anti-fibrotic effects of this miRNA, through the downregulation of the TGF-β1 pathway via TGF-βRII and NOX4 silencing and suggest a new strategy for the prevention or treatment of skin fibrosis.

P107

Antioxidant enzyme activity and protein expression of heat stress in a model of hypoxic preconditioning

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Oxidative stress is caused by increased production of reactive oxygen species (ROS). To protect cells against oxidative stress, living organisms have developed an antioxidant defense system including catalase, superoxide dismutase (SOD), glutathione synthase (GST) and glutathione peroxidase (GPX).

Expression of heat-shock proteins (HSPs) occurs due to various factors that may act independently or simultaneously. Energy depletion, hypoxia situations or ROS production can induce transcription of HSPs, such as HSP70.

The aim of this study was to investigate whether an experimental model of intermittent hypobaric hypoxia (IHH) preconditioning could modulate the expression of HSPs (27 and 70) or the activity of SOD, the main line of antioxidant defense. Adult rats were separated randomly into a control group (CG), a group exposed for 6h to severe acute hypoxia (7% oxygen) in a normobaric chamber (HS), a group exposed to Pb 462 Torr (equivalent to an altitude of 4000 m) in a hypobaric chamber for 4 h/day during 8 days (IHH) and finally, a combined IHH HS group.

The results obtained in the study allowed us to observe that treatment with IHH causes an increase in antioxidant defenses, including SOD activity levels, which were increased 33% in the IHH HS group compared to HS group. Similar results were observed for HSP27 protein expression, which experienced a significant increase in expression in the IHH HS group relative to the levels observed in the HS group. On the other hand, no significant changes between IHH HS and HS groups were evident for HSP70.

These findings demonstrate that IHH improves enzymatic activity of antioxidant defenses where the significant increase in SOD and HSP27 may be implicated in the cyto-protection phenomenon known as preconditioning, whereas HSP70 is apparently not involved in neuroprotection. Nevertheless, this requires further investigation of the role of these proteins and their participation as neuroprotectors.

P108

Nrf2 deletion increases basal apoptosis *via* a caspase-independent mechanism involving monoamino oxidase activity and mitochondrial permeabilization

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Nuclear factor E2-related factor-2 (Nrf2) is a cap'n'collar/basic leucine zipper (b-ZIP) transcription factor which acts as sensor of oxidative and electrophilic stress. Low levels of Nrf2 predispose cells to chemical carcinogenesis but a dark side of Nrf2 function also exists because its unrestrained activation may allow the survival of potentially dangerous damaged cells. Since Nrf2 inhibition may be of therapeutic interest in cancer, and a decrease of Nrf2 activity may be related with degenerative changes associated with aging, it is important to investigate how the lack of Nrf2 activates those molecular mechanisms mediating cell death. The expression of antiapoptotic Bcl-2 and Bcl-xL proteins is under direct transcriptional control of Nrf2, and inhibition of Nrf2-dependent transcription is linked to the potentiation of etoposide and radiation-induced apoptosis in mouse and human hepatic tumor cells. We have investigated if the lack of Nrf2 modifies basal apoptotic signaling and cell death. Murine Embryonic Fibroblasts (MEFs) bearing a Nrf2 deletion (Nrf2KO) displayed diminished cellular growth rate and shortened lifespan compared with wild-type MEFs, indicating increased damage accumulation. In accordance, basal rates of DNA fragmentation and histone H2AX phosphorylation were increased in Nrf2KO MEFs. Enhanced rates of apoptosis were confirmed in tissues from Nrf2KO mice. Apoptosis was associated with a decrease of Bcl-2 (but not Bax) levels and with the release of the mitochondrial pro-apoptotic factors cytochrome *c* and AIF. Procaspase-9 and Apaf-1 were also increased in Nrf2KO MEFs but caspase-3 was not activated. Inhibition of XIAP increased cells death in Nrf2KO but not in wild-type MEFs. Interestingly, inhibition of monoamine oxidase B (MAO-B) diminished DNA fragmentation in Nrf2KO MEFs. Our results support that Nrf2 deletion results in mitochondrial dysfunction associated with MAO-B activation and a caspase-independent apoptotic pathway through AIF.

P109

Melatonin stimulates PKC phosphorylation and Ca^2 influx, and increases the expression of Nrf2-regulated antioxidant enzymes in mouse pancreatic acinar cells.

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We have studied the potential activation of the cellular antioxidant response in the presence of melatonin in freshly prepared mouse pancreatic acinar cells. Single cell fluorescence analysis was employed to monitor changes in intracellular free-Ca^2 concentration. Western blot analysis was used to study activation of PKC. Quantitative reverse transcription-polymerase chain reaction was employed to detect the expression of Nrf2-regulated antioxidant enzymes. Immunocytochemistry was employed to determine nulclear location of phosphorylated-Nrf2. And the cellular redox state was monitored following CM-H_2DCFDA-derived fluorescence. The results show that incubation of cells with melatonin (1 μM to 1 mM) induced a slow and progressive increase of $[Ca^2]_c$ towards a stable level. This response could only be noted in the presence of Ca^2 in the extracellular medium. Incubation of cells with the PKC activator PMA (1 μM) evoked a response similar to that induced by melatonin (100 μM). Conversely, in the presence of the PKC inhibitor Ro31-8220 (3 μM), stimulation of cells with melatonin failed to evoke changes in $[Ca^2]_c$. Employing an antibody specific for phospho-PKC, we noted that melatonin induced PKC activation. Melatonin also induced the phosphorylation and translocation towards the nucleus of the transcription factor Nrf2. A concentration-dependent increase in the expression of the antioxidant enzymes NAD(P)H-quinone oxidoreductase 1, catalytic subunit of glutamate-cysteine ligase and heme oxygenase-1 could also be noted. Finally, incubation of CM-H_2DCFDA-loaded pancreatic acinar cells in the presence of 1 nM CCK-8 induced a statistically significant increase in CM-H_2DCFDA-derived fluorescence, that was abolished by pretreatment of cells with melatonin (100 μM) or PMA (1 μM). Nevertheless, pretreatment of cells with Ro31-8220 (3 μM) blunted the effect of melatonin. We can conclude that melatonin stimulates Nrf2-surveillance of cellular oxidative state involving activation of PKC and Ca^2 signaling.

P110

First evidence of a glutathione S-transferase P-dependent feedback mechanism of the Nrf2 activation response.

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Glutathione S-transferase p (GSTP), a phase II gene downstream of the Nrf2-ARE/EpRE transcription pathway, plays a key role in both the detoxification and signaling response to Se-organic molecules with thiol peroxidase activity. The role of GSTP signaling on the Nrf2 activation response by these peroxidases remains unexplored and it was investigated using a new class of diselenides with expected hormetic activity that share the structure of $(PhSe)_2$. These diselenides, and particularly DSBA, behave as mild thiol peroxidases and cellular stressors by a limited depletion of cellular thiols, generation of H_2O_2 and NOx, and unbalanced activity of stress activated and survival-promoting kinases (namely JNK and ERK), which ultimately control the mitochondrial pathway of apoptosis. Such a mild or sub-maximal stressogenic effect produced an increased nuclear translocation of Nrf2 protein in murine embryonic fibroblasts (MEFs) with resulting induction of phase II genes such as the same GSTP and other GSTs and HO-1; this transcriptional response to the novel diselenides was also confirmed in HepG2 human hepatocarcinoma cells in which HO-1 and ALDH-1 protein levels were assessed. The knockout of GSTP gene in MEFs increased the toxicity of these Se-organic compounds increasing NOx generation and activating SAPK-JNK pathway. Noteworthy, $GSTP^{-/-}$ MEFs also showed a markedly increased nuclear translocation of Nrf2. Immunoprecipitation and affinity purification experiments provided early evidence of the existence of a complex between this transcriptional factor and GSTP protein that was absent in $GSTP^{-/-}$ MEFs. In conclusion, GSTP protein expression influences the Nrf2-dependent response to the hormeticdiselenides investigated in this study. Possible mechanistic interpretation for this GSTP-dependent effect may include a direct interaction of GSTP with Nrf2 protein.

NOTES

P111

Decreased human dental pulp stem cells proliferation at 21% O2 through P38/P21/NRF2 signaling pathway

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High rates of stem cell proliferation are an important tool in regenerative medicine. Ambient oxygen tensions (21% O_2) are normally used for *in vitro* culture, but physiological levels *in vivo* range between 3-6% O_2.

The aim of this study was first to characterize our human Dental Pulp Stem Cells (hDPSC), and then to analyze their proliferation under different oxygen conditions.

Stem cell properties include the ability to differentiate to specialised cells. After osteogenic and adipose induction, our hDPSC showed mineralized nodules and lipid droplets. We assessed further characterization based on stem cell surface markers, such as STRO1, OCT4, CD34, CD133 and Nestin.

We compared proliferation of hDPSC cultured under 21% versus 3% O_2. The rate of hDPSC proliferation is significantly lower at 21% O_2 compared to physiological oxygen levels due to enhanced oxidative stress. Under 21% O_2, increased p38 phosphorylation led to activation of p21, known to interact with cell cycle proteins to decrease proliferation. Increased generation of reactive oxygen species and p21 led to activation of the Nrf2 signaling pathway. The up-regulation of Nrf2 antioxidant defense genes under 21% O_2 may interact with cell cycle related proteins involved in regulating cell proliferation. Activation of p38/p21/Nrf2 in hDPSC cultured under ambient oxygen tension inhibits stem cell proliferation and up-regulates Nrf2 antioxidant defenses.

DPSC proliferation rate is significantly lower at 21% O_2 compared to 3% O_2. This is due to increased levels of oxidative stress at 21% O_2 which induced the activation of the cell signaling pathway p38/p21/Nrf-2.

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P112

Role of Nrf2 in regulating cellular antioxidant responses to chemical toxicity in fish

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The Nrf2-Keap1 pathway is present in fish, but its functionality as a master regulator of antioxidant defense is less characterized than in higher vertebrates. Obstacles to characterizing fish Nrf2 responses include the lack of molecular tools, as well as the duplication of Nrf2 and of Nrf2-regulated genes that resulted from the teleost-specific whole genome duplication. Zebrafish (*Daniorerio*) are an excellent model to understand the role of nrf2 in regulating teleost cellular defenses against oxidative stress due to the availability of transgenics, imaging capabilities, and molecular tools. We have shown that cadmium (Cd), an Nrf2 activator and inhibitor of olfactory function, elicits a dose-dependent induction of nrf2-regulated genes associated with adaptation to oxidative stress (e.g., *hemeoxygenase 1*, *glutathione S-transferase pi*, and *glutamate-cysteine ligase catalytic subunit*) in zebrafish larvae, and in isolated olfactory tissues of adult zebrafish. The increased antioxidant gene expression by Cd was attenuated by morpholino knockdown of *nrf2a*, which further disrupted olfactory mediated behaviors and increased the loss of olfactory sensory neurons by Cd. A recessive loss-of-function zebrafish mutant of nrf2a(*nrf2*^{fh318}) is being used to better define the role of nrf2 in maintaining zebrafish sensory behaviors under chemical exposures, and to evaluate the safety of emerging chemicals. Studies of prototypical Nrf2-regulated genes, including those with consensus electrophile responsive elements (EpREs), which mediate Nrf2-gene induction in higher vertebrates, are not as definitive in other fish. We are approaching this data gap by reporter studies of cloned promoters of fish genes containing EpREs (e.g. *gstp2* and *gst rho)* in transfected cells. Collectively, our studies indicate an evolutionarily conserved role of Nrf2 in protection against cellular oxidative stress in zebrafish, but care should be exercised in extrapolating Nrf2 responses across aquatic species. Supported by NIEHS P42-004696 and NSF 339637.

P113

Dominant Role of Peroxiredoxin/JNK Axis in Stemness Regulation during Neurogenesis from Embryonic Stem Cells

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Redox balance has been suggested as an important determinant of "stemness" in embryonic stem cells (ESCs). In this study, we demonstrate that peroxiredoxin (Prx) plays a pivotal role in maintenance of ESC stemness during neurogenesis through suppression of reactive oxygen species (ROS)-sensitive signaling. During neurogenesis, Prx I and Oct4 are expressed in a mutually dependent manner and their expression is abruptly downregulated by an excess of ROS. Thus, in Prx I–/– or Prx II–/– ESCs, rapid loss of stemness can occur due to spontaneous ROS overload, leading to their active commitment into neurons; however, stemness is restored by addition of an antioxidant or an inhibitor of c-Jun N-terminal kinase (JNK). In addition, Prx I and Prx II appear to have a tight association with the mechanism underlying the protection of ESC stemness in developing teratomas. These results suggest that Prx functions as a protector of ESC stemness by opposing ROS/JNK cascades during neurogenesis. Therefore, our findings have important implications for understanding of maintenance of ESC stemness through involvement of antioxidant enzymes and may lead to development of an alternative stem cell-based therapeutic strategy for production of high-quality neurons in large quantity.

P114

Testosterone production by Leydig cells is suppressed by hyperthermia-induced endoplasmic reticulum stress in mice.

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Testicular spermatogenesis requires a temperature lower than that of the general body. Leydig cells are endocrine secretory cells characterized by their ability to produce testosterone, which in turn is essential for normal spermatogenesis and fertility in mammals. When the Leydig cells are unable to produce enough testosterone, spermatogenesis fails completely. Considering this, it is of great interest to investigate whether the expression of steroidogenic enzymes (required for testosterone production in Leydig cells) is affected by testicular heat stress. In this study, the expression of the steroidogenic enzyme 3β-HSD was decreased by heat stress and human chorionic gonadotropin (hCG) treatment. While the levels of the endoplasmic reticulum (ER) stress markers GRP78/BiP and CHOP were increased by ER stress inducers, those of the steroidogenic enzyme and progesterone were decreased. In contrast, an ER stress inhibitor rescued the progesterone levels, even under heat stress conditions. Moreover, the clusters of Leydig cells were randomly scattered, and severely damaged upon repetitive testicular heat treatment. Additionally, immunohistochemical analyses revealed that GRP78/BiP, cleaved caspase-3, and TUNEL-positive staining were all elevated in the Leydig cells when compared with the controls. Thus, repetitive testicular heat treatment in mice promotes excessive ER stress, thereby leading to apoptosis of the Leydig cells and thus, decreased testosterone production in the mouse serum. Taken together, we demonstrate that heat-induced ER stress significantly influences steroidogenic enzyme expression and testosterone production in the Leydig cells. Therefore, our findings help to provide a mechanistic explanation to the impairment of spermatogenesis upon elevation of the testicular temperature.

P115

Progesterone production is affected by unfolded protein response (UPR) signaling during the luteal phase in mice

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AIMS:
We examined whether the three unfolded protein response (UPR) signaling pathways, which are activated in response to endoplasmic reticulum (ER)-stress, are involved in progesterone production in the luteal cells of the corpus luteum (CL) during the mouse estrous cycle.

MAIN METHODS:
The luteal phase of C57BL/6 female mice (8 weeks old) was divided into two stages: the functional stage (16, 24, and 48 h) and the regression stage (72 and 96 h). Western blotting and reverse transcription (RT)-PCR were performed to analyze UPR protein/gene expression levels in each stage. We investigated whether ER stress affects the progesterone production by using Tm (0.5 μg/g BW) or TUDCA (0.5 μg/g BW) through intra-peritoneal injection.

KEY FINDINGS:
Our results indicate that expression of Grp78/Bip, p-eIF2α/ATF4, p50ATF6, and p-IRE1/sXBP1 induced by UPR activation was predominantly maintained in functional and early regression stages of the CL. Furthermore, the expression of p-JNK, CHOP, and cleaved caspase3 as ER-stress mediated apoptotic factors increased during the regression stage. Cleaved caspase3 levels increased in the late-regression stage after p-JNK and CHOP expression in the early-regression stage. Additionally, although progesterone secretion and levels of steroidogenic enzymes decreased following intra-peritoneal injection of Tunicamycin, an ER stress inducer, the expression of Grp78/Bip, p50ATF6, and CHOP dramatically increased.

SIGNIFICANCE:
These results suggest that the UPR signaling pathways activated in response to ER stress may play important roles in the regulation of the CL function. Furthermore, our findings enhance the understanding of the basic mechanisms affecting the CL life span.

KEYWORDS:
Corpus luteum; ER stress; Luteal phase; Progesterone production; Unfolded protein response.

P116

Repeated superovulation via PMSG/hCG administration induces 2-Cys peroxiredoxins expression and overoxidation in the reproductive tracts of female mice

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Superovulatory PMSG/hCG treatment is known to affect oocyte quality, maternal environment, which may result from PMSG/hCG treatment-induced oxidative stress. 2-Cys peroxiredoxins (2-Cys Prxs) act as antioxidant enzymes that protect cells against oxidative stress. This study investigated the effects of repeated PMSG/hCG treatment on 2-Cys Prx expression in female reproductive tracts of mice. Repeated PMSG/hCG stimulation induced histomorphological changes in the ovaries and uteri. The protein expression levels of 2-Cys Prxs after PMSG/hCG treatment were increased most significantly in the ovaries, while that of Prx1 was most affected by PMSG/hCG stimulation in all reproductive organs.Repeated PMSG/hCG treatment eventually led to 2-Cys Prxsoveroxidation in all reproductive organs. Our finding suggest that excessive oxidative stress caused by repeated PMSG/hCG stimulation induces histomorphological changes and increases expression and overoxidation of 2-Cys Prxs in the female reproductive organs. Intracellular 2-Cys Prxs are crucial to maintaining the reproductive organ environment upon exogenous gonadotropin treatment.

P117
SOD2 systemic deficiency leads to tumor aggressive and relapse in TRAMP mice

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The manganese superoxide dismutase (SOD2/MnSOD) is the first antioxidant barrier due to its location in the matrix mitochondria. Its role during tumor progression is controversial. It was first reported as a tumor suppressor protein whereas more recent results suggested its implication in tumor progression. In prostate cancer, previous data indicated that SOD2 is upregulated in patients both at mRNA and protein levels and H_2O_2 levels were increased. This result was confirmed in other 2 tumor types, i.e. colon and lung. The TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model shares several features with the human disease. SOD2 expression increased in 24- and 32-week-old TRAMP mice, and it significantly decreased after castration. SOD2 knockout heterozygous mice (SOD2 +/-) were mated with TRAMP animals to compare the prostate tumor formation in the TRAMP and TRAMPSOD2+/- offspring. TRAMPSOD2+/- males showed lower genitourinary tract weight than their TRAMP siblings until 24 weeks, but up to 32 weeks they experimented a quicker growth and showed more aggressive tumors being in the 50% of cases poorly-differentiated tumors compared to 12.5% in TRAMP mice. Also seminal vesicles showed a 3-fold higher incidence of tumors in TRAMPSOD2+/- mice than in TRAMP animals at 32 weeks. TRAMP and TRAMPSOD2+/- mice were castrated at 12 weeks of age and sacrificed at 24 and 32 weeks. None castrated TRAMP mice exhibited a tumor relapse after castration and prostates were completely atrophic, however 2 out of 15 TRAMPSOD2+/- castrated mice developed a poorly-differentiated relapse. Surprisingly, tumor relapses showed stronger and wider androgen receptor (AR) staining and it was both nuclear and cytoplasmic conversely what happened in TRAMP castrated mice which only showed a cytoplasmic AR staining. SOD2 systemic deficiency in TRAMP mice leads to higher AR expression which causes an initial growth retardation but promotes more aggressive tumors and relapse after castration.

P118
SOD2 imbalance disrupts spermatogonial stem cell differentiation

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Redox regulation by reactive oxygen species (ROS) has a key role in cell signaling, participating in decisions of proliferation-differentiation cell fate. Spermatogenesis is a complex process involving self-renewal of spermatogonial stem cells as well as their differentiation to mature haploid spermatozoa. Differentiation signaling of this process includes modulation of ROS levels. Thus, expression of antioxidant enzymes including superoxide dismutases (SOD), which control H_2O_2 production, might have a physiological role in spermatogenesis. Additionally, it is assumed that uncontrolled ROS levels might also affect spermatozoa production. In order to study the participation of MnSOD/SOD2 in this process, WT C57BL6/J as well as $SOD2^{+/-}$ knock down and overexpressing $SOD2^{+/++}$ mice were employed. Mice were sacrificed at 4, 6, 10 and 24 weeks of age and testis were dissected and collected for immunohistochemistry or for molecular biology studies. Testis sections were stained with DAPI and observed under confocal microscopy for spermatogenic cells counting. Additionally, RNA was extracted and used to perform a semi-quantitative PCR to analyse the expression levels of PGP9.5, Sycp3 and Shippo-1 as markers for spermatogonia, spermatocyte and haploid cells, respectively. qPCR for androgen receptor (AR) was also carried out. $SOD2^{+/-}$ mice show a higher AR expression than WT and $SOD2^{+/++}$. A significant decrease in the amount of both, spermatocytes and haploid cells, was also found in $SOD2^{+/-}$ mice. Accordingly, $SOD2^{+/++}$ mice show a higher number of these spermatogenic cells. An increase in the number of spermatogonia and a decrease in both spermatocytes and haploid cells in $SOD2^{+/-}$ and $SOD^{+/++}$ mice between 6 and 10 and 24 weeks of age was also observed. Therefore, we demonstrate that an imbalance in SOD2 levels and a subsequent modulation of H_2O_2 levels alters the spermatogenesis likely through a redox regulation of AR signaling in Sertoli cells.

P119

Evaluation of the effects of anti-angiogenic compounds on cell redox state

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The proper functioning of cells in our oxidant atmosphere requires a tight control of redox homeostasis. Aerobic metabolism gives rise to the emergence of reactive oxygen species (ROS) as byproducts. An unbalance of the redox homeostasis can induce oxidative stress. Cells have several lines of defense against oxidative stress, including a set of anti-oxidant enzymes, small anti-oxidant compounds, damage repair enzymes and cell death control.

In our lab we have identified and characterized a number of natural bioactive compounds able to inhibit angiogenesis. This communication will show data regarding the ability of some of these compounds to modulate the redox state of both endothelial and tumor cells.

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P120

p38α Deficiency causes oxidative stress and delays liver regeneration after partial hepatectomy

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p38 MAPK is a redox-sensitive kinase that may play a novel role in cytokinesis promotion, the last step of mitosis. Cytokinesis failure generally occurs in hepatocytes leading to plolyploid cells, and increases upon age. Interestingly, hepatocyte ploidy is a reversible feature: hepatocytes are able to change from polyploid to diploid, and from binucleated to mononucleated by a phenomenon called somatic 'reductive mitoses'. Furthermore, oxidative stress may induce binucleation.

Since p38**α** knock out mice exhibited higher GSSG/GSH ratio after partial hepatectomy (HPx), and oxidative stress could be mediating failed cytokinesis, we worked with liver specific p38**α** knock out mice in a context that hepatocytes need to efficiently proliferate. In our model, p38**α** deletion significantly reduced liver proliferation 72h after HPx. Nevertheless, deletion of p38**α** did not completely block the priming of hepatocytes. The binucleation rate was reduced after HPx, but HPx p38**α** knock out livers exhibited higher binucleation rates when compared to HPx wild-type livers. Regarding cell cycle, the G_1/S checkpoint was markedly altered by p38**α** deletion: a significant increase in mRNA levels of *CyclinD1* was found in HPx p38**α** knock out livers in comparison to wild-type mice. The progression G_2/M checkpoint was blocked in p38**α** knock out mice, which exhibited significant rise in mRNA levels of *CyclinA1*, *Cdc25* and *CyclinB2*, as well as mitotic delay. Furthermore, the p38**α**-mediated signaling pathway during cytokinesis showed a significant decrease in MNK1 phosphorylation upon p38**α** deficiency, which may impair cell abscission.

In conclusion, p38**α** modulates intracellular redox status and mitotic transit, which makes p38**α** necessary for complete recovery of liver mass after HPx. p38**α** is not essential for ploidy reversal but it is likely to contribute to ploidy reversal's coupled cytokinesis and to S-phase followed cytokinesis by activating MNK1.

P121

Rapid mobilization of intracellular "labile iron" modulates MAP kinase signal transduction, following exposure of cells to H_2O_2

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It has been shown that hydrogen peroxide (H_2O_2) can act as a second messenger in signal transduction, participating in redox regulated pathways, including cytokine and growth factor induced signals. However, the exact molecular mechanism(s) underlying these processes remain poorly understood and need further investigation. Using Jurkat T-lymphoma cells and primary endothelial cells (HUVECs), we investigated the capacity of "intracellular labile iron" to modulate signal transduction in H_2O_2-mediated apoptotic pathways. Exposure of cells to H_2O_2, induced a rapid elevation of intracellular labile iron level, most probably by promoting mobilization of lysosomal iron. At the same time ferritin expression increased. On the other hand, modulation of "intracellular labile iron" by desferioxamine (DFO) induced profound effects on mitogen activated protein kinase (MAPK)-mediated cell signals. In order to identify the points of action and to elucidate the molecular mechanisms of these iron effects, we investigated selected steps in H_2O_2-induced apoptosis. It was observed that labile iron was implicated in the sustained activation of stress activated MAPKs, JNK1/2 and p38, but not ERK1/2. It is concluded that the level as well as the temporal and spatial distributions of "intracellular labile iron" represents a key factor in redox regulated signaling pathways, able to determine the final decision of cells about survival or death. Unraveling the role of iron in redox regulated signaling pathways may open the road for potential interventions in serious pathological conditions.

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P122

Molecular mechanisms responsible for the antiproliferative effects of Bergamot Essential Oil and its extractive fractions on SH-SY5Y human neuroblastoma cells

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In the area of cancer, essential oils have drawn the attention of researchers because some molecules present in the phytocomplexes, including monoterpenes and coumarins, may have antitumor property.

Bergamot essential oil (BEO) is obtained by rasping the fruit peel of *Citrus bergamia* (bergamot), a small tree cultivated almost exclusively along the southern coast of Calabria region (Italy). BEO is very appreciated for its organoleptic properties so as to be widely used in the manufacture of perfumes.

The goals of our study were to investigate the mechanisms underlying the antiproliferative effects of BEO on human neuroblastoma SH-SY5Y cells and to identify the compounds mainly responsible for its cells growth rate inhibition.

Five BEO extractive fractions (BEOs) differing in their chemical composition were used. Cell proliferation was determined by MTT and cell count assays. Both trypan blue exclusion test and Annexin V/PI staining were performed to assess their cytotoxic activity. Genotoxicity was detected by comet assay. It was also checked the cell cycle cytofluorimetrically. ROS and $\Delta\psi m$ were measured fluorimetrically. Western blotting analyses for some apoptosis-related proteins were carried out.

Treatment of SH-SY5Y cells with some types of BEOs decreased cell growth rate by a mechanism correlated to both apoptotic and necrotic cell death. Colored BEOs act by increasing ROS generation, responsible for the drop in $\Delta\psi m$, and modulate p38 and ERK ½ MAPKs, p53, Bcl-2 and Bax signaling pathways.

Finally, we identify bergamottin and 5-geranyloxy-7-methoxycoumarin as the bioactive molecules that play a pivotal role in the antiproliferative effects exerted by coloured BEOs.

Our study provides novel insights into the field of the antiproliferative effects of BEO, which could be exploited in the context of a multitarget pharmacological strategy.

P123
The activation of PGC-1α protects the liver from IR damage

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The study is focused on an important clinical problem, liver transplant. The number of donor livers is insufficient to cover the needs for liver transplants, which drives the use of suboptimal livers, like those that have an important accumulation of fat (fatty liver/ steatotic liver). The steatosis makes the liver more sensitive to the damage that results from the ischemia-reperfusion process (IR) but also makes it less responsive to preconditioning protocols that normally make the livers more resistant to IR damage.

Our study shows that PGC-1α in response to IR is necessary to protect the liver from IR damage and mediates the activation of protection mechanisms following the ischemic preconditioning protocol. It also suggests that the reduced activity of PGC-1α in the steatotic liver could be responsible for its increased sensitivity to IR damage and poor response to preconditioning protocols. These observations allow us to propose PGC-1α as a relevant therapeutic target whose activation in the donor livers, could facilitate the clinical use of steatotic livers for transplantation. Therefore, we believe this study has adequately addressed the objectives initially proposed and it has identified PGC-1α as a key element in hepatic physiology, providing relevant insight into the mechanisms involved and opening new therapeutic venues.

P124

Estrogen replacement therapy induces antioxidant and longevity-related genes in women after medically induced menopause

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Previous studies from our laboratory demonstrate the ability of oestrogens to up-regulate longevity-related genes in rodents.

The aim of the current study was to ascertain whether estrogen replacement therapy (ERT) also induces the expression of antioxidant and longevity-related genes in women.

For this purpose, 16 women of reproductive age (18-42 years), underwent a medically-induced menopause with GnRh analogues, and were afterwards replaced with estrogens and progesterone. Blood samples were obtained at four different times (basal, after induced menopause, after estrogen replacement and after progesterone replacement). Whole blood was collected in VACUTAINER®CPT™ tubes with heparin, obtaining mononuclear cells by centrifugation, or in heparin-tubes, to get the whole blood. RNA was extracted from mononuclear cells using the TRIZOL®Reagent, following the manufacturer's instructions. Gene expression (P53, P21, sestrins (SENS2), manganese superoxide dismutase (MnSOD), glutathione peroxidase (GPx) and 16s rRNA) was determined through real time polymerase chain reaction (RT-PCR). Blood levels of reduced glutathione (GSH) were determined by high performance liquid chromatography (HPLC) after derivatization of the sample.

P21, GPx and 16s rRNA gene expression, as well as blood GSH levels decreased with menopause, when compared to basal levels. After estrogen replacement, we found an increase in P53, P21, GPx, MnSOD, and 16s rRNA mRNA expression, as well as an increase in blood GSH levels. Progesterone replacement resulted in a significant increase in SENS2 and P53 mRNA expression, when compared to basal expression.

ERT induces antioxidant and longevity-related gene expression in women suffering from a medically-induced menopause. This, therefore, may confirm results previously seen in rodents.

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P125

Homocysteine treatment increases total redox capacity in both endothelial and tumor cells

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Homocysteine is a non-proteinogenic amino acid playing key roles in two interconnected metabolic pathways, namely, the activated methyl cycle and the linear trans-sulfuration pathway that allows the conversion of methionine to cysteine. A dysregulation of intracellular homocysteine metabolism could yield an increased export of this amino acid, leading to hyperhomocysteinemia, which has been associated with an increased risk of cardiovascular diseases. In spite of decades of experimental effort, there is no definitive consensus on what could be the molecular mechanisms whereby hyperhomocysteinemia could contribute to cardiovascular disease. The redox active nature of homocysteine has favored the idea of an induction of oxidative stress as the underlying mechanism of homocysteine toxicity. In contrast, homocysteine can also behave as an anti-oxidant. The present work is aimed to further analyze the capacity of homocysteine to modulate the redox capacity of both endothelial and tumor cells.

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P126

Resveratrol induces PTEN-mediated antioxidant gene expression in MCF-7 cells

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Antioxidant properties of resveratrol have been intensively studied for the last years, both *in vivo* and *in vitro*. Its bioavailability after an oral dose is very low and therefore it is very important to make sure that plasma concentrations of free resveratrol are sufficient enough to be active as antioxidant.

In the present study, using nutritionally relevant concentrations of resveratrol, we aimed to confirm its antioxidant capacity on reducing peroxide levels and look for the molecular pathway involved in this antioxidant effect.

For this purpose, we used mammary gland tumor cells (MCF-7), which were pretreated with different concentrations of resveratrol for 48 h, and/or a PTEN inhibitor (bpV: bipy). Hydrogen peroxide levels were determined by fluorimetry, PTEN levels and Akt phosphorylation by Western Blotting, and mRNA expression of antioxidant genes by real-time reverse transcriptase-polymerase chain reaction (RT-PCR).

Resveratrol treatment for 48 h lowered peroxide levels in MCF-7, even at low nutritional concentrations (1 nM). This effect was mediated by the activation of PTEN/Akt pathway, which resulted in an up-regulation of catalase and MnSOD mRNA levels.

As a conclusion, resveratrol acts as an antioxidant at nutritionally relevant concentrations by inducing the expression of antioxidant enzymes, through a mechanism involving PTEN/Akt signaling pathway.

This work was supported by grants SAF2010-19498 and SAF2013-44663-R,ISCIII2012-RED-43-029 from (RETICEF);RS2012-609 INCLIVA Intramural Grant and EU Funded CM1001 and FRAILOMIC-HEALTH.2012.2.1.1-2. The study has been co-financed by FEDER funds from the EU.

P127

SIRT3 silencing increases the effect of treatment in MCF-7 breast cancer cell line

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Breast cancer is the most common malignancy diagnosed among women worldwide. Reactive oxygen species (ROS) and mitochondrial dysfunction are key factors for the development, progression and treatment of this disease. Sirtuin 3 (SIRT3), the major deacetylase in mitochondria, has been reported to participate in the regulation of mitochondrial metabolism and the antioxidant response to ameliorate oxidative stress. Thus, SIRT3 could have a protective role allowing the cell to counteract the effect of anticancer therapies, which target mitochondria and increase ROS levels.

The aim of this study was to analyze whether SIRT3 knockdown in breast cancer cells could increase oxidative stress and therefore make them more sensitive to cytotoxic treatments. MCF-7 breast cancer cell line was treated with cisplatin (CDDP) or tamoxifen (TAM) alone or in combination with a siRNA against SIRT3. Parameters such as ROS production, cell viability, apoptosis and autophagy were studied.

Results showed that SIRT3 silencing significantly raised both mitochondrial membrane potential and ROS levels. ROS production was increased by 96% with SIRT3 silencing alone, and this increase was even more significant, higher than 200%, when cells were treated with CDDP or TAM. Cell viability showed an important decrease when the cytotoxic treatment was combined with SIRT3 knockdown. This reduction in viability was due to an increment in the apoptotic process in CDDP-treated cells, while in TAM-treated cells there was an induction of the autophagic cell death.

In conclusion, these results suggest that SIRT3 is a key factor regulating mitochondria when cells are exposed to oxidative stress. SIRT3 could be a therapeutic target against breast cancer, since it improves the effectiveness of cytotoxic treatments.

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P128
RCAN1: implication in oxidative stress and apoptosis

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Introduction: Rcan1 (regulator of calcineurin1), originally named as DSCR1 (Down Syndrome Candidate region gene 1), belongs to a highly conserved family of proteins that regulate Calcineurin's activity. Calcineurin is a serine/threonine phosphatase playing an important role in cellular responses to extracellular signals and to environmental stresses and it is important in the regulation of memory processes, in differentiation and in apoptosis. It is known that RCAN1 might play a central role in pathological processes because of its increased expression after exposure to damaging stimuli associated with Ca2 overloading, such as oxidative stress.

Materials and Methods: The following animals were used: 6 WT mice, 6 RCANKO mice, 6 WT mice treated with Paraquat and 6 RCANKO mice treated with Paraquat. After the treatment they were sacrificed and their organs extracted. We used homogenated tissue to perform western blots and plasma to measure oxidation markers.

Results: RCAN1 is overexpressed in paraquat treated WT mice. MDA levels, oxidized glutathione and protein oxidation decreased in paraquat-treated RCAN KO mice, suggesting that those animals suffered less oxidative stress. Antioxidant enzymes were overexpressed in cortex of RCANKO mice treated with paraquat.

Conclusions: RCAN KO mice show less oxidative stress after paraquat injection, probably due to increase of antioxidant levels.

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P129

Intestinal anti-inflammatory properties of an anthocyanin-rich fraction from Portuguese blueberries (*Vacciniumcorymbosum* L.) in a TNBS colitis rat model: comparison with 5-aminosalicylic acid

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Inflammatory bowel disease (IBD) is a chronic inflammatory disorder characterized by periods of remission and relapses whose etiology needs to be clarified. This disease does not have cure yet and among the therapeutic strategies, 5-aminosalicylic acid (5-ASA) is a well-established drug, unfortunately not devoid of adverse effects, requiring the need to explore alternative therapeutic strategies. In this context, anthocyanins have been a focus of intensive research, as they are the most common dietary polyphenols and show strong antioxidant activity and ability to modulate inflammatory pathways. The poor intestinal absorption and high intake of these compounds make the intestinal tract the compartment with the highest concentrations which may play a key role in modulating IBD.

Thus, the aim of this work was to assess the intestinal anti-inflammatory action of an anthocyanin-rich fraction (ARF) isolated from Portuguese blueberries (*Vacciniumcorymbosum* L.) in a colitis rat model, as compared to 5-ASA.

The ARF showed a very high content and diversity of anthocyanins, as studied by HPLC-DAD analysis. Its anti-inflammatory role was evaluated in a well characterized trinitrobenzenesulfonic acid (TNBS) rat model of colitis, after daily administration by intragastric infusion (10 mg anthocyanin/kg/day) during 8 days, as compared with 5-ASA (100 mg/kg/day) alone or combined with the fraction. ARF significantly counteracted the TNBS-induced inflammatory markers, namely the body weight loss and colonic tissue damage, and the increases in either myeloperoxidase and alkaline phosphatase activities or COX-2 and iNOS expressions in colon homogenates, in a more effective way than 5-ASA. Also, the anti-inflammatory effect of this drug was improved by its association with anthocyanins.

In conclusion, blueberry anthocyanins show a relevant intestinal anti-inflammatory action improving the 5-ASA efficacy, suggesting its potential role in IBD management.

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P130

The absence of the *B. cinerea bcsod1* reduced fungal virulence altering plant responses.

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Plants activate response signals during infection by pathogens to halt their advance and an oxidative burst may take place. The necrotrophic pathogens can produce reactive oxygen species (ROS) to contribute to the oxidative imbalance in plants that benefits the colonization process (Heller and Tudzynski, 2011). In a previous work, we demonstrated that tomato plants challenged with the necrotroph*Botrytis cinerea* accumulated ROS and callose, along with the induction of genes involved in defense, signaling and oxidative metabolism (Finiti et al, 2014). In this work, we studied the infection phenotype of the *B. cinereaΔbcsod1* strain (Rolke et al., 2004) in both tomato and Arabidopsis plants. This mutant lacks the gene *bcsod1*, which encodes the superoxide dismutase Cu-Zn-SOD that catalyzes the conversion of superoxide ion in hydrogen peroxide. These ROS species play a protective role and act as signaling compounds in plants. *Δbcsod1* displays a reduced virulence relative to the wild type in both species. Plants infected with the mutant accumulate less H_2O_2 and a higher amount of O_2^- in the infection area than those infected with the wild type. This was associated to an increased accumulation of the defensive polymer callose. The early induction of the callose synthase gene *PMR4* suggests that changes in ROS metabolism alter plant defensive responses leading to a reduced virulence of *B. cinerea.* Gene analysis and metabolites involved in signaling and ROS detoxification showed significant alterations in the complex signaling networks of the plant responses against *B. cinerea*, supporting this connection.

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NERVOUS SYSTEM & NEURODEGENERATION (P131-P161)

P131

Regulation of Bcl_{xL}-ATP synthase interaction by mitochondrial cyclin B1-Cdk1 determines oxidative complex I damage and neuronal survival

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The survival of post-mitotic neurons needs continuous degradation of cyclin B1, a mitotic protein aberrantly accumulated in the damaged brain areas of Alzheimer's disease and stroked patients. Degradation of cyclin B1 takes place in the proteasome after ubiquitylation by the anaphase-promoting complex/cyclosome (APC/C)-Cdh1, an E3 ubiquitin ligase that is highly active in neurons. However, during excitotoxic damage – a hallmark of neurological disorders– APC/C-Cdh1 is inactivated causing cyclin B1 stabilization and neuronal death through an unknown mechanism. Here, we show that an excitotoxic stimulus in rat cortical neurons in primary culture promotes cyclin B1 accumulation in the mitochondria, where it binds to –and activates– cyclin-dependent kinase-1 (Cdk1). Cyclin B1-Cdk1 complex in the mitochondria phosphorylates the antiapoptotic protein Bcl-xL leading to its dissociation from the ß subunit of F_1F_o-ATP synthase. The subsequent inhibition of ATP synthase activity causes complex I oxidative damage, mitochondrial inner membrane depolarization and apoptotic neuronal death. These results unveil a previously unrecognised role for mitochondrial cyclin B1 in the oxidative damage associated with neurological disorders.

P132

A new pathway to excitotoxicity in alzheimer's disease by inactivation of the anaphase promoting complex/cyclosome-Cdh1 E3 ubiquitin ligase

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The anaphase promoting complex/cyclosome (APC/C) is a large protein complex forming an E3 RING finger ubiquitin ligase that has a canonical role as cell cycle regulator in proliferating cells. Yet, in the last decade a new role of APC/C in neurons has been described. APC/C-Cdh1 regulates the degradation of key glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase-3. Thereby it controls the bioenergetics and antioxidant status in neurons.

Here we describe a new pathway by which amyloid beta (Aβ), a peptide abundantly present in Alzheimer 's disease, causes an elevated generation of glutamate in neurons, mediated by the down-regulation of E3 ubiquitin ligase APC/C-Cdh1 activity. Our results show that Aβ decreases the protein level of cdh1 in neurons in primary culture and this leads to an accumulation of the APC/C-Cdh1 degradation target glutaminase. This enzyme converts glutamine to glutamate, and we observed that increased glutaminase levels lead to an elevation of glutamate concentration in the extracellular medium and subsequently to higher intracellular Ca^2 levels in neurons. We have tested these findings in vivo using the transgenic APP/PS1 model of AD. We have observed a decreased protein level of cdh1 and an increase in glutaminase in the transgenic animals. These results indicate that the cell cycle related ubiquitin ligase APC/C-Cdh1 plays a central role in excitotoxicity, and could therefore be an interesting target against neurodegeneration in AD.

P133

PI3K/Akt/FoxO signaling mediated neuroprotective effects of nicotinamide in Aβ(1-42) induced experimental alzheimer disease's model

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Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline and associated with widespread senile plaques (SP) and neurofibrillary tangles (NFT) that ultimately lead to progressive synaptic dysfunction and neuronal death. In some animal models intracerebral administration of Aβ(1-42) induces neurodegenerative disorders that mimic AD. Mitochondrial dysfunction, oxidative stress and cell death mechanisms have been proposed as important contributing factors in AD. Protein kinase B (PKB/Akt) is an intracellular signal transduction protein which is activated by various neurotrophic factors and cytokines. PKB/Akt, downstream target of phosphatidylinositol-3-kinase (PI3K), plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration. Activation of PI3K/Akt signaling mediates neuroprotection in *in vivo* and *in vitro* models of neurodegenerative diseases.

In the present study we searched the neuroprotective effects of nicotinamide (100/500 mg/kg NA) on PI3K/Akt pathway in experimental model of Alzheimer's Disease (AD) induced by Aβ(1-42). For *in vivo* AD model; stereotaxic intrahippocampal Aβ(1-42) injections were made bilaterally. The effects of Aβ(1-42) and nicotinamide on PI3K/Akt pathway were investigated by real-time PCR in hippocampus through the mRNA expression levels of PI3K, Akt1, Akt2, FoxO1, FoxO3a, Bim and Puma. Aβ(1-42) treatment only caused significant increases in the mRNA expressions of FoxO1, FoxO3a, Bim and Puma while decreased PI3K, Akt1 and Akt2 mRNA expressions. NA treatments against Aβ(1-42) significantly elevated PI3K, Akt1 and Akt2 expressions and decreased the mRNA levels of FoxO transcription factors and proapoptoticBim and Puma. In conclusion, nicotinamide may be helpful as a therapeutic agent in neurodegenerative diseases like AD through the activation of PI3K/Akt pathway.

P134
Delirium and oxidative stress

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Delirium is a common manifestation of brain dysfunction in critically ill patients. According the Diagnostic and Statistical Manuel of Mental Disorders (DSM-IV), it is an acute and fluctuant alteration of perception and consciousness due to a somatic serious disorder. Data from 15 consecutively admitted intensive care unit (ICU) delirium patients (10 men, 5 women, mean age : 83 ± 4.7 years) were examined to determine whether oxidative stress (OS) existed within 24 hours after admission to ICU. Sixteen markers of OS including antioxidants (vitamin C, alpha and gamma - tocopherol, beta - carotene, thiol proteins, SOD and GPx, ubiquinone, total glutathione), trace elements (selenium, copper, zinc, ratio, copper/zinc) and markers of lipid peroxidation (lipid peroxides, oxidized LDL and isoprostanes) were investigated and compared to those of 38 older but robust adults (19 men and 19 women, mean age : 73 ± 4.2 years) and to normal reference values (see poster Pincemail et al. Oxidative stress or not in healthy older subjects ?).

We observed a significant decrease ($p < 0.05$) in vitamin C, alpha - tocopherol, gamma - tocopherol, ubiquinone, total glutathione, thiol proteins, zinc and selenium concentrations respectively by 45%, 25%, 40%, 30%, 10%, 17%, 23% and 39% in delerium patients when compared to robust subjects. In fact, we evidenced a total collapse of plasma non enzymatic antioxidant defenses since some of these parameters (vitamin C, thiol proteins, zinc and selenium) were largely below normal reference values. By contrast, there was a significant increase of the copper/zinc ratio by 75% and of lipid peroxides by 35%. Surprisingly both concentrations of oxidized LDL and isoprostanes tended to decrease in delirium patients.

In conclusion, delirium was associated with an important increase of OS. This may therefore suggest the implementation of an antioxidant therapy since the admission of delirium patients in ICU.

P135

Altered mitochondrial function, oxidative stress and leukocyte/ endothelium interactions in leukocytes of anorexia nervosa patients

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Background: Anorexia nervosa is a common illness among adolescents and is related to cardiovascular complications and characterized by oxidative stress. Our aim was to study the effect of this condition on metabolic parameters, mitochondrial function and redox state, leukocyte-endothelium interactions, adhesion molecules and proinflammatory cytokines.

Materials and Methods: The following parameters were evaluated in 24 anorexic patients and 36 controls: anthropometric and metabolic factors, interactions between polymorphonuclear neutrophils (PMN) and human umbilical vein endothelial cells (HUVEC), proinflammatory cytokines (TNFα and IL-6) and cellular adhesion molecules, including E-selectin, VCAM-1 and ICAM-1. Mitochondrial function and redox state in PMNs were evaluated by measuring mitochondrial O_2 consumption, membrane potential, reactive oxygen species production, glutathione levels, mitochondrial mass, and complex I and III activity.

Results: Anorexia nervosa induced a decrease in PMN rolling velocity and an increase in PMN rolling flux and PMN adhesion. Increases in IL-6, TNFα and VCAM-1 were also observed. Mitochondrial function was impaired in leukocytes from the anorexic patients, we observed decreases in mitochondrial O_2 consumption, mitochondrial membrane potential and GSH levels, and an increase in ROS production. Furthermore, a reduction of mitochondrial mass was detected, while the activity of mitochondrial complex I, but not that of complex III, was inhibited.

Conclusions: Our results support the hypothesis of an association between anorexia nervosa, inflammation and the induction of leukocyte-endothelium interactions, which might explain, in part, the increased risk of vascular disease among these patients. Moreover, oxidative stress is produced in the leukocytes of anorexic patients and is closely related to mitochondrial dysfunction. We propose that the oxidative stress that occurs in anorexia takes place at mitochondrial complex I

P136

Effects of natalizumab, dimethyl-fumarate and N-acetylcysteine in an experimental model experimental of autoimmune encephalomyelitis

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Aim:
The main aim was to analyze the role played by oxidative stress in experimental autoimmune encephalomyelitis (EAE)

Material and Methods:
The study was performed on 60 *Dark agouti* rats aged 8 weeks. These rats were divided into 6 groups of 10 as followsg: i) control; ii) vehicle; iii) EAE; iv) EAE Nata; v) EAE DMF and vi) EAE NAC. The EAE inductor agent was myelin oligodendrocyte glycoprotein (MOG) emulsified in Freund´s adjuvant supplemented with heat-inactivated Mycobacterium tuberculosis. N-acetyl-cisteine (NAC) and dimethyl-fumarate (DMF) were administered through a gastric catheter, while Natalizumab was administered by intraperitoneal injection. The treatments were applied during 21 days starting 14 days after inoculation with MOG. Animals were scored on days 1, 14 and 35 for signs of EAE. On day 35 the animals were sacrificed and biomarkers of oxidative damage and mitochondrial viability were determined.

Results:
EAE showed an increase in limb paralysis together with increases in oxidative damage biomarkers and decrease in mitochondrial viability. The treatment with Natalizumab, DMF and NAC decreased limb paralysis, reversed toward normality oxidative damage and increased mitochondrial viability.

Conclusions:
Our date show that

1- EAE reproduces a good experimental model of relapsing-remitting multiple sclerosis.
2- The important role played by oxidative stress in this disease
3- Oxidative stress maybe an important target to design and develop a new therapeutic strategy

However, more experimental and clinical studies in this line are required.

P137

Loss of mitofusin 2 links beta-amyloid-mediated mitochondrial fragmentation and Cdk5-induced oxidative stress in neuron cells.

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Mitochondrial dysfunction is implicated in age-related degenerative disorders such as Alzheimer's disease (AD). Maintenance of mitochondrial dynamics is essential for regulating mitochondrial function. Ab oligomers (AbOs), the typical cause of AD, lead to mitochondrial dysfunction and neuronal loss. AbOs have been shown to induce mitochondrial fragmentation, and their inhibition suppresses mitochondrial dysfunction and neuronal cell death. Oxidative stress is one of the earliest hallmarks of AD. Cyclin-dependent kinase 5 (Cdk5) may cause oxidative stress by disrupting the antioxidant system, including Prx2. Cdk5 is also regarded as a modulator of mitochondrial fission; however, a precise mechanistic link between Cdk5 and mitochondrial dynamics is lacking. We estimated mitochondrial morphology and alterations in mitochondrial morphology-related proteins in Neuro-2a (N2a) cells stably expressing the Swedish mutation of amyloid precursor protein (APP), which is known to increase AbO production. We demonstrated that mitochondrial fragmentation by AbOs accompanies reduced mitofusin 1 and 2 (Mfn1/2) levels. Interestingly, the Cdk5 pathway, including phosphorylation of the Prx2-related oxidative stress, has been shown to regulate Mfn1 and Mfn2 levels. Furthermore, Mfn2, but not Mfn1, over-expression significantly inhibits the AbO-mediated cell death pathway. Therefore, these results indicate that AbO-mediated oxidative stress triggers mitochondrial fragmentation via decreased Mfn2 expression by activating Cdk5-induced Prx2 phosphorylation.

P138

Dominant role of peroxiredoxin/JNK axis in stemness regulation during neurogenesis from embryonic stem cells

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Redox balance has been suggested as an important determinant of "stemness" in embryonic stem cells (ESCs). In this study, we demonstrate that peroxiredoxin (Prx) plays a pivotal role in maintenance of ESC stemness during neurogenesis through suppression of reactive oxygen species (ROS)-sensitive signaling. During neurogenesis, Prx I and Oct4 are expressed in a mutually dependent manner and their expression is abruptly downregulated by an excess of ROS. Thus, in Prx I(-/-) or Prx II(-/-) ESCs, rapid loss of stemness can occur due to spontaneous ROS overload, leading to their active commitment into neurons; however, stemness is restored by the addition of an antioxidant or an inhibitor of c-Jun N-terminal kinase (JNK). In addition, Prx I and Prx II appear to have a tight association with the mechanism underlying the protection of ESC stemness in developing teratomas. These results suggest that Prx functions as a protector of ESC stemness by opposing ROS/JNK cascades duringneurogenesis. Therefore, our findings have important implications for understanding the maintenance of ESC stemness through involvement of antioxidant enzymes and may lead to development of an alternative stem cell-based therapeutic strategy for production of high-quality neurons in large quantity.

P139

Oxidative stress affects FET protein localization and alternative pre-mRNA processing in cellular models of ALS

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FUS/TLS, EWS and TAF15 are members of the FET family of DNA and RNA binding proteins, involved in multiple steps of DNA and RNA processing and implicated in the regulation of gene expression and cell-signaling. All members of the FET family contribute to human pathologies, such as neurodegenerative diseases. Mutations in *FUS/TLS,* in *EWSR1* and in *TAF15* genes cause Amyotrophic Lateral Sclerosis (ALS), a fatal human neurodegenerative disease that affects primarily motor neurons and is characterized by the progressive loss of motor neurons and degradation of the neuromuscular junctions. ALS-associated FET mutations cause FET protein relocalization into cytoplasmic aggregates, thus impairing their normal function. Cytoplasmic mislocalization of FET proteins contributes to the formation of cytoplasmic aggregates that may alter RNA processing and initiate motor neuron degeneration. Interestingly, oxidative stress, which is implicated in the pathogenesis of ALS, triggers the accumulation of mutant FUS in cytoplasmic stress granules where it binds and sequester wild-type FUS. In order to evaluate the role of FET proteins in ALS and their involvement in the response to oxidative stress, we have developed cellular models of ALS expressing ALS-related FET mutants in neuroblastoma cell lines. Upon treatment with sodium arsenite, cells were analysed by immunofluorescence to monitor the localization of wild-type and mutated FET proteins. Furthermore, we have characterized signal transduction pathways and cell survival upon oxidative stress in our cellular models of ALS. Interestingly, we found that EWS protein display a different localization from FUS. Moreover, we identified EWS regulated alternative splicing events in genes with critical role in ALS. Remarkably these events are also affected by oxidative stress. Collectively, our data provide a new link between the oxidative stress response and RNA metabolism in ALS disease.

P140

Role of *S*-nitrosoglutathione reductase (GSNOR) as new molecular player in amyotrophic lateral sclerosis.

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Amyotrophic lateral sclerosis (ALS) is a neuromuscular disease characterized by motor neuron degeneration and skeletal muscle atrophy. The 2% of ALS cases are related to genetic mutations (familial form, fALS), affecting the gene coding for SOD1. To date, 100-point mutations have been identified. Among them, the G93A substitution ($SOD1^{G93A}$) has been suggested to confer a new toxic function to the enzyme, e.g. the capability to produce reactive oxygen species. Oxidative stress condition can be exacerbated by the accumulation of nitric oxide (NO), whose role in ALS still waits to be clarified.

In this work we enter this issue focusing on the denitrosylase*S*-nitrosoglutathione reductase (GSNOR), the enzyme responsible for the maintenance of *S*-nitrosylated protein (PSNO) homeostasis. *S*-nitrosylation is an NO-mediated posttranslational modification occurring on cysteine residues.

By analyzing $SOD1^{G93A}$ overexpressing mice, we observed a general accumulation of cellular proteins, which increased from early to late symptomatic stages, arguing for ALS being included among protein aggregation diseases. Indeed, coupled with this condition, we got evidence of a general impairment of autophagy, as demonstrated by the accumulation of p62/sequestosome 1 (SQSTM1). However, despite this scenario, GSNOR mRNA and protein levels were significant reduced in both spinal cord and skeletal muscle (gastrocnemius), this being associated with PSNO increase. Interestingly, such alterations were much more evident in gastrocnemius. Therefore, we analyzed gastrocnemius from conditional transgenic mice overexpressing $SOD1^{G93A}$ only in skeletal muscle ($MLC/SOD1^{G93A}$) and found the same alterations. These data confirm that GSNOR is modulated in ALS etiopathogenesis, but mostly give strength to the new theory according to which ALS is mainly a muscular disease. In vitro experiments provide evidence for a direct role of GSNOR in ALS onset.

P141

Novel modulators of redox homeostasis for schizophrenia.

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In a proof of concept clinical trial with schizophrenia patients, the administration of the glutation (GSH) precursor N-acetylcysteine (NAC) lead to an improvement in negative symptoms (Berl 2008), as well as in auditory evoked potentials (Lavoie 2008) and in cortical synchronization (Carmeli 2012). NAC however has several major drawbacks; It has biodistribution issues, specifically at the blood-brain barrier level, and it is an inefficient precursor when the synthesis of GSH is impaired, as it is presumable for the patients. We thus aim at developing compounds specifically designed to overcome NAC issues. We set up a panel of cell-based mechanistic assays and we are currently using this panel for both lead-oriented and high throughput compound library screening. We are developing and testing β-amino acids analogs, biologically more stable than the natural α-amino acids, identified in many natural products with some traditional antioxidant indication. As example, the first assay of this panel monitors the capability of the test compounds to preserve neuronal GSH levels following an oxidative stress challenge. It enables us to estimate effective concentrations of compounds and to rank their potency with NAC. The full assay panel ranges from monitoring endogenous antioxidant defense compounds, oxidant-trigged cellular response pathways, redox cellular state, to end consequences of oxidative stress as mitochondrial dysfunction, DNA and lipid oxidation. We also plan to translate some of these readouts to rodent paradigms, so we can monitor the same biological process in our animal models. The ultimate goal is to a) deploy a pre-clinical translational platform to assess the effectiveness and benefit of these analogues for the treatment of schizophrenia and b) Then to select those that most efficiently improve the physiological redox balance and potentially alleviate schizophrenia related phenotypes. These compounds might represent potential candidates for clinical trials.

P142

Characterization of Oxidative Damage in Brains of Rats Subjected to Acute Hypoxia and Reoxygenation

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Hypoxia exposure results in reduction of oxygen of tissues and increased formation of reactive oxygen species (ROS), which causes oxidative damage to DNA, proteins and lipids. The effects of hypoxia depend on level and duration as well as tissue status. Exposure to hypoxia generates a decrease in the activity of antioxidant enzyme system, which is lower in brain compared to other tissues, making it more susceptible to hypoxic stress. The aim of this study was to investigate the oxidative stress impact in brain following a treatment of hypoxia/reoxygenation. Rats (~250g males) were divided into non-hypoxic and hypoxic groups. The experimental animals were subjected to 6h of hypoxic exposure (a humidified mixture of gases consisting of 93% nitrogen and 7% oxygen) and then sacrificed at 0, 24 or 48h of reoxygenation. The levels of oxidants (thiobarbituric reactive substances, TBARS), antioxidant enzymatic activities of superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase, tissue content of reduced glutathione (GSH), oxidized glutathione (GSSG) and GSH/GSSG ratio, and tissue damage indicators of protein oxidation (AOPP) were measured. Hypoxia caused an increase in the oxidative stress indicators (TBARS 60% and AOPP 71%) and a decrease in antioxidant activities (SOD 29% and GR 33%); and also caused a diminution in GSH content and GSH/GSSG ratio in brain tissue. In summary, oxidative stress markers increased with hypoxia; although basal values were recovered after 24-48h reoxygenation, indicating that the hypoxic insult was not enough to cause long-term effects on the activity of these oxidative stress markers. These observations suggest that hypoxia is responsible for the production of ROS, which overhead the antioxidant system causing oxidative stress in rat brain.

P143

Preconditioning with intermittent hypobaric hypoxia protects against glutathione depletion and oxidative damage in hypoxic rat brain

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Oxidative stress damage is an important factor in the pathogenesis of brain injury due to hypoxia. However, we previously demonstrated (Coimbra Costa et al., 2013) that intermittent hypobaric hypoxia (IHH) exposure can increase brain antioxidant capacity and induce neuroprotection.

The aim of the present work was to use IHH as a preconditioning model against acute hypoxic induced damage. Adult rats were divided into three groups: 1) Acute Hypoxia group (AH) subjected to 6 h of an acute hypoxic exposure of 7% oxygen; 2) Intermittent Hypobaric Hypoxia group (IHH) exposed to 380 mmHg (equivalent to an altitude of 4000 m) in a hypobaric chamber for 4 h/day for 8 days; and 3) a combined IHH AH group that after the last IHH exposition was subjected to acute severe hypoxia (7% oxygen) for further 6 h. Brains were extracted and results compared to the AH group.

AH induced oxidative stress in the brain as indicated by increased levels of oxidized proteins, lipid peroxidation, inducible nitric oxide synthase (iNOS) expression and nitric oxide metabolites. Glutathione depletion and glutathione peroxidase increased activity also resulted from acute hypoxic exposure. The use of IHH as a preconditioning model against acute hypoxic damage was able to reduce by a 28% the nitric oxide levels, by a 30% the content of oxidized proteins and by 48% the lipid peroxidation values, and to better preserve the ratio of oxidized/reduced glutathione in brain tissue.

This study demonstrates that IHH can be used as a model for preconditioning to reduce damage from oxidative stress. The mechanisms by which this protection occurs and the time frame of that protection are currently being investigated.

P144

Efavirenz alters mitochondrial respiratory function in cultured neurons and glial cells

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The non-nucleoside analogue reverse transcriptase inhibitor Efavirenz (EFV) is among the most widely employed antiretroviral drugs. Although generally considered safe, EFV has been linked with several adverse effects including neurological manifestations which appear in the majority of the patients on EFV-including regimens and often require discontinuation of the therapy. The molecular mechanisms responsible for these manifestations are not understood but mounting *in vivo* and *in vitro* evidence points to the presence of altered brain bioenergetics. Here, we evaluate the effect of short-term (1h) EFV (10 and 25μM) treatment on the mitochondrial respiratory function in cultured glial and neuron cell lines by employing the Seahorse Extracellular Flux Analyzer. Incubation with EFV provoked a significant and concentration-dependent decrease in the basal respiration. Glial cells were more susceptible to EFV-exposure than neurons and displayed a reduction of roughly 50% of basal Oxygen Consumption rate (OCR) with EFV 25μM. Specifically, the ATP production-coupled O_2 consumption of both SH-SY5Y and U-251MG cells was undermined with the effect being more pronounced again, in glial cells. On the contrary, EFV did not alter the mitochondrial proton leak in neither of the cell types. EFV led to a decrease in the respiration control ratio, and reduced maximal respiration rate and spare respiratory capacity in both glial cells and neurons, being the former cells more susceptible. Importantly, neuronal exhaustion can have fatal consequences, as while resting neurons utilize approximately 6% of their maximal respiratory capacity, firing neurons utilize up to 80%. These data reveal that mitochondria in cells under EFV treatment operate closer to their bioenergetic limit. In sum, our findings reveal that EFV specifically alters mitochondrial respiration, which is of relevance for a better understanding of the molecular mechanisms responsible for the EFV-associated neurological effects recorded in the clinics.

P145

Finding biomarkers in plasma of patients suffering from mild cognitive impairment or Alzheimer's disease

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Alzheimer's disease is the most common form of primary dementia. This disease, due to its high prevalence in elderly people requires a lot of inversion and the improvement of the social system to adapt to the patient's needs. Therefore, identifying new biomarkers of early AD has turned into a priority for the scientific community. This study aims to identify biomarkers of Alzheimer's disease (AD) and its previous stage, mild cognitive impairment (MCI) either from blood samples and cerebrospinal fluid (CSF).

Determination of aβ, total tau and phosphorylated tau in CSF is included as criteria for AD diagnosis. In this study we took samples from 25 healthy people (controls), 25 patients with diagnosed MCI and 25 patients with AD. CSF was extracted from all of them by lumbar puncture and blood samples were also taken to obtain plasma and serum. From these samples we determined levels of aβ, total tau, phosphorylated tau, cluterin, regulator of calcineurin (RCAN1), protein kinase RNA-activated (PKR), calcineurin, receptor of advanced glycation end-products (RAGE) and ApoE genotype.

Patients diagnosed with MCI or AD showed lower levels of aβ in CSF and higher levels of total tau and phosphorylated tau than control patients. In addition, AD patients had lower plasmatic levels of clusterin and increased levels of RAGE. Finally, both groups (MCI and AD) showed decreased levels of PKR.

Finding blood biomarkers will be promising for early diagnosis of AD in the development of successful therapies, in this study we conclude that clusterin, RAGE and PKR could be used as biomarkers for AD. Both, clusterin and RAGE are involved in aβ clearance from the brain since they correlate with aβ levels. Also we want to remark that aβ, total tau and phosphorylated tau correlate with the clinical diagnosis of patients.

P146

Neuroprotective expression of markers in the brains of rats treated with intermittent hypobaric hypoxia against oxidative damage caused by severe acute hypoxia

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The vascular endothelial growth factor (VEGF) is an angiogenic protein induced by hypoxia and, with neurotrophic effects. Some neurotrophic factors can protect neurons from hypoxic or ischemic damage; thus, the expression of this marker was studied in an experimental model with the aim of using intermittent hypobaric hypoxia (IHH) as precondition to improve oxidative damage caused by severe acute hypoxia. Moreover, the expression of the neuroprotector agent, Erythropoietin (EPO), was also estimated in the same model.

Neurotrophic factors such as the levels of oxidants, thiobarbituric reactive substances (TBARS), and tissue damage indicators for protein oxidation (AOPP) and activity in the oxid nitric complex (NOx), were measured.

The rats were randomly divided into four groups: Severe Normobaric Hypoxia (HS) subjected for 6h to severe acute hypoxia (7% oxygen) in a normobaric chamber; Intermittent Hypobaric Hypoxia (IHH) maintained at Pb 462 Torr (equivalent of 4000 m) 4 h/day during 8 days; Preconditioning (IHH HS); and, Control in normoxic environment.

The results demonstrate that the IHH reduces the oxidative stress indicators (TBARS, AOPP and NOx) in the rats of the IHH HS group compared with the HS group. The neuroprotector factor induced by IHH was also observed via the increased expression of VEGF and EPO in the IHH HS group in comparison with the HS group.

This study demonstrates that IHH sessions can be used as preconditioning in order to reduce tissue damage caused by oxidative stress in response to severe acute hypoxia. The neuroprotective mechanisms need to be further studied.

P147

Oxidative stress during age-related dementia induced by D-galactose and treated by Simvastatin on rat: *In vivo* magnetic resonance study

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Recent studies suggest that chronic administration of D-galactose (D-gal) induced generation of reactive oxygen species (ROS) and increasing age with ROS that results in oxidative stress have been proposed to be an important step leading to neuronal death in neurodegenerative disorders and dementia such as Alzheimer disease. To test this hypothesis, male Wistar rats were subjected to a model of age-related dementia induced by D-gal (120 mg/kg) and treated with Simvastatin (40 mg/kg), a drug with various properties including anti-oxidant effects which pass through the hemetoencephalic barrier. The technique of proton and phosphorus magnetic resonance spectroscopy (^{1}H and ^{31}P MRS) was used to evaluate a neurochemical profile and energy metabolism of rats. Cognitive features (learning and memory) were evaluated by the Morris water maze test and thiobarbituric acid reactive substances plus total coenzyme Q in plasma were established to determine possible oxidative stress.

Our results suggest that, by chronic administration of D-gal to the rat, we could induce the early stages of age-related dementia, accompanied by changes in cytosolic metabolite concentrations (^{1}H MRS), especially N-acetylaspartate (NAA), which is believed to be a marker for neuronal number and health. Additional, NAA was remedied after Simvastatin treatment. Further, we have shown that long-term administration of D-gal created conditions for systemic oxidative stress, manifested by increased plasma lipid peroxidation and decreased concentration of coenzyme Q, and administration of Simvastatin returned values to a normal and also standardized brain CK reaction, a sensitive indicator of oxidative stress. Our results also show a slight decrease in cognitive features, where Simvastatin improves cognitive capacity. We concluded that D-gal alone is capable of inducing neurodegenerative symptoms, and that administration of Simvastatin is able to eliminate it.

P148

PEGylated Cerium oxide nanoparticles improves hypobaric hypoxia induced cognitive deficit in rat by augmenting hippocampus plasticity

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Cerium oxide nanoparticles (CNPs) are emerging class of Nanotherapeutics especially as a potential regenerative antioxidant. Several *in vivo* and *in vitro* studies suggest the beneficial effects of CNPs. Recently new routes of protective activities of CNPs have been revealed that include SOD and catalase mimetic activity, improvement of mitochondrial membrane potential and other bioenergetics roles. Neuroprotection by CNPs has also been attributed to its antioxidant capacities in neuronal cell lines. However its inability to cross BBB and larger size of particles restrict its *in vivo* applications. We, encapsulated the particles with polyethylene glycol (PEG) and restricted them to size ~4 nm thereby enabling a significant proportion to cross BBB. We then analysed if previously known intrinsic properties of CNPs could ameliorate the neuronal damage reportedly caused by hypobaric hypoxia. Interestingly, we found that these particles PEG-CNPs were not only reducing RNOS and related oxidants in brain but also augmented the hippocampus plasticity by neurogenesis which was reflected in the Morris water maze based memory function tests in rat. We further investigated the mode of protection and found that particles tend to upregulate the AMPK-aPKC mediated signaling causing the neurogenesis in rat hippocampus. We therefore report a PEG-CNPs as brain permeating Neuroprotective particles with wide applications in neurodegenerative diseases.

P149

Neuroprotective effects of a fermented grain powder: calcium homeostasis and HO-1 activation in a neuronal model of DTT-induced stress

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According to WHO predictions by 2040 neurodegenative diseases (NDs) will overtake cancer to become the second leading cause of human death after cardiovascular diseases. Increase of oxidative stress and aggregation of misfolded proteins are among the main features of NDs.

Even if the mechanisms leading to oxidative stress in the brain are multiple, many studies suggest an important role of Nrf2(Nuclear factor erythroid-derived 2-like 2) pathway; in particular, aNrf2-mediated-heme-oxygenase (HO-1) has a marked effect against oxidative damage and neuronal death.

Recently, natural or synthetic neuroprotective compounds acting as Nrf2 activators have been identified, such as resveratrol, sulforaphane and epicatechin.

In our work we evaluated the potential neuroprotective action of Lisosan G on murine hippocampal cells (HN9.10e) exposed to dithiothreitol (DTT). Lisosan G is a fermented grain powder which already showed hepatoprotective action and beneficial effects on endothelial dysfunction. These positive results are due to its high content of bioactive compounds, generated by the fermentation process. DTT is a well-known endoplasmic reticulum (ER) stress inducer, which not only causes a depletion of ER calcium, but also an oxidative stress increase. Perturbation in calcium homeostasis directly correlates with the occurrence of ER stress and with the extent of protein misfolding.

Treatment with Lisosan G ameliorates viability and reduces ROS content, both in the cytosol and in the mitochondria of HN9.10e exposed to DTT, by scavenge activity. The treatment also inhibits calcium loss from ER, thus allowing its correct functioning. Moreover, gene expression of antioxidant enzymes such as Gpx-1, SOD-2 and HO-1, is modulated by treatment with Lisosan G, in particular HO-1 is strongly induced suggesting an involvement of the Nrf2 pathway.

Our results indicate a remarkable potential of LisosanG asneuroprotective agent, very useful in NDs prevention.

P150

Age-dependent changes in the NMDAr-NO pathway in the hippocampus of a triple transgenic mouse model of Alzheimer´s disease: implications for neurometabolic regulation.

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Alzheimer´s disease (AD) is a multifactorial disease in which early alterations include changes in neuromolecular plasticity, synaptic deterioration, oxidative damage to cellular constituents and impaired energy metabolism. Nitric oxide (•NO) is linked to the activation of glutamatergic transmission and acts as a neuromodulator in synaptic plasticity as well as an effector of neurometabolic and neurovascular coupling. It has also been linked to neuronal toxicity and degenerative processes. As such, changes in the glutamate:nNOS:NO signaling pathway may be implicated in AD progression.

We performed a longitudinal study using the triple transgenic model of AD (3TgAD). We evaluated changes in nitrergic signaling and activity-dependent O_2 consumption rates using carbon fiber microelectrodes to record •NO and O_2 in hippocampal slices challenged with NMDA. We assessed changes in oxidative phosphorylation using high-resolution respirometry with intact hippocampal slices. Furthermore, we investigated the putative shift in •NO bioactivity towards increased peroxynitrite formation by determining the pattern of 3-nitrotyrosine staining in the hippocampus.

Using this approach we found: (1) age-dependent decrease in NMDA-evoked •NO peak concentrations with increased signal ½ width in NonTg and 3TgAD; (2) decrease of activity-induced O_2 consumption in 3TgAD when compared NonTg; (3) age-dependent decrease in basal and maximal resporatory rates in both genotypes, as well as decrease in sparing capacity; (4) increase 3-NT staining in the hippocampus of 3TgAD when compared to age-mathedNonTg controls.

Our data supports the notion of aging as the major risk factor in synaptic and bioenergetics degeneration in AD. Also, changes in NMDAr-dependent •NO may contribute not only to degradation of synaptic plasticity, but also regulation of mitochondrial function in AD progression.

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P151

Neurometabolic Changes in the Central Nervous System of Awake-Behaving Rodent Models of Epilepsy

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Temporal lobe epilepsy (TLE) is a form of acquired epilepsy in which humans and animals experience recurrent epileptic seizures arising from one or both lobes of the brain. The initial trauma can induce complex biochemical, physiological and structural changes that contribute to the development of epileptogenisis and onset of spontaneous and recurrent seizures.

The extreme change in neuronal excitability during seizures perturbs energetic demand (observed as an initial decrease in focal O_2) and produces a vascular response (observed as increased cerebral blood flow, CBF, and volume, CBV).

The spatiotemporal dynamics of neurovascular and neurometabolic coupling during the evolution of an ictal event are poorly understood. *In vivo* electrochemistry combining fast electrochemical techniques (amperometry) with micro(bio)sensors allow us to measure O_2, glucose and glutamate molecules directly in the brain extracellular space with high spatial and temporal resolution. The measurement of changes in [O_2] in the interstitial space using multisite platinum-based microelectrodes arrays is particularly attractive because it reports relevant information regarding both neurometabolic and vascular function.

Here we investigate changes in O_2 in the hippocampus of awake-behaving rats treated with pilocarpine to induce TLE. The *in vivo* measurements were performed using the FAST16mkIII electrochemical system (Quanteon, LLC, USA). Our results show that seizure activity is accompanied by drastic changes in O_2. Onset of a seizure is accompanied by an increase O_2 consumption followed by an increase in interstitial O_2 concentration resulting from typical increase in CBF at epileptic focus.

Combining these observations with recordings of glutamate, glucose and electrical activity (local field potentials) at the epileptic focus affords us a unique tool to investigate neurochemical and metabolic events associated with seizures in TLE.

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P152

Microelectrode-based sensors and biosensors for high resolution measurements of neurochemicals in the brain

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Direct measurements of neurochemicals in the brain extracellular space with high resolution tools is of paramount importance to understand both chemical signaling and metabolite trafficking between cells.The use of micro(bio)sensors associated with fast electrochemical methods fulfill the most strict requirements for neurochemicals measurements in complex environments, due to their high spatial and temporal resolution of tens of micrometers and milliseconds to seconds, respectively. Other important key features include very high sensitivity, selectivity, biocompatibility and minimal damage to tissue.

Over the past years we have developed electrochemical micro(bio)sensors based on carbon fiber microelectrodes (CFMs) and ceramic-based multisite platinum microelectrodes arrays (MEAs) for *in vivo* measurement of electroactive (e.g. dopamine, ascorbate, nitric oxide, oxygen and hydrogen peroxide) as well as non-electroactive neurochemicals (e.g. glutamate, choline, glucose and lactate). Microelectrode surface modification for measurement of electroactive neurochemicals has been accomplished by exclusion layer coatings and/or nanomaterials in order to enhance the analytical performance. A rational design of microbiosensors for measuring non-electroactive substances has been accomplished through the immobilization of oxidase enzymes in a suitable polymeric film onto the microelectrode surface combined with the amperometric detection of hydrogen peroxide. Micro(bio)sensor surface morphology and analytical evaluation have been assessed by SEM and electrochemical techniques, respectively. Moreover, *in vivo* measurements have been performed by the FAST16 electrochemical system (Quanteon, LLC, USA).

In this work, high resolution measurements of relevant neurochemicals *in vivo* in rat brain by using electrochemical micro(bio)sensors will be presented.

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P153

Neonatal exposure to low-dose ionizing radiation induces a long term inflammatory response in adult mice Hippocampus

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The brain may be exposed to ionizing radiation (IR) following nuclear accidents, atomic weapon testing and use, and medical treatment. Epidemiological and animal studies reported that low doses of IR to the human brain during infancy may induce cognitive dysfunctions. Although the underlying mechanisms involved in the development of radiation-induced cognitive deficit are not fully understood, evidence suggests that inflammation in the hippocampus plays a key role. The aim of this study was to evaluate the levels of pro-inflammatory cytokines in the hippocampus of mice exposed to internal radiation (^{137}Cs) during neonatal brain development. At the postnatal day 10 (PND10), mice (C57BL/6J) received subcutaneous injection of ^{137}Cs at activities of 0, 500, 100, 200, 4000 or 8000 Bq/kg. At the age of 20, 40 and 60 weeks, the levels of pro-inflammatory cytokines (IL-1β, IL-2 and IFN-γ) in hippocampus were studied. Results showed that the lower cesium activities studied induced Th1 Cytokines rather than the higher ones. These results correlate to behavioural impairment seen in this group of animals. A better understanding of the mechanism of cognitive and neurological dysfunction at low radiation doses is pivotal in minimizing radiation-associated health risks.

P154

Effect of electrical pulse stimulated media and thymoquinone on the neuronal toxicity of acrolein, hydrogen peroxide, and amyloid-β

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Electrical Pulse Stimulation (EPS) of myotubes is a reliable method to recapitulate the physiological effect of exercise *in-vitro*. Using EPS, we conditioned the media in the presence of myotubes. In order to fundamentally investigate the effect of exercise on neurons, we utilized the conditioned media to culture neuronal cells.

Two groups of 144 hours of differentiated C2C12 were divided into chronic or acute EPS groups and a control group with no EPS was kept as a control. Each of the groups was further divided to two subgroups of high glucose (15mM for 48 hours) or control. The parameters for acute EPS consisted of 12V, 50Hrtz during 1 second -1 second of pause, for the entire duration of 90 minutes. Chronic EPS was conducted at 12V, 1Hrtz during 6mSec - 1 second of pause, for the entire duration of 24 Hours.

Following EPS the conditioned media were collected. The human neuroblastoma cell line (SK-N-SH) was cultured in each of the conditioned media. Furthermore, SK-N-SH cells were separately treated with different toxins or oxidants, namely acrolein, amyloid-β, or and hydrogen peroxide, all of which are involved in the cellular stresses of neurodegeneration. Finally the effect of thymoquinone (TYM) as an antioxidant was investigated. Cell viability and LD50 were measured following each treatment.

The results indicate that none of the conditioned media are toxic to the SK-N-SH cells. The conditioned media were not able to protect against hydrogen peroxide with or without addition of TYM. All conditioned media protected against acrolein toxicity until 2 micromolarconcentration was reached and TYM did not show any additional protection in this regard. Conditioned media did show evidence promising of protection against amyloid-β toxicity, which was more prominent in the presence of 40 micromolar TYM.

Our findings suggest that exercise may help protect against the neuronal toxicity of acrolein and amyloid- β, which play crucial roles in the onset of Alzheimer's disease.

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Neuroprotective effect of anthocyanins against the amyloid-β peptide toxicity on neuronal cell line: relevance to Alzheimer's disease.

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder of aging. It is a multifactorial disease with several overlapping pathways. Therefore, successful treatment will rely on simultaneously targeting multiple pathological features. Polyphenols have proven to exert neuroprotective effects through different mechanisms. In this study, we tested the hypothesis that a formulation based on a mixture of anthocyanins may mitigate the amyloid-ß peptide (Aβ) toxicity. Anthocyanins are a class of polyphenols able to cross the blood brain barrier and their intake is associated to a reduced risk of some several chronic diseases.

Our results showed that the formulation MAF14001, based on 4 anthocyanins, was more efficient than individual compounds in the protection against Aβ-induced cell toxicity. From 5 µM, MAF14001 protected SK-N-SH cells against Aß toxicity by preventing oxidative stress, mitochondrial dysfunction and apoptosis. Furthermore, MAF14001 might directly interact with Aß to prevent its aggregation process, believed to play a key role on Aß-induced oxidative stress. Indeed, in the presence of MAF14001, Aß was less susceptible to fibrillization. Finally, MAF14001 decreased the tau phosphorylation (Ser-202) induced by Aβ. Altogether, these results demonstrated that MAF14001 could target multiple mechanisms involved in the etiology of AD and could be useful in preventing and treating AD.

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P156

Effects of palmitoyl ethanolamide on Alzheimer's disease: focus on mitochondrial function

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BACKGROUND. Alzheimer's disease (AD) is characterized by early intracellular accumulation of amyloid-beta (Abeta) peptide mainly in the hippocampus (H) and cortex (C). These pathological alterations are associated with mitochondrial dysfunction and oxidative stress. Palmitoylethanolamide (PEA) is an endogenous lipid that may exert a combination of neuroprotective and anti-inflammatory effects in Abeta-induced toxicity. We aimed at studying the potential efficacy of PEA on mitochondrial impairment in a triple transgenic model of AD (3xTg-AD).

METHODS. 3 months-old 3xTg-AD mice and lean littermates were subcutaneously infused up to 90 days with 10 mg/kg PEA or vehicle by osmotic mini-pumps. Mitochondria were isolated from H and C to measure respiratory activities, membrane potential and proton leak.

RESULTS. Mitochondrial respiration and membrane potential were slightly modified in the 3xTg-AD mice with respect to lean littermates, both in H and in C. These findings were associated with small changes in mitochondrial proton leak. PEA treatment did not improve mitochondrial impairment.

CONCLUSIONS. Abeta pathology in AD initiates early modifications in mitochondrial function, even though a severe mitochondrial impairment occurs only in the late stage of the disease, suggesting that early alterations may be adaptive. PEA does not show any effect on mitochondria both in H and in C of 3 months-old 3xTg-AD mice, but further studies are required to establish whether it could be effective in aged mice.

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Dual protective effect of tideglusib in neurodegeneration

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Tideglusib, a thiadiazolidinone compound, is a non-ATP competitive inhibitor of glycogen synthase kinase-3β (GSK-3β) and an agonist of peroxisome proliferator-activated receptor gamma (PPARγ). It is accepted as a good candidate for the treatment of neurodegenerative diseases such as Alzheimer's disease and stroke. NMDA receptor overactivation has been reported to disturb prooxidant and antioxidant balance in the brain leading to oxidative stress. The aim of the present study was to evaluate the protective effects of tideglusib against NMDA receptor overactivation induced by NMDA and D-serine in neural stem cells (NSCs). Moreover, we aimed to compare its effects with PPARγ agonist, pioglitazone, and GSK-3β inhibitor, LiCl. Cell viability or cell proliferation was measured by MTT assay and GSK-3α/β protein levels were analyzed using Western Blot following treatments. NMDA treatment, alone or with the combination of D-serine, significantly triggered cell death. The viability of cells was significantly increased with different concentrations (0.25-100 μM) of tideglusib, alone, or with NMDA/D-serine treatments. The decreased cell loss was observed following cotreatment with tideglusib and LiCl or pioglitazone against NMDA/D-serine. However, GW9662 (a PPARγ antagonist) treatment significantly inhibited the protective effect of tideglusib. After treating cells with NMDA/D-serine for 24h, GSK-3β was significantly overactivated as indicated by decreased phosphorylation levels. Tideglusib or LiCl treatments significantly increased the levels of phospho-GSK-3β both in the absence or in the presence of NMDA/D-serine in NSCs. Our study provides the evidence that GSK-3β and PPARγ may be directly involved in pathways leading to NMDA receptor-induced cell death and that the inhibitors including tideglusib may exert neuroprotective effect against these receptors overactivation.

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Ethanol is metabolized in the human retinal pigment epithelium: role of the cytochrome P450-2E1

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Cytochrome p450 2E1 (CYP2E1) is a detoxifying enzyme found in several tissues with particular affinity for ethanol (EtOH). Although, CYP2E1 has been identified in human retinal pigment epithelium (RPE), nothing is known about its metabolic activity. CYP2E1 expression and its metabolism after EtOH exposure has been studied in human RPE and ARPE-19 cells. CYP2E1 mRNA expression was analyzed in a human donor RPE as well as in EtOH-treated and non-treated ARPE-19 cells, by RT-PCR and qPCR. Protein expression was examined by immunocytochemistry and Western Blot. Cell viability was studied by the colorimetric assay XTT. The selective CYP2E1 inhibitor diallyl sulfide (DAS) was used to study mRNA and CYP2E1 protein expression after ethanol exposure. The microsomal CYP2E1 activity assay was determined by quantification of 4-nitrocatechol (4NC) formation through HPLC. Relevant CYP2E1 mRNA levels are present in human RPE. Ethanol in ARPE-19 cells augmented the formation of reactive oxygen species (ROS). CYP2E1 mRNA expression, CYP2E1 protein activity and ROS production where induced by ethanol in a concentration-dependent manner. Interestingly, the treatment with DAS reduced all the aforementioned increased values.These novel findings support the hypothesis of a local ethanol metabolism in the RPE. The meaning of this finding may explain some retinal alterations induced by ethanol.

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P159

Autophagy and oxidative stress in human retinal pigment epithelium-derived ARPE-19 cells

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Retinal pigment epithelial (RPE) cells play a central role in retinal physiology, due to its localization and function. In fact, RPE pathological degeneration leads to photoreceptor damage, and subsequent blindness. Among RPE-related diseases age-related macular degeneration (AMD) is especially relevant, and the main cause of blindness worldwide. Reactive Oxygen Species (ROS) may induce damage in RPE cells and also lead to activation of autophagy. Autophagy is a catabolic process that engulfs cytosolic material and transports it to the lysosome for its final degradation. Thus we treated ARPE-19 cells with three different pro-oxidant reagents (H_2O_2, Rotenone and Ethanol) in order to increase ROS levels. We observed a massive increase in ROS and 4-HNE adducts (a lipid peroxidation product). Oxidative stress induces an accumulation of 4-HNE deposits in every condition. Furthermore, we demonstrated that 4-HNE intracellular deposits presented hallmarks of aggresomes (misfolded proteins aggregated in the vicinity of the nucleus). Under ethanol and H_2O_2 exposure, mTOR-dependent autophagy was activated to selectively degrade 4-HNE aggresomes and reestablish cellular homeostasis. However, rotenone addition leads to an increase in tubulin acetylation that impairs autophagy flux. Therefore, autophagy disruption blocks 4-HNE aggresomes clearance. Data are provided herein that help understanding of the mechanisms underlying selective aggresome autophagy under ROS-induced damage in ARPE-19. Our data suggest that mitochondrial selective damage disrupts autophagy, which can be extremely harmful for the cell.

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P160

Paraoxonase-1 and dementia-related diseases

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Aim: The aim of our research was to investigate whether the serum serum levels of paraoxonase (PON-1) and ferroxidase (FeOx) activities might be associated with the occurrence of late Alzheimer's disease (LOAD) and Vascular Dementia (VAD). **Methods:** To address this issue we evaluated arylesterase and paraoxonase activities of PON-1 and Ferroxidase I and II activities in a sample including 260 mild cognitive impairment (MCI, so-called prodromal phase of LOAD), 173 LOAD, 67 VAD and 151 older normal cognitive controls. Moreover, the clinical progression of 104 MCI patients was taken into observation for a period of 2 years. **Results:**Arylesterase was the only enzymatic activity that significantly changed among the groups (ANOVA: $p<0.001$), with similar lower levels in MCI, LOAD, and VAD compared to controls. Considering the subset of the MCI patients longitudinally followed, the 19 that eventually converts to VAD showed a significantly lower level of both paraoxonase and arylesterase (ANOVA; $p>0.05$ for both) compared to the 58 remaining stable. **Conclusions:** Overall, our results suggest that depression of enzymatic activity of PON-1 might be an early feature of dementia-related diseases.

P161

Tramadol influence on lipid profile, sexual hormones and paraoxonase activity in healthy volunteers

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Tramadol is a centrally acting synthetic opioid used as analgesic. It is known that opioids can influence sexual hormones secretion by acting on hypothalamus hypophisis axe and also by inhibiting directly their synthesis in gonads. Sexual hormones are known to influence lipid profile and also oxidative stress paramaters. Paraoxonase is an HDL associated enzyme known to be responsible of HDL associated antioxidant activity.

The aim of our work was to evaluate the influence of tramadol administration on sexual hormones profile and paraoxonase activity in healthy volunteers.

29 healthy men volunteers participated to the study. Lipid profiles, paraoxonase activity – towards paraoxon and phenylacetate and the levels of estradiol, 17-OH-progesteron and progesterone were evaluated before and after tramadol treatement.

!7- OH – progesterone and progesterone were lower but not statistically significant at the end of the study while estradiol levels were significantly higher ($p<0.05$) in the study group. There were no significant differences for HDL cholesterol, LDL cholesterol and trigycerides levels. Paraoxonase activity towards phenylacetate has significantly increased at the end of the study suggesting increased oxidative stress induced by tramadol.

OBESITY, METABOLIC SYNDROME & DIABETES (P162-P171)

P162

The nitroxide radical TEMPOL prevents obesity, hyperlipidemia, elevation of inflammatory cytokines, and modulates atherosclerotic plaque composition in apoE$^{-/-}$ mice

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Objective: The nitroxide compound TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl radical) has been shown to prevent obesity-induced changes in adipokines in cell and animal systems. In this study we investigated whether dietary supplementation with TEMPOL inhibits obesity, inflammation and atherosclerosis in apoE$^{-/-}$ mice fed a high fat diet (HFD).

Methods: ApoE$^{-/-}$ mice were fed for 12 weeks on standard chow diet or a high-fat diet. Half the mice were supplemented with 10 mg/g TEMPOL in their food. Plasma samples were analysed for triglycerides, cholesterol, low- and high-density lipoprotein cholesterol, inflammatory cytokines and markers (interleukin-6, IL-6; monocyte-chemotactic protein, MCP-1; myeloperoxidase, MPO; serum amyloid A, SAA; adiponectin; leptin). Plaques in the aortic sinus were analysed for area, and content of collagen, lipid, macrophages and smooth muscle cells.

Results: High fat feeding resulted in marked increases in body mass and plasma lipid levels. Dietary TEMPOL decreased both parameters with these levels reduced to the levels seen in chow fed animals. In the high-fat-fed mice significant elevations in plasma lipid levels and the inflammatory markers IL-6, MCP-1, MPO, SAA were detected, along with an increase in leptin and a decrease in adiponectin. TEMPOL supplementation reversed these effects. The HFD also induced significant atherosclerotic lesions in the aortic sinus. When compared to HFD-fed mice, TEMPOL supplementation increased atherosclerotic plaque collagen content, decreased lipid content and increased macrophage numbers.

Conclusions: These data indicate that in a well-established model of obesity-associated hyperlipidaemia and atherosclerosis, TEMPOL decreased body mass, atherosclerosis, hyperlipidaemia and inflammation in a significant manner. TEMPOL may therefore be of value in suppressing obesity, metabolic disorders and increasing atherosclerotic plaque stability.

P163

A novel selenium-containing sugar enhances wound closure and improves microvascular perfusion: implications for diabetic wound healing

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Background:Selenoproteins (e.g. glutathione peroxidases, thioredoxin reductases, some methionine sulfoxide reductases) play a key role in protecting biological systems against oxidative stress and damage. Diabetes is associated with increased oxidative and carbonyl stress, impaired wound healing, and increased infection and tissue morbidity.

Hypothesis: We hypothesized that a novel water-soluble selenium sugar with established antioxidant activity would reduce oxidative damage, chemotactic signals and inflammatory mediators, and improve wound healing in both wild-type and diabetic mice.

Methods: Pairs of circular incision wounds on the backs of wild-type C57/BL6 and diabetic ($db^{-/-}$) mice (n=12 each) were treated daily with a novel seleno-sugar (Se) or vehicle, topically for 10 days. Wound closure, vascular perfusion (as assessed by Doppler imaging) and tissue histology were assessed.

Results: Se-treated diabetic mice showed significantly better wound closure from day 3 post-surgery when compared to controls ($p < 0.01$). Se-treated wild-type mice showed significant improvement from day 4 post-surgery when compared to controls ($p < 0.05$). Improvements in wound closure in the Se-treated diabetic mice were more marked than those in the wild-type mice. Doppler imaging showed improved vascular perfusion ($p < 0.05$) in diabetic, but not wild-type wounds treated with Se. Tissue histology showed decreased levels of MPO ($p < 0.001$), MCP-1 ($p < 0.001$), IL-6 ($p < 0.05$) and vWF ($p < 0.01$), and increased elastin levels ($p < 0.01$) in diabetic wounds treated with Se, compared to vehicle.

Conclusions: Enhanced wound healing was observed on treatment with the selenium sugar, with this being more marked in diabetic compared to wild-type mice. The decrease in monocyte chemotactic activity and IL-6 expression, and improvement in tissue elasticity and tensile strength, suggest that this compound may have therapeutic potential in wound healing.

P164

Nitric oxide and mitochondrial biogenesis in heart of diabetic rats

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Diabetic cardiomyopathy described as ventricular dysfunction occurring in diabetic (DM) patients may not be attributable to underlying coronary artery disease and hypertension. This cardiac contractile dysfunction could be associated to mitochondrial dysfunction. The aim of this work was to study heart mitochondrial function of diabetic rats, 4 weeks after streptozotocin injection (60 mg/kg, i.p.), focusing on the role of nitric oxide (NO) in mitochondrial biogenesis. Diabetic hearts showed lower contractile response (40%) after a β-adrenergic stimulus than control animals and an attenuated decrease in the isovolumic relaxation time (Δt_{50C} = -26%; Δt_{50DM} = -11%) indicating an impairment in the lusitropic reserve (55%). Tissue O_2 consumption was also declined (15%) in DM in comparison with control animals. Cytochrome oxidase activity measured in heart homogenates indicates that the mitochondrial mass per heart mass was 45% higher in DM than in control animals. This effect was confirmed by electron microscope images that showed an increase in the mitochondria number (94%) and in the mitochondrial area per total area (33%) in diabetic hearts, suggesting that DM leads to mitochondrial biogenesis. However, mitochondrial state 3 O_2 consumption (22%), respiratory control ratio (25%), mitochondrial respiratory complexes activities (I-III, 22%; II-III, 30% and IV, 22%) and ATP production (40%) were decreased in DM hearts. Moreover, an enhancement in mitochondrial NO production (23%) and mtNOS expression were observed in diabetic rat hearts. These results show a mitochondrial dysfunction with an enhancement in mitochondrial biogenesis, in which NO may be involved. This process could be triggered in response to hyperglycemia and could explain the cardiac energy metabolism impairment that occurs in hearts of diabetic rats.

P165

Oxidative stress levels in morbid obesity before and after bariatric surgery

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Obesity prevalence has increased over the last decade in developed countries, being associated with important comorbidities such as cardiometabolic syndromes or cancer. As it is estimated to increase in next decades, analysis of OE values as new clinical markers could give useful information about its pathogenesis and possible neoplastic complications, thereby contributing to improve the clinical diagnosis and treatment of these patients.

Morbid obesity (BMI>40) is considered as an inflammatory process, which is related with the production of reactive oxygen species (ROS) and alteration of antioxidant systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and reduced/oxidised glutathione (GSH/GSSG) as well as high levels of 8-isoprostanes, malondialdehyde (MDA) and 8-oxo-dG, characteristic of the oxidative stress (OE) that induces damage to lipids, proteins and DNA.

The aim of this study is to evaluate changes at OE level by ELISA and HPLC in 21 patients with morbid obesity before and after bariatric surgery by measuring the activities of SOD, CAT, GPx and GSH/GSSG (plasma) as well as 8-isoprostanes (urine), MDA (urine) and 8-oxo-dG (serum and urine) levels during 1 year period (1, 3, 6 and 12 months), comparing with healthy non-obese controls.

After surgery, all patients lost weight progressively over the studied period, with progressive decrease of OE values and improvement of antioxidant systems, being the final values of OE similar to controls.

Obtained results were concordant with prior reports on obese humans, supporting the utility of OE values as risk predictors in morbid obese patients, highlighting the use of 8-isoprostanes and urine 8-oxo-dG marker for its rapid, easy (non invasive) and cost-effective use in clinical practice.

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P166

Lactonase paraoxonase 2 enzymatic activity and anthropometric markers in newly diagnosed type 2 diabetic patients with obesity and metabolic syndrome

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Background: Metabolic syndrome (MS), invariably associated with obesity, central distribution of body fat, dyslipidemia and hypertension is often characterized also by an imbalance between the production and inactivation of free radicals. Our aim was to investigate the association of MS with some oxidant and antioxidant markers in newly diagnosed type 2 diabetic patients (T2D) with obesity.

Methods: 219 newly diagnosed T2D were divided into two groups according to the absence (MS-) or presence (MS) of MS and further taking into account their body mass index (BMI) in overweight and obese. Anthropometric measurements, routine blood tests, total oxidant and antioxidant status (TOS/TAS) and ELISA measurements for insulin, proinsulin, leptin and adiponectin were included. The peripheral blood mononuclear cells (PBMC) capacity to release free radicals and to neutralize them was also determined by measuring the respiratory burst (RB) together with the lactonase activity of the intracellular antioxidant enzyme paraoxonase 2 (PON2).

Results: Comparing diabetic patients with MS with those MS-, the RB of the PBMC was significant higher ($p<0.05$) while lactonase PON2 enzymatic activity was decreased ($p<0.001$). The differences were maintained also in the overweight compared to the obese only for MS . Univariate correlation analysis revealed significant negative correlation of RB with TAS (r -0.416, $p < 0.05$) and negative corelations of PON2 with glycaemia (r –0.275, $p<0.001$), HbA_{1c} (r –0.308, $p<0.001$), weight (r -0.183; $p<0.05$), waist circumference (r -0.353, $p<0.001$) and body mass index (r -0.290, $p<0.001$)**.**

Conclusion: PON2 lactonase activity is negatively associated with anthropometric markers in newly diagnosed T2D with MS.

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P167

Protective effect of carnosine on oxidative stress injury in pancreatic β–cells

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Oxidative stress plays a key role in β-cells apoptotic induction, responsible, in most cases, for type 1 and type 2 diabetes as well in the failure of islets transplantation procedure. Pancreatic β-cells, in fact, are markedly susceptible to oxidative stress for their low intrinsic level of antioxidant response. Thus, protection from oxidative stress of islet graft appears to be a crucial factor to achieve a successful islet transplantation.

The aim of our study is to evaluate the effect of L-carnosine (β-Alanyl-L-histidine) (L-car) and its derivate D-carnosine (β-Alanyl-D-histidine) (D-car) on oxidative stress protection using as experimental model rat and murine insulin-producing pancreatic β-cells (INS-1E and MIN6). First of all, we studied their effects on cell growth, cell death, insulin production, reactive oxygen and nitrogen species (ROS and RNS) production and NF-κB expression/activation. To further characterize the molecular mechanisms underlying carnosine action, we measured the nitrite production and the protein modification by glycosylation and nitration.

We found that both L-car and D-car clearly inhibited hydrogen peroxide (H_2O_2)-induced cytotoxicity, with a decrease of intracellular ROS and RNS formation. Both L-car and D-car prevented H_2O_2-induced apoptosis/necrosis, nitrite production and the reduction of glucose-induced insulin secretion. Furthermore, NF-κB expression/activation, induced by H_2O_2-treatment, was significantly reduced in L- and D-carnosine-treated cells. L- and D-car protective effect reduced also the presence of glycated and nitrated protein, markers of oxidative stress, induced by H_2O_2-treatment.

These results clearly indicated that both L-car and D-car have a significant cytoprotective activity through the reduction of the oxidative stress in insulin producing β-cells, suggesting their potential use to improve islet survival during islet transplantation.

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N-acetyl cysteine inhibits kinase phosphorylation during 3T3-L1 adipocyte differentiation

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Antioxidants in obesity have provided contradictory results. We used 3T3-L1 preadipocytes (undifferentiated cells: CC), which are capable of differentiating into mature adipocytes (differentiated cells: DC), which accumulate triglycerides (Tg) and express proteins related to oxidative stress such as monoamine oxidase A (MAOA). Following the differentiation of preadipocytes, an increase in the levels of phospho-kinases (AKT, JNK and ERK½) is observed, with mitotic cellular expansion (MCE) in the first days of cellular differentiation. We have demonstrated that N-acetylcysteine (NAC) inhibited Tg accumulation in DC (Redox Reports, 2013). Here we determined that 10 µM NAC treatment did not affect AKT phosphorylation, but significantly inhibited JNK phosphorylation (DC: 10.02 ± 0.45 vs DC 10 µM NAC: 5.53 ± 1.04 arbitrary units (AU), $p< 0.01$); higher doses of NAC (up to 1mM) provoked similar inhibition. 10 µM or 1mM NAC provoked 80% decrease on ERK½ phosphorylation, evaluating pERK½/ERK (DC: 2.24 ± 0.50 vs DC 10µM NAC: 0.40 ± 0.07 AU; $p< 0.01$). At day 2 of adipogenic differentiation, MCE was observed in DC (DC: $1.3x10^5 \pm 0.18x10^5$ cells/ mL vs CC: $0.51x10^5 \pm 0.09x10^5$ cells/ mL, $p<0.01$), NAC significantly inhibited MCE (DC vs DC 10 µM NAC: $0.49x10^5$ cells/ mL ± $0.03x10^5$ cells/ mL, $p<0.01$; DC vs DC 1mM NAC: $0.65x10^5$ cells/ mL ± $0.04x10^5$ cells/ mL, $p<0.01$). NAC significantly decreased mitochondrial MAOA expression in a dose dependent manner (DC: 1.00 ± 0.84 vs DC 10 µM NAC: 0.70 ± 0.03 AU, $p< 0.01$; DC vs DC 1mM NAC: 0.34 ± 0.01 AU $p<0.01$). We postulated that NAC could inhibit lipid accumulation and mitochondrial protein expression by inhibiting both mitotic cellular expansion and kinase phosphorylation as a part of a MAPK pathway during adipogenic differentiation.

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Effect of *Hibiscus sabdariffa* extracts and polyphenolic compounds and metabolites on high glucose induced metabolic stress on hypertrophied 3T3-L1 adipocytes

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Dietary high-sugar foods cause hypertrophy in adipocytes, which may compromise their function. This dysfunction is associated with oxidative stress and inflammation, which lead to obesity-associated metabolic disturbances. Recent data suggest that *Hibiscus sabdariffa* (HS) extracts might ameliorate high glucose induced metabolic stress.

The objective of this study was to determine the effect on triglyceride accumulation, oxidative stress and inflammation of HS extracts as well as some polyphenolic compounds and metabolites derived from these extracts (quercetin, quercetin-3-glucoside and quercetin-3-glucuronide) on hypertrophied 3T3-L1 adipocytes, a model of diet-induced obesity obtained from preadipocytes differentiation in high glucose conditions. Furthermore, the antioxidant effect of HS extracts was validated in a model of glucolipotoxicity using INS832/13 β-cells.

The results suggest that polyphenol-enriched HS (PEHS) extract was more efficient in reducing oxidative stress than aqueous HS (AHS) extract in both hypertrophied 3T3-L1 adipocytes and INS832/13 β-cells. Likewise, PEHS extract and quercetin derivatives inhibited triglyceride accumulation and oxidative stress and modulated adipokine secretion in hypertrophied adipocytes, being quercetin-3-glucuronide the most active compound.

Therefore, the polyphenolic extract of HS is able to alleviate high glucose-induced metabolic stress in adipocytes and pancreatic cells. Among the most representative polyphenolic compounds in the extract, quercetin derivatives seem to be partially responsible for such effects. Quercetin-3-glucuronide, a quercetin metabolite, was the most active compound among the quercetin derivatives utilized. Therefore, HS polyphenolic extract might have potential in the management of obesity-associated metabolic disturbances.

P170

Incretins modulate antioxidant status via ghrelin in diabetic patients

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Introduction: Ghrelin regulates food intake and plays an important role in the control of cellular pathways involved in glucose and lipid metabolism. Incretin-resistance has been defined as main component of the type 2 diabetes affecting food intake and weight gain. Diabetic patients show lower circulating levels of ghrelin. Since oxidative stress is part of the pathophysiology of diabetes mellitus, we aimed at investigating the impact of incretins on ghrelin and antioxidant status in patients affected by type 2 diabetes mellitus (DM2).

Methods: Serum level of acylated ghrelin was determined after overnight fasting in sixteen DM2 patients treated with the association of metformin and an inhibitor of dipeptidyl peptidase-4 (M DPP-4 group) and sixteen with metformin alone (M group). The antioxidant status was evaluated by measuring mRNA expression of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in peripheral blood mononuclear cells.

Results: Although both therapies were effective in the glycemic targeting, circulating level of acylated ghrelin and antioxidant enzyme expression were differently regulated by incretins. Serum ghrelin was significantly lower in the Metformin and DPP-4 group as compared to Metformin alone. Moreover, the mRNA expression of SOD and CAT was higher in metformin-DPP patients while GSH-Px was not different.

Conclusion: DPP4-inhibitors restore glucagon-like peptide 1 (GLP-1) level in diabetic patients. The reduction of active ghrelin observed in DPP group associated with CAT and SOD increase suggests that ghrelin may be part of the fine regulation of food intake, energy metabolism and redox balance. The role of GLP-1 on circulating levels of ghrelin and antioxidant enzymes merits further investigation.

P171

Reduction of oxidative stress and genomic damage, after weight loss either by gastric bypass or caloric restriction alone in a rodent model of obesity

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Morbid Obesity is a risk factor for cardiovascular disease, Type 2 Diabetes and cancer. Bariatric surgery - with the Roux-en-Y gastric bypass (RYGB) being the gold standard -has become the therapeutic option of choice as a sustained weight loss. There is, however, a lack of evidence focusing on bariatric surgery induced sustained weight loss and its possible impact on cancer risk.

We aimed to investigate the association between obesity, oxidative stress and genomic damage in a rodent model of obesity (male Zucker fa/fa rats) after weight loss induced either by caloric restriction or by RYGB. Therefore male Zuc-Lrpfa (aged 12 weeks) rats were randomly divided into four groups: Lean control, Sham-operated, RYGB and CR. Body weight and food intake were monitored before and after the intervention. After an oral glucose tolerance test (OGTT) was performed rats were sacrificed to collect kidney, liver and colon for further analysis.

The sham rats showed elevated insulin levels and insulin resistance response. The RYGB and CR rats showed elevated insulin levels, but decreased insulin resistance. Evaluation of DNA damage was performed with γ-H2AX staining of paraffin-embedded tissue sections. DHE staining was used to detect oxidative stress in kidney, liver and colon. Additionally HSP70 and HO-1 expressions were measured by western blot to evaluate cellular stress response. Lipid oxidation was monitored by measuring urinary malondialdehyde. To quantify the oxidation of DNA and RNA urinary 8-oxoGuo and 8-oxoGua measurements were performed by LC/MS/MS.

The obese rats showed elevated oxidative stress and genomic damage in comparison to lean rats. After bodyweight loss, a decrease in oxidative stress level and genomic damage was observed. The response of the gastric bypass surgery group and the caloric restriction group were slightly different in their pattern. This might be due to a difference in metabolism.

VASCULAR DISEASE (P172-P179)

P172

Cyanidin-3-*O*-glucoside inhibits palmitate-induced endothelial insulin resistance via blocking IKKβ and serine phosphorylation of IRS-1.

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Endothelial insulin resistance is tightly associated with diabetic cardiovascular complication such as coronary heart disease, peripheral vascular disease, and stroke. In addition to crucial metabolic actions, insulin exerts desirable effects on the maintenance of physiological endothelial function through its ability to stimulate NO release, which is mainly mediated by a cascade of signalling that involves activation of the IRS1-PI3K-Akt axis and the downstream activation of NO synthase (eNOS). Under insulin resistance conditions, PI3K-dependent signaling pathways are selectively impaired, leading to endothelial dysfunction, which is characterized by decreased insulin-mediated NO production and increased secretion of ET-1 from the endothelium.

This study aims to clarify whether Cyanidin-3-*O*-glucoside (C3G), an anthocyanin belonging to the flavonoid family and commonly present in food and vegetables from Mediterranean Diet, contributes to amelioration of insulin resistance in the endothelium. Palmitate (PA) stimulation markedly reduced insulin-mediated PI3K-Akt axis, and then eNOS expression in HUVECs. Interestingly, C3G pretreatment effectively reversed the effects of palmitate on PI3K-Akt axis and restored NO and ET-1 mRNA levels.Furthermore, we observed that PA induced IRS-1 phosphorylation at serine residues and reduced tyrosine phosphorylation of IRS-1 in response to insulin. However, in HUVECs pretreated with C3G, tyrosine phosphorylation of IRS-1 was restored.

In conclusion the present study aimed to understand the actions of C3G on PA-induced endothelial dysfunction, providing insight into the potential mechanisms, and suggesting interesting possible applications for this molecule in the prevention of cardiovascular complications in insulin resistance.

P173

Aberrant redox signaling in hypertension and the effect of a PPAR-gamma agonist treatment on spontaneously hypertensive rats

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Aberrant redox regulation in hypertension is characterised by changes in ROS sources, different redox signaling and antioxidant response. In the develompent of hypertension, vascular endothelium balance and PI3K/Akt/NO pathway are changed. The PPAR-gamma is a nuclear receptor that takes part in the regulation of lipid - energy metabolism and cell signaling. PPAR gamma ligands can influence aberrant redox regulation (1), antioxidant response (2), PI3K/Akt/NO pathway in vessels (3), and/or neuronal regulation (4). Our study was focused on radical signaling triggered via the Ang 1 receptor (AT1R) - NADPH-oxidase - superoxide production and antioxidant response of SOD and HO-1. We also studied the involvement of Akt kinase and beta-catenin. Pioglidazone (PIO 10 mg/kg/day) was administered by gavage to young and adult spontaneously hypertensive rats (SHR) during two weeks. Blood pressure was measured by plethysmography. Superoxide was detected by Lucigenin-enhanced chemiluminiscence. Gene expression was observed by qPCR and protein levels by western blots. The most pronounced changes were observed in young rats. Administration of PIO significantly suppressed blood pressure increase and positively influenced vessel relaxation in young (1), but not in adult rats. In the brain stem (BS), a significant increase in mRNA PPAR-gamma and SOD2 was observed. We found that PIO treatment had tissue-dependent effects on SOD activities. The treatment of young SHR with PIO differentially affected the levels of intracellular signaling proteins Akt kinase and beta-catenin. Our data suggest that PIO is more effective in young SHR than in adults, and that PPAR - gamma regulation depends on the type of agonist as well as on the type of experimental model of hypertension (4).

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P174

Oxidative stress and inflammatory early biomarkers as determinants of risk of first cardiovascular events

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The prevalence of obesity has significantly increased in recent decades. The increase in body weight has been associated with increased mortality and morbidity related to cardiovascular disease, cancer, diabetes and depression. The nutrition and life style intervention would be effective on primary prevention, such as cardiovascular events, and on the weight loss. The aim was to assess several biomarkers in order to evaluate the differences between healthy subjects and patients with cardiovascular risk factors. After signing the written informed consent, 24 participants (55-80 years old) were included in the study. One group was constituted by participants without cardiovascular disease, but with type 2 diabetes mellitus and almost three of these factors: smoking, hypertension, hypercholesterolemia, high LDL levels, overweight/obesity, and family history of premature coronary heart disease. The control group did not meet these criteria. Plasma and lymphocyte fraction were obtained. The analytical analysis showed significant higher concentrations of glucose, urate and the number of neutrophils in patients than in controls. No significant differences in malondialdehyde levels in plasma and lymphocytes were found. However, the catalase (CAT) and myeloperoxidase activities in plasma were higher in the patients than in the control group. In lymphocytes, glutathione reductase and superoxide dismutase were increased in patients respect to the control group, whereas no changes were observed in CAT and glutathione peroxidase. Additionally, lymphocyte nuclear factor κB (NF-κB) gene expression was increased in patients. In conclusion, the patients with higher cardiovascular risk showed increased antioxidant mechanisms, and a rise in the immune and inflammation responses as it was evidenced by neutrophil levels in blood and in NF-κB.

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P175

A predictive model for the evolution of stroke patients using oxidative stress markers and antioxidant capacity

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The aim of this research is to establish a predictive model to indicate the evolution of stroke patients using oxidative damage markers.

Among the oxidative stress markers approached here F2-isoprostanes are of particular interest. They are considered the best available biomarkers of oxidative stress and lipid peroxidation "*in vivo*". Other fatty acids are also oxidized as a result of oxidative damage and can therefore be used as markers for this pathology. DHA and EPA, which were studied in this research, are two of the most important fatty acids. Phospholipid damage disturbs and modifies cellular membranes and consequently cellular function. Concentration of molecules generated by lipid peroxidation such as MDA and HNE were measured. Low molecular weight antioxidants were determined. So GSH (glutathione tripeptide reduced form) as well as many other low molecular weight antioxidants such as vitamin C (ascorbic acid) and Vitamin E (α-tocopherol) were measured.

We approached a new method for measuring the total antioxidant activity, since there is a lack of an efficient method for this assay. The method proposed here shows a much broader measure of antioxidant status, thus reflecting the actions of all antioxidants present in the sample and not only a specific molecule.

A comparative study between control subjects and patients suffering a stroke in terms of oxidative stress was performed. Values of oxidative stress parameters such as DHA, vitamin C or vitamin E, decrease in patients suffering stroke, while parameters that indicate oxidative stress such as LPO and isoprostanes were significantly higher in the same group of patients.

Finally, in order to establish a predictive model to indicate the evolution of patients, a statistical analysis was performed. In this way we were able to discriminate between two groups of patients with different forecast three days after the cerebrovascular damage. ROC analysis was also performed, showing the predictive efficacy of vitamin C and LPO in patients after 72h after ischemic stroke.

P176

The influence of cardiac rehabilitation on oxidative stress parameters in plasma of patients with ischemia heart disease

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Cardiac rehabilitation (CR) of patients with ischemia heart disease (IHD) after coronary heart interventions is based largely on physical activity. CR evokes various effects, however, they have not been fully elucidated. This study examined the plasma parameters after physical exhaustion of patients with IHD before and after CR. The properties of plasma such as its viscosity, the level of carbonyls, glutathione (GSH) and ascorbic acid (ASC) were studied before and after four weeks of rehabilitation. Parameters were investigated before, just after and 1 hour post exercise.

Cycling on an ergometer with a load gradually increasing in 4 min steps from 30W to 75W with return to 30W has been taken as a model rehabilitation. Cycling was performed three times a week for two months and adapted to the particular patient. Experimental setting was ratified by appropriate Local Ethics Committee.

Blood of male volunteers with IHD (age (52.2±6.4 years), BMI (28.3±4.2) was collected before, immediately after and 1h after cycling test.

The standard parameters of blood were determined. No changes in plasma viscosity were detected using Tempamine in EPR spectroscopy.

On the other hand, significant increase in carbonyl content in plasma just after exercise, which persisted on the same level an hour later both before and after CR was found.

Before CR, 1 hour after exercise a decrease in GSH concentration in plasma of patients was found. However, we did not observe changes in GSH concentration in patients subjected to exercise after CR.

Similar results were obtained for ASC, we did not find alterations in its concentration subjected to exercise in patients after CR. Noteworthy, ASC concentration was significantly lower after exercise and 1 hour later before CR.

The results suggest that CR improves antioxidant defense in the plasma of cardiovascular disease patients.

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P177

Cardiac function in neonatal rats with hyperthyroidism: interrelation between nitric oxide and gender

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Objetives: to clarify the mechanism mediating the effect of hyperthyroidism on cardiac function during the second month of life in rats.

Design and methods: male and female Sprague-Dawley rats were assigned to a control or to a triiodothyronine (T_3)-treated group. Treatment of each group was started on the third day after birth. Control rats (Eut) received 0.9% NaCl (0.1 ml/100 g body weight (BW)) every second day during 60 days and T_3-treated rats (Hyper) received subcutaneous (SC) T_3 injections every second day during 60 days.

Results: Hyperthyroidism decreased left ventricle volume only in male rats. Female euthyroid rats presented higher atrial nitric oxide synthase (NOS) activity than male rats and hormonal treatment decreased this enzyme's activity in both sexes. Euthyroid male and female rats had similar atrial NOS protein levels, but females had higher caveolin (cav) 3 protein levels. T_3 treatment increased this protein only in males. Female rats had lower ventricular NOS activity than male rats; hyperthyroidism increased NOS activity in both sexes but this effect was associated with lower cav 3 protein levels. Hyperthyroidism did not change cav 1 protein levels in both male and female rats.

Conclusions: The results of this study demonstrating clinically relevant sex-related differences in the pathophysiology of the hyperthyroid heart have raised new questions regarding the mechanisms responsible for the observed differences. This study suggests that sex-related intrinsic factors such as nitric oxide may modulate the response to hyperthyroidism that leads to cardiovascular dysfunction.

P178

Determination of the role of superoxide in acute pulmonary vascular hypoxic signaling by electron spin resonance spectroscopy

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Reactive oxygen species (ROS) have been suggested to play an important role in acute hypoxic signalling processes in the pulmonary vasculature leading to hypoxic pulmonary vasoconstriction (HPV). However, it remained unclear whether ROS are up- or downregulated during hypoxia, which ROS species are involved, and which cell types and cellular organelles take part in these processes. Previous measurements of ROS production are limited by the use of fluorescent probes that may detect ROS unspecifically or are prone to auto-oxidation.

We thus aimed to measure superoxide production in pulmonary arterial smooth muscle cells (PASMC) during acute hypoxia. For detection of ROS in PASMC isolated from mice electron spin resonance spectroscopy was applied. Using the spin probe CMH and CMH in combination with polyethylened superoxide dismutase (pSOD), superoxide radical signals were acquired separately. After incubation with CMH or CMH pSOD the PASMC were either subjected to acute hypoxia (1% O_2, 5 min) or normoxia (21% O_2, 5 min), respectively. For determination of the HPV, isolated ventilated and perfused mouse lungs were ventilated with hypoxic gas (1% O_2, 10 min) alternating with normoxic ventilation (21% O_2, 15 min). The mitochondria-targeted antioxidant MitoTempo was used to determine the effect of mitochondrial ROS scavenging on HPV.

PASMC showed a significantly increased superoxide production during acute hypoxic (1% O_2, 5 min) incubation, but not during normoxia. Application of MitoTempo resulted in decreased HPV whereas the potassium chloride induced vasoconstriction remained unchanged.

Increased mitochondrial superoxide is involved in acute pulmonary vascular oxygen signalling leading to HPV.

P179

Hydroxycinnamic acids regulate biomarkers of cellular oxidative stress, inflammatory mediators and foam cell formation in the atherogenic oxLDL-macrophage interaction

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Hydroxycinnamic acids (HAs) are bioavailable metabolites of chlorogenic acids from coffee drink. This beverage has been associated with the prevention of diseases related with oxidative stress such as cardiovascular disease. However, the molecular mechanisms of action of those plasmatic compounds have not been yet clarified. The atherogenic process involves a chronic interaction of oxidized low-density lipoprotein (oxLDL) with macrophages (MΦ), which triggers a lipid metabolism alteration, reactive oxygen species (ROS) production and inflammation into the arterial wall. The aim of this study was to determine the regulatory effect of HAs on ROS production, proatherogenic receptors (CD36, SR-A1 and LOX-1) expression, foam cell formation and eicosanoid induction (isoprostanes (IsoPs) and prostanoids (PNs)) in MΦs treated with oxLDL. THP-1 macrophages were activated with human oxLDL and the morphological, phenotypic and functional changes were evaluated by microscopy, flow cytometry and LC/MS-MS, this last one by a targeted lipidomic approach. Further, the cells were pretreated independently with HAs (caffeic, ferulic, 4-coumaric, dihydrocaffeic and dihydroferulic acids). We established that oxLDL is a strong ROS inductor and its chronic exposure increases proatherogenic receptors and foam cell formation. In the same manner, the lipidomic analysis for 37 eicosanoids (20 PGs; 1 thromboxane; 16 IsoPs) showed that oxLDL-MΦ interaction induce 22 of them, which are the result of oxidation or cyclooxygenase activity on arachidonic, eicosapentanoic and dihomo-γ-linolenic acids. When cells were pretreated with HAs, most of them can inhibit ROS production, but only dihydroferulic and 4-coumaric acids inhibit more effectively proatherogenic receptors and foam cell formation respectively. Most of HAs reduced significantly the release of PGs and IsoPs. Bioavailable chlorogenic acids metabolites such as HAs can modulate cellular events involved in the atherogenic process.

OXIDATIVE STRESS AND OTHER DISEASES (P180-P199)

P180

Alteration of serum lipid profile, SRB1 loss and impaired Nrf2 activation in *CDKL5* disorder

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CDKL5 mutation is associated with an atypical Rett syndrome (RTT) variant. Recently, cholesterol homeostasis perturbation and oxidative-mediated loss of HDL receptor SRB1 in typical RTT has been suggested. Here, we demonstrated an altered lipid serum profile also in *CDKL5* patients with decreased levels of SRB1 and impaired activation of the defensive system Nrf2. In addition, *CDKL5* fibroblasts showed an increase in 4-hydroxy-2-nonenal- and nitrotyrosine-SRB1 adducts that lead to its ubiquitination and probable degradation. This study highlights a possible common denominator between two different RTT variants (*MECP2* and *CDKL5*) and a possible common future therapeutic target.

P181

Acid sphingomyelinase activity but not de novo ceramide synthesis is implicated in the pro-apoptotic actions of melatonin on HepG2 hepatocarcinoma cells

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Background: Hepatocellular carcinoma is the most common primary liver cancer. Apoptosis is defective in cancer, leading to uncontrolled proliferation. Ceramides modulate apoptosis and endoplasmic reticulum stress through ROS formation. Melatonin shows anti and pro-oxidant capacity and exerts anti-proliferative actions on HepG2 cells, but its role in ceramide metabolism has not been tested. The aim of this study was to evaluate the possible implication of melatonin in the regulation of ceramide metabolism and the effect of these messengers in HepG2 cell viability.

Material and Methods: Ceramide levels were measured by HPLC. Acid sphingomyelinase (ASMase) activity was analyzed with a fluorescent sphingomyelin analog. RT-PCR and Western blot were utilized to determine protein expression. Cytometry allowed cell death analysis.

Results: Melatonin administration (2 mM) increased ceramide levels after 1 to 4 hours. Expression of serine palmitoyltransferase (SPT) and ASMase raised from 4 hours after exposure to melatonin, as well as ASMase activity, whose induction was observed from 1 hour. Cytometry revealed a decrease in cell viability that was restored by ASMase inhibitor imipramine (10 μM) but not by myriocin (2.5 μM) SPT inhibitor. Western blot analyses showed an increase in the apoptotic markers cleavaged-PARP and BAX after 24 and 48 hours by melatonin. Imipramine but not myriocin reversed the pro-apoptotic effect of melatonin. Moreover, melatonin increased ER stress and JNK phosphorylation, but imipramine was able to decreased p-JNK levels in presence of melatonin.

Conclusions: Melatonin induces ceramide levels and enhances *de novo* synthesis of ceramide as well as sphingomyelin degradation in HepG2 cells. ASMase activity increased by melatonin is related to apoptotic cell death in HepG2. ER stress observed in melatonin-treated cells could be associated to pro-apoptotic effects of ASMase through JNK signaling pathway.

P182

Role of oxidative and endoplasmic reticulum stress in polycystic ovary syndrome and their relation with obesity

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Polycystic ovary syndrome (PCOS) is a metabolic disorder characterized by irregular menstruation, infertility, polycystic ovaries and hirsutism. A high percentage of PCOS patients develop obesity. Oxidative stress and endoplasmic reticulum (ER) stress have been implicated in the pathophysiology of obesity. In addition, reactive oxygen species (ROS) production has been related to the pathogenesis of PCOS. However, it is unknown whether levels of myeloperoxidase (MPO) (as a source of ROS), and ER stress could be increased during PCOS and be related to obesity in these patients.

The study population consisted of 4 groups of 10 women: healthy with normal weight (Body Mass Index (BMI) <25 kg/m^2), PCOS with normal weight, healthy obese (BMI >30 kg/m^2) and PCOS obese. Anthropometric, clinical and metabolic parameters were assessed. Leukocytes were isolated by Ficoll gradient centrifugation to evaluate ROS production and expression of the ER stress markers sXBP1 and CHOP was measured by RT-PCR. Plasma MPO levels were evaluated by ELISA.

Insulin levels and insulin resistance (HOMA-IR) were higher in obese PCOS patients than in the other groups ($p<0.001$). ROS production and MPO levels increased significantly in PCOS patients independently of BMI, and were more pronounced in those who were obese ($p<0.01$). Regarding ER stress markers, an increase in sXBP1 was observed in both PCOS groups ($p<0.05$) with respect to their BMI-matched controls. However, CHOP levels were elevated specifically in obese PCOS patients ($p<0.05$), suggesting a proapoptotic state. Furthermore, CHOP levels correlated positively with BMI ($r=0.38$, $p<0.05$) and insulin levels ($r=0.35$; $p<0.05$).

Conclusion: Oxidative stress, measured in terms of ROS production and MPO levels, is enhanced in PCOS patients and aggravated by the presence of obesity. ER stress is increased in PCOS, with an adaptive response activated in general and a proapoptotic response when obesity coexists. In addition, this proapoptotic state seems to correlate with obesity and insulin resistance in our population.

P183

Potential new markers to monitor oxidative stress in acute exacerbations in COPD

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Chronic obstructive pulmonary disease (COPD) represents a global health problem and is characterized by chronic airway inflammation and oxidant-antioxidant imbalance. Recent studies have demonstrated increased production of oxidants in exhaled air, breath condensate or induced sputum. The aim of this study was to identify potential new oxidative stress markers such as erythrocyte superoxide dismutase (SOD) and prostaglandin E2 (PGE2) for monitoring disease activity in COPD.

Methods: Sputum samples from ex-smoker COPD patients (n=23, GOLD I-IV stage) hospitalized for severe acute exacerbation as well as from ex-smoker stable COPD patients (n=24) and healthy non smoker controls (n=23) were collected. SOD activity in sputum supernatant was measured by enzyme activity assay and PGE2 level was determined by immunoassay, data was correlated with respiratory parameters and sputum cell counts.

Results: SOD activity was slightly elevated in stable COPD compared to controls (0.06 vs. 0.03 U/ml, $p>0.05$). In acute exacerbations SOD activity was markedly increased (0.109 U/ml, $p<0.05$). Treatment of the acute condition resulted in a slight decrease in SOD activity (0.08 U/ml). PGE2 level was similar in controls and stable COPD patients (7.1 vs. 6.1 pg/ml, p=NS). In exacerbations PGE2 levels sharply increased (160.7 pg/ml, $p<0.001$), and upon recovery a significantly reduction was observed (48.87 pg/ml, $p<0.05$). SOD and PGE2 did not correlate with lung function and clinical parameters, but exhibited good correlation with sputum neutrophil and lypmphocyte counts.

Conclusion: Activity of SOD and levels of PGE2 in sputum are increased in COPD patients during an episode of severe exacerbation. While clinical recovery from exacerbation is paralleled by a marked reduction in PGE2 levels, SOD activity remains elevated for a prolonged period of time. PGE2 is a promising candidate for monitoring exacerbations.

P184

Consumption of tetracycline and omeprazol induced by hypochlorous acid

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The reaction between omeprazol, tetracycline and HOCl was studied and the effect of polyphenols. The study estimated consumption of drugs, mediated by reactive species in the presence and absence of antioxidants. The addition of HOCl to drugs consumption resulted in decrease in intensity of bands corresponding features. Large differences were found when comparing reaction rates, showing that the HOCl-Tetracycline interaction is much faster than HOCl-Omeprazol, depending the product on the reaction time and concentration of HOCl provided. The behavior described for each drug was confirmed by HPLC, confirming the appearance of a reaction product associated with tetracycline and two for omeprazole. For tetracycline reaction stoichiometry was 0,5. It was evidenced by stopped-flow method that the reaction occurs in two steps. First a product P1, which reacts immediately with HOCl resulting product P2 (equations 1 and 2) would be the one that is actually being recorded originates. In addiiton, at least six reaction products could be identified by HPLC-mass.

Tetracicline HOCl -> P1 [1]
P1 HOCl -> P2 [2]

Finally it is noted that, in the presence of phenol, tetracycline consumption inhibition occurs. These results would be an indication of the beneficial role of antioxidants against phenomena associated with gastric ulcer.

P185

Detection of paraoxonases 1, 2 and 3 in human ovarian follicular fluid

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Female infertility affects many women from around the world, and is considered in many cultures as a social stigma. The assisted reproduction techniques are used to overcome the problem of infertility, being essential the improvement of these techniques and the finding of biomarkers of dysfunction and fertility. Previous studies in infertile women have suggested a possible role for ROS in the occurrence of conception in *In Vitro Fertilization*. In this context the control of the redox balance of follicular fluid becomes essential for reproduction, so that the presence of enzymes with antioxidant activities would play a role in maintaining this balance. The human paraoxonase (PON) gene family consists of three members, PON1, PON2 and PON3, which exhibit properties against oxidative damage. The aim of this study was to determine the expression of PON1, PON2 and PON3 in follicular fluid from women undergoing a controlled ovarian stimulation. Follicular fluid was separated from the retrieved oocytes by centrifugation and used as the protein source. The presence of the proteins was studied by immunoanalysis using specific antibodies. No cross-reactivity was detected under the conditions assayed. The results showed the presence of all the three proteins in the follicular fluid. The relative amount of these proteins was in the order PON1>>PON3>>PON2. These relatively newly identified antioxidant enzymes could have a role in fertility, probably preventing oxidative stress during oogenesis.

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P186

Effects of prenatal testosterone on oxidative status in rat testes

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Testosterone as one of steroid sex hormones mediates a wide range of physiological functions. The prenatal exposure to testosterone is associated with infertility in females as well as in males. On the other hand, oxidative stress is also associated with decreased fertility, mainly in males. The aim of study was to analyze the impact of prenatal testosterone on steroidogenesis in rat testes, morphological description and analysis of the oxidative status in the testes of offspring males.

Twenty females were randomly assigned into one of the following groups: control group, testosterone, testosterone flutamide and flutamide group. These females were receiving either oil, testosterone, flutamide, or both from the 14th day of pregnancy. Offsprings were analyzed during the adulthood, i.e. between 130-135 postnatal days. Markers of oxidative stress (malondialdehyde, advanced oxidation protein products, advanced glycation-end products, fructosamine) and antioxidative status (total antioxidant activity, ferric reducing activity of plasma) were measured by spectrophotometrical methods.

In our study, a trend of lower testosterone levels of offsprings in the testosterone group compared with the control group was observed. However, the testosterone concentrations were comparable to control group in the testosterone flutamide and flutamide group. All measured markers of oxidative stress and antioxidant status showed a trend towards a higher concentration in the testosterone group. Similarly as testosterone, these markers were comparable to the control group in flutamide and testosterone flutamide group.

In conclusion, the results showed that the prenatal testosterone may contribute to increased oxidative stress in testes of adult male rats, and that this effect might be at least partially mediated through androgen receptor. A causal link to male infertility, however, has to be studied and proved in further studies.

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P187

8-oxo-7'8-dihydro-2'-deoxyguanosine (8-oxo-dG) in human gastric carcinoma. Beyond oxidative stress

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Gastric cancer causes the death of approximately700.000 people every year along the world. Despite of being a frequent disease in the developed countries, good markers for its detention have not been well defined. The principal markers currently used for determination of gastric cancer such CRP (C reactive protein), CEA (carcinoembryonic antigen), CA19.9 (carbohydrate antigen) have both low sensibility and specificity. The effort to find new markers to achieve easier and more reliable diagnostic is an important area in translational research.

Base of the pivotal role of oxidative stress (OS) in human diseases, it is thought that its by-products could be used as possible clinical markers. The damaged base 8-oxo-7'8-dihydro-2'-deoxyguanosine (8-oxo-dG) is present in a very low number in normal cells DNA. However, their levels can be increased upon different OS conditions. Besides its recognized value as an OS indicator, 8-oxo-dG could be used as a potential clinical marker.
We found that 8-oxo-dG was significantly increased in the gastric carcinoma compared with the normal mucosa of affected patient. DNA oxidation in these tumour tissues was accompanied by an enhanced mRNA expression of DNA repair proteins. Recently, we observed that gastric carcinoma patients excrete an extraordinary amount of 8-oxo-dG by the urine compared with the normal levels in healthy control subjects. Surgical tumor resection in these patients results in a progressively decrease of urine 8-oxo-dG levels to values close to those found in the healthy population. Statistical analysis revealed that a significant difference exists between both studied groups allowing to predict a cut-off value of 14.4(mmols 8-oxo-dG/nmols creat) with a specificity and sensitivity of 95% and 92% respectively. We suggest that 8-oxo-dG is a reliable tumour marker, which may be used to improve the diagnosis efficiency and clinical monitoring of gastric cancer disease.

P188

Decreased glutathione and low catalase activity contribute to increased oxidative stress in children with alpha-1 antitrypsin deficiency

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Background: Alpha-1 antitrypsin deficiency (AATD) is an hereditary condition that leads to decreased circulating AAT levels, increasing the risk of serious lung and liver disease. Recent investigations in animal models have revealed oxidative stress (OS) in the pathogenesis of AATD.

Rationale and aims: We have previously shown that chronic systemic OS characterizes AATD. However, the mechanisms leading to the OS observed in these patients are unknown. In addition, since OS shorten telomeres, we hypothesized that telomere shortening would be accelerated in AATD patients and be associated with higher risk of developing lung disease.

Methods: Biomarkers of OS, antioxidant enzymatic activity and telomere length (TL) were prospectively measured in serum and PBMC of 51 clinically healthy children diagnosed with AATD and 38 control individuals.

Results: Intermediate- (MZ;SZ) and high-risk (ZZ) patients showed significantly higher GSSG/GSH ratio ($p=0.001$,$p<0.0001$; respectively), MDA ($p=0.004$;$p<0.0001$), 8-OHdG ($p<0.0001$;$p<0.0001$) and protein carbonyls ($p=0.01$,$p=0.002$) than the control group (MM), whereas total glutathione ($p<0.0001$;$p<0.0001$) and GSH levels ($p<0.0001$;$p<0.0001$) were significantly diminished. Catalase activity was significantly decreased ($p=0.003$;$p<0.001$) leading to an accumulation of hydrogen peroxide ($p=0.04$;$p=0.001$), which would explain the significantly increased levels of OS biomarkers observed in these patients. TL was significantly shorter in AATD patients than in control individuals ($p<0.001$) and an association between TL and AATD phenotypes such as high-risk patients showed shorter TL than intermediate-risk and low-risk groups. No differences were observed between the control and the low-risk (MS;SS) groups.

Conclusions: Increased OS together with reduced antioxidant defense are involved in the pathophysiology of AATD. An association between TL and risk of developing lung/liver disease is observed indicating that TL could be a promising biomarker for AATD disease progression.

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P189

Characterization of antioxidant systems in different complementation groups of Dyskeratosis Congenita

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The telomerase complex and the telosome regulate, maintain and repair human telomeres. The first is formed by a set of proteic (TERT, Dyskerin, GAR, NHP2, NOP10) and nucleic acid (TERC) subunits, which together work as a reverse transcriptase complex; the second comprises a network of proteins (TRF2, TRF1, TIN2, RAP1, TPP1 and POT1). Dyskeratosis congenita (DC; ORPHA1775) is a rare disease with similar characteristics to premature aging. DC is a genetically heterogeneous pathology caused by mutations in the genes that encode for different subunits of the telomerase and telosome complexes.

In this study we evaluate the cellular effects related to the production of oxidative stress, alteration of antioxidant systems, and DNA damage in relation to the activity of telomerase and telosome components. Through the technology of RNA interference (siRNA), we have independently silenced the DKC1, NOP10 and TINF2 genes, all of them components involved in telomere maintenance and each one of them related to different subtypes of DC (X-linked DC, autosomic recessive DC and autosomic dominant DC, respectively) in HeLa cells. Also, we have evaluated Reactive Oxygen Species (ROS) by flow cytometry, and oxidative stress marks (carbonylation and oxidized peroxiredoxin protein levels) by western blotting. An increase in oxidative stress marks was observed, as well as a deregulation in the levels of the antioxidant enzymes MnSOD, Trx1 and Trx2. Finally, we have analyzed by flow cytometry DNA damage marks (□H2AX and PARP), detecting elevated levels of PARP. In addition, measurement of the levels of DNA repair enzymes (Rad51, Rad53, oGG1, PARP1 and WRN), show PARP1 and RAD51 deregulation. These results suggest a tight relationship between antioxidant systems and DNA damage response mechanisms with the activity of specific telomerase and telosome proteins, pointing to interesting possibilities for the molecular dissection of the pathophysiology of specific DC subtypes.

P190

Progesterone reduces oxidative stress and slows photoreceptor cell death in an animal model of retinitis pigmentosa

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Purpose: The aim of this study is to study the neuroprotective role of progesterone (PG) in an animal model of retinitis pigmentosa (RP), the rd10 mice, and elucidate its possible mechanism. RP results in progressive death of photoreceptors and vision impairment and there is no effective treatment for this disease.

Methods: PG was administered to rd10 and control mice at postnatal days 15, 17, 19 and 21. At the last day of treatment, animals were sacrificed; eyes were enucleated and sectioned or homogenized.

Histological evaluation was performed with hematoxilyn-eosin and cell death was assayed using terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end-labeling (TUNEL).

Alterations of different transcription and neurotrophic factors, autophagy markers and enzymes (c-AMP response element binding (CREB), pCREB, brain-derived neurotrophic factor (BDNF), (tumor necrosis factor alfa) TNFα, LC3, inducible nitric oxide synthase (iNOS) were investigated by Western Blot. Oxidative stress markers such as reduced and oxidixed glutathione (GSH and GSSG) and malondialdehyde (MDA) were determined by HPLC. Finally, glutamate-cysteine ligase catalytic (GCLC) subunit, the key enzyme in glutathione syhthesis was studied by immunohistochemistry.

Results: After PG treatment amelioration on cell death was observed, mostly at the periphery of the retina. No differences were found in retinal levels of pCREB/CREB ratio, BDND, TNFα and LC3III between control and rd10 mice. Retinal GSH/GSSG ratio was decreased and MDA increased in rd10 mice compared with the control group, and PG was able to restore these alterations. GCLC was increased in the retina of rd10 mice, though progesterone was not able to induce any change in this enzyme.

Conclusion: Results suggest that progesterone has a protective role in RP, its possible mechanism could be related with its antioxidant ability.

P191

Involvement of oxidative stress in 7-ketocholesterol-induced intestinal barrier dysfunction

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Decreased integrity and increased permeability of barrier tissues may lead to serious disturbances of local homeostasis, causing various pathologies, including inflammatory bowel disease (IBD), food allergy, and bacterial infections.

The intestinal epithelium is exposed to a wide range of products of cholesterol oxidation present in foodstuffs, among which 7-oxygenated sterols, such as 7-ketocholesterol (7-Keto), are the most common. 7-Keto has been demonstrated to affect intestinal epithelial barrier functions and to exert pro-oxidant and pro-inflammatory effects in various cell types.

The aim of this study was to evaluate the damage to intestinal mucosa, in relation to the pro-oxidant action of 7-Keto, using differentiated human Caco-2 cells on transwell inserts as a model system of the human intestine. The disruption of epithelial integrity, was measured as transepithelial electrical resistance (TER), and as markers of the alteration of cellular redox status, the generation of reactive oxygen species (ROS) was determined, using the fluorescent probe 2', 7'-dichlorofluorescein diacetate, together with the ratio GSH/GSSG, by HPLC analysis.

7-keto treatment caused the loss of the epithelial integrity and TJ ion selectivity, as indicated by the decrease of the TER value with time. Such barrier dysfunction seemed to be linked to a significant alteration of the cellular redox status, as indicated by ROS increase and GSH depletion.

Our data strengthen the hypothesis that oxysterols of dietary origin may significantly interfere with the homeostasis of the human intestine, being involved in the pro-oxidant and pro-inflammatory processes linked to the onset and development of major chronic diseases.

P192

The antiproliferative effects of *Rosmarinus officinalis* extract in colon cancer cells

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Carnosic acid, carnosol and rosmarinic acid are the main compounds present in *Rosmarinus officinalis* extract (RE) and have shown anti-proliferative effects on various tumor cell lines. Previous studies of our research group have shown that a rosemary extract obtained by CO_2-supercritical fluid extraction has the capacity to inhibit cell proliferation in colon cancer human cell models.

The goal of this study was to elucidate if the observed anti-proliferative activity of RE on different colon human cancer cells (HGUE, HT29 and SW480) was related to free radical generation and the modulation of oxidative stress-related pathways. For this purpose, intracellular free radical generation, mitochondrial viability and modulation of NRF2 pathway was explored.

Cells were treated with RE (10-60µg/mL) for 24h at 37°C. Cell viability was determined by MTT assay. Reactive oxygen species (ROS) production was measured with DCF-DA. Mitochondrial functionality was tested by MitoTracker Red (CMXRos) and MitoTracker Green, FM (MTG) fluorescent probes. In addition, cells were transfected with *Nrf2*-targeting *siRNA* to determine the potential role of this transcription factor. Fluorescence was analyzed using the Cytation microscope, Imaging Multi-Mode Reader of Bio-Tek.

RE significantly reduced cell viability starting at 10 µg/mL in HGUE, HT29 and SW480 colon cancer cells. The inhibition of cell proliferation was correlated to an increase of intracellular ROS content and a decrease of the mitochondrial membrane potential. Furthemore, the molecular mechanism seems to involve activation Nrf2/ARE pathway as a response for cell survival since NRF2 silencing decreased cell viability in SW480 cells. The antiproliferative effects of a RE are mediated by the intracellular generation of ROS and decrease of mitochondrial viability, which lead to activation of NRF2 as a response for cancer cell survival.

P193

Oxidative stress is increased in Prader-Willi syndrome patients

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Introduction: Prader-Willi's syndrome (PWS) is a rare genetic disease characterized by hypotonia and feeding difficulties in early infancy, followed by progressive obesity in later infancy and adult age.

Aims and objectives: Since systemic oxidative stress (OS) has been linked to obesity in several diseases, we hypothesized that OS is increased in PWS patients. However, the OS status of PWS patients has not been previously determined. This study was aimed to analyze the OS profile in patients with PWS.

Materials and methods: Oxidative stress biomarkers, ROS production and mitochondrial parameters were prospectively measured in leukocytes obtained from 6 control subjects and 6 patients with PWS.

Results: Higher protein carbonylation levels were observed in PWS patients (PG) compared to control individuals (CG) (189.50±65.76 vs. 264.08±143.95, respectively; p=0.521). Mitochondrial mass is increased in PG compared to CG (2321.00±137.18 vs. 4296.67±2312.00, respectively; p=0.095), while membrane potential is decreased in patients respect to the controls (4356.00±189.50 vs. 2997.00±856.30, respectively; p=0.078). Mitochondrial O_2^- is increased the PG compared to the CG (21.00±8.63 vs. 68.22±56.86, respectively; p=0.096). NO levels are increased in lymphocytes of PWS patients (86.45±33.30 vs. 138.40±16.41, respectively; p=0.03). No differences in GSH, O_2^- and H_2O_2 levels were observed between groups.

Conclusions: Although additional work, including a higher number of patients is needed to determine the clinical relevance of this observation, our preliminary results indicate that OS parameters are increased in PWS patients, indicating that it could play an important role in the pathogenesis of the disease.

P194

Comparison of airway and systemic malondialdehyde levels for assessment of oxidative stress in cystic fibrosis

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Oxidative stress plays an important role in the pathogenesis of cystic fibrosis (CF). In this study airway and systemic oxidative stress was investigated in CF using malondialdehyde (MDA), an established by-product of polyunsaturated fatty acid peroxidation.The study included 40 stable CF patients and 25 healthy controls. Exhaled breath condensate (EBC), sputum and plasma were collected from during routine clinical visits. The concentration of MDA was measured by HPLC. The ROC analysis was used to determine the discriminatory power of the MDA measurement. The relationships between the levels of MDA and clinicopathological factors were also assessed. MDA levels in sputum (279.8±14.7 vs. 92.7±9.2 nmol/L, $p<0.0001$), EBC (139.9±6.7 vs. 71.5±4.3 nmol/L, $p<0.0001$) and plasma (176.1±15.9 vs. 129.6±12.9.nmol/L, $p<0.05$) were increased in patients with CF compared to controls. MDA measurement in sputum (area under ROC curve [AUC]: 0.977, $p<0.0001$) or EBC (AUC: 0.94, $p<0.0001$) distinguished between patients and controls with greater accuracy than in plasma (AUC: 0.677, $p<0.05$). There were significant associations between sputum MDA levels and the serum concentration of C-reactive protein ($r=0.42$, $p<0.05$) and the erythrocyte sedimentation rate ($r=0.47$, $p<0.05$). Sputum and EBC MDA levels were elevated in patients with severe pulmonary dysfunction (forced expiratory volume in 1 sec [FEV1]<50% predicted) compared to those with mild-to-moderate functional impairment (FEV1>50% predicted) ($p<0.05$).MDA concentrations in CF patients colonized either with Pseudomonas aeruginosa or with other bacteria were similar (p=NS). Inhaled corticosteroid treatment did not affect MDA levels (p=NS).The intra- and inter-assay repeatability of MDA measurements was similar in all three types of samples, while the between-visit variability was higher in plasma. MDA is a potential new airway marker of oxidative stress in patients with CF. Sputum MDA differentiates best between patients and healthy subjects.

P195

Antioxidant effects of melatonin in chronic periodontitis

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Periodontitis is a chronic inflammatory disease affecting oral mucosa and teeth supporting tissues. Together with inflammation, oxidative stress is considered to play a crucial role in pathogenesis of periodontitis. Melatonin is well-known for its anti-inflammatory and antioxidant properties. This could help to regulate the immune response in periodontitis. The aim of our work was to analyze the effects of melatonin in treatment of chronic periodontitis in animal experiment and in a study of human volunteers.

The periodontitis was induced by threefold ligature 7-0 around the first right lower molar neck area and left there for 4 weeks. Forty adult male rats were randomly divided into CTRL – rats without ligature drinking water, CTRL MEL – rats without ligature treated with melatonin, LIG – rats with ligature drinking water and LIG MEL group – rats with ligature treated with melatonin. Afterwards, animals in CTRL MEL and LIG MEL group were treated with melatonin (10 mg/kg in water) for 2 weeks. Macroscopic score and markers of oxidative and antioxidative status were analyzed in the gingival and salivary samples along with salivary melatonin. In human study, 20 patients with chronic periodontitis were randomly assigned into placebo (CTRL) or melatonin (MEL) group. Saliva was collected before and 2 weeks after the treatment.

In the LIG MEL group, observed gingival tissue parameters improved after two weeks of melatonin treatment. Additionally, increased salivary levels of melatonin, a trend towards higher total antioxidant capacity and lower advanced glycation end-products, IL-1 and TNF-α levels were found when compared to LIG group. These positive effects of melatonin treatment were, however, not confirmed in our human study.

In conclusion, melatonin improved periodontitis in our model. In humans, studies should evaluate the melatonin effect on larger population sample or longer treatment.

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Anti-oxidative effect of Squalene on doxorubicin treated mice

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Objective: Squalene (SQ) is a precursor of cholesterol synthesis. Although SQ has been used for total health promotion, little scientific information is available about the antioxidant and antitumor activities. We previously demonstrated the anti-tumor effect of SQ on mice through the modulation of DNA damage response induced by anti-tumor drugs. However, anti-tumor drugs occasionally impair the redox balance and anti-inflammatory responses during the treatment. In the present study, we examined the anti-oxidative and anti-inflammatory effects of SQ on tumor transplanted mice.

Methods: Colon26 mouse tumor cells were transplanted into the left paw of BALB/c mice. Mice were orally administrated with PBS (CT) or SQ (2 mol/kg) during Doxorubicin (DOX) (2mg/kg, i.p.) treatment. Prostaglandin E_2 (PGE_2), a pro-inflammatory mediator, was analyzed by ELISA. The lipid peroxidation was measured by TBARS. To analyze the effect of SQ on fatty acids (FA) composition of cell membrane, a normal human skin fibroblast (NB1-RGB) was treated with or without SQ.

Results and Conclusion: The plasma TBARS level in SQ administrated mice was lower than that of CT. SQ decreased the plasma concentration of PGE_2 when the mice were treated with DOX. The plasma EPA of mice was tended to increase by SQ administration but not arachidonic acid (AA). Meanwhile, EPA and DHA were time-dependently increased by the SQ treated NB1-RGB cells, though not AA. As a result, ω3/ω6-FA balance was increased by SQ treatment. These results suggest that the SQ reduces the oxidative stress of anti-tumor drug treated mice through the modulation ω3-FA composition.

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Reduced antioxidant response and elevated oxidative stress in patients with retinitis pigmentosa

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Retinitis Pigmentosa (RP) is a common form of hereditary retinal degeneration characterized by loss of retinal photoreceptor cells (rods and cones). RP constitutes the largest single cause of blindness in the developed world. It has been suggested that after rod death, cone die due to several causes including oxidative damage, depletion of trophic factors and release of toxic factors by apoptotic rods and surrounding cells. Oxidative stress probably plays a major role in the pathogenesisi of RP. We evaluated whether RP is associated with oxidative damage in eye, and whether the damage is reflected in peripheral blood. We also investigated the relation between visual function and oxidative stress in these patients.

Forty-one patients diagnosed with typical RP and fifteen patients diagnosed with Usher syndrome were included in this study. We included sixty participants without oxidative stress-related disease, including thirteen patients diagnosed with cataract as controls. Aqueous humor and peripheral blood were collected to measure markers of antioxidant response (superoxide dismutase (SOD) activity, total antioxidant activity) and markers of oxidative stress (nitrotyrosine, thiobarbituric acid reactive sbustances (TBARS), nitrites). Ophthalmic test included were best-corrected visual acuity and visual field.

MANCOVA revealed that RP reduces antioxidant response on eye and also produces an imbalance redox status in blood independent of sex and visual impairment. Patients with RP present low total antioxidant capacity including reduce SOD activity in aqueous humor. In blood RP also induces lower SOD activity, and higher TBARS and nitrotyrosine content than in control situation.

We suggested that the eyes of patients with RP could have less capacity to cope with toxic oxygen intermediates, that in turns, could contribute to the progression of the retinal degeneration.

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Preliminary report on DNA damage among IBD patients

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Chronic inflammation is the hallmark in inflammatory bowel disease (IBD). Research has identified genetic predisposition and environmental factors as key elements in the development of IBD; nevertheless, its etiology remains unknown. The characteristic exorbitant inflammatory response seen in patients' bowel may arise from various mechanisms at the cellular and subcellular level and in active periods of disease the gut mucosa is massively invaded by inflammatory cells. This uncontrolled and excessive immune response renders the inflammatory cells to produce reactive oxygen species (ROS) and free radicals which are known to trigger mucosal injury and induce inflammation. In this scenario of chronic inflammation, when sustained production of reactive oxygen species (ROS) is achieved and antioxidant defenses weaken or are depleted, oxidative stress occurs. Oxidative stress can cause injury to many cellular macromolecules and in part be responsible for some of the clinical manifestations of IBD such as ulcers, sores, mucosal frailty and for the perpetuation of inflammation. Oxidative DNA damage has been associated with cancer promoting mechanisms. High rates of DNA damage may promote loss of homeostasis and faulty DNA repair capacity. We can hypothesize that in a context of IBD there may be an impairment regarding the repair capacity of cells as they are under continued stress and their function deregulated. Currently there is limited knowledge regarding the contribution of oxidative modification of macromolecules, and their subsequent repair, to the IBD pathogenesis.Our preliminary results to evaluate and compare DNA damage and oxidative damage between patients (343) and healthy controls (293) showed increased values for DNA damage among patients – 9,01 AU vs 5,45 AU ($p<0,001$) and for oxidative damage also – 2,25 AU vs 1,76 AU ($p<0,001$). DNA damage and oxidative DNA damage were assessed using medium troughput*comet assay*. We aim to enlighten the importance of oxidative stress over IBD pathogenesis and disease course.

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The heme and radical scavenger α_1-microglobulin (A1M) confers protection of the periventricular white matter following preterm intraventricular hemorrhage (IVH)

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Background

Cerebral IVH is a major cause of severe neurodevelopmental impairment and mortality in very preterm infants. To date, no therapy is available that prevents or reduces serious neurological disability. Following hemorrhage, extravasation of blood and release of hemoglobin (Hb) generates heme and free radicals that constitute pivotal events in the development of brain damage. Hb and its downstream metabolites are toxic and causal in inflammation, oxidative stress and cell death. We propose that the use of the heme and free radical scavenger A1M might confer protection of the immature brain following preterm IVH.

Material/Methods

Using a preterm rabbit pup model of IVH, the structural and functional integrity, cellular, inflammatory and oxidative response of the periventricular white matter was evaluated at 24 and 72 hours following hemorrhage. Animals were injected with A1M and sham and evaluated using mRNA and protein expression, immunohistochemistry and electron microscopy. The protective effects of A1M was further studied in primary oligodendrocytes exposed to heme and free radicals.

Results

Results show that IVH in premature rabbit pups leads to an increased mRNA and protein expression of inflammation (TNFα, IL-1β and CCL2), tissue injury mediators (MMP9) and cellular activation (TLR-4, COX-1, COX-2 and IL1R1) in periventricular brain tissue both 24 and 72 hours after bleeding has occurred. Addition of A1M and congruent scavenging of heme and free radicals caused a significant reduction in inflammation, tissue injury and cellular response. *In vitro* studies showed that oligodendrocytes are highly sensitive to heme and radical exposure and that addition of A1M displayed a very protective effect.

Conclusion

Following IVH there is an increase of inflammation, tissue injury and cellular activation in the periventricular white matter. Administration of the heme- and radical scavenger A1M almost completely inhibited these effects. Our studies present an efficient means of decreasing the damage to the periventricular white matter following IVH.

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