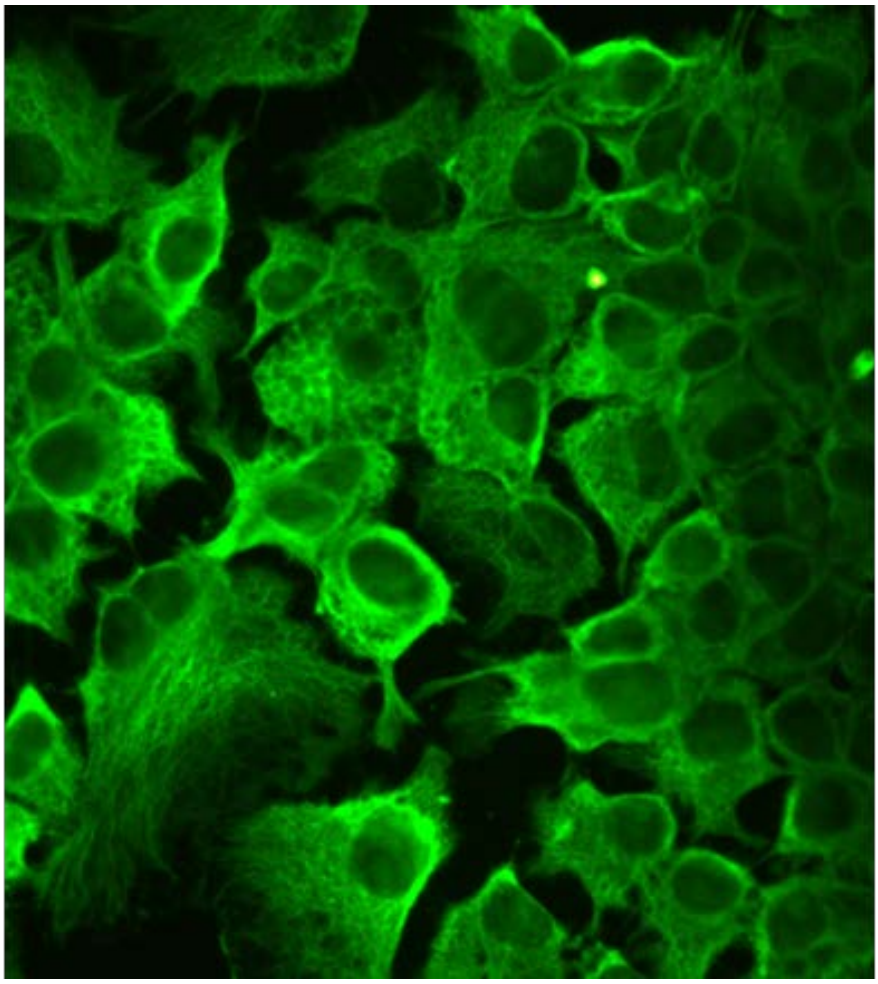
GEIRLI GrupoEspañol de Investigación en Radicales Libres

X MEETING of the SPANISH GROUP FOR RESEARCH ON FREE RADICALS (GEIRLI)

"SYMPOSIUM ON OXIDATIVE STRESS AND REDOX SIGNALING IN BIOLOGY AND MEDICINE"

Homage to
Luis Alfonso del Río and Pere Puig-Parellada

2nd-4th June 2014
Venue: Aula Magna –Faculty of Medicine- Valencia



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X MEETING of the Spanish Group for Research on Free Radicals (GEIRLI)

"SYMPOSIUM ON OXIDATIVE STRESS AND REDOX SIGNALING IN BIOLOGY AND MEDICINE"

Homage to Luis Alfonso del Río and Pere Puig-Parellada

VALENCIA, 2nd-4th June 2014

Venue: Aula Magna, Faculty of Medicine, University of Valencia

SCIENTIFIC PROGRAMME

2nd June 2014

Morning session

Redox signaling in Biomedicine: from epigenetics to disease

Chairs: **Nadezda Apostolova** *(Department of Medicine, University Jaume I, Castellón)* and **Francisco Dasí** *(INCLIVA & Department of Physiology, University of Valencia)*

9:00-9:30 **José Luis García-Giménez** *(CIBERer & Department of Physiology, University of Valencia)*
Unlocking the redox control of the epigenome

9:30-10:00 **Francisco Dasí** *(INCLIVA & Department of Physiology, University of Valencia)*
Decreased glutathione and low catalase activity contribute to increased oxidative stress in early stages of alpha-1 antitrypsin deficiency

10:00-10:30 **Consuelo Borrás** *(Department of Physiology, University of Valencia)*
Hormone replacement therapy: it is critical to provide it on time

10:30-11:00 **Carmen Gómez** *(Department of Physiology, University of Valencia)*
Redox regulation of E3 ubiquitin ligases in skeletal muscle and its role in muscular atrophy

11:00-11:15 Break

Chairs: **Carmen Gómez** *(Department of Physiology, University of Valencia)* and **José Luis García-Giménez** *(Department of Physiology, University of Valencia)*

11:15-12:15 **Oral communications**

O1 **Ana Blas-García** *(Department of Pharmacology, School of Medicine, University of Valencia, FISABIO, University Hospital Doctor Peset, Valencia)*
The anti-HIV drugs Abacavir and Didanosine induce oxidative stress and enhance acetaminophen-induced hepatotoxicity

O2 **Vanesa Carmona** *(Department of Pharmacology, School of Pharmacy, Universidad Complutense de Madrid, Madrid)*
Multiple targets involved in Alzheimer's disease are affected by morin and isoquercitrin

O3 **Susana Rovira-Llopis** *(Service of Endocrinology, FISABIO, University Hospital Doctor Peset, Valencia)*
Oxidative and endoplasmic reticulum stress are influenced by glycaemic control in leukocytes of type 2 diabetic patients

O4 **Pablo Hernansanz-Agustín** *(Servicio de Inmunología, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa, Madrid, Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Madrid and Instituto de Investigaciones Biomédicas Alberto Sols, Madrid)*
Acute hypoxia produces a superoxide burst in cells

O5 **Celia Vived** *(Dept. Ciències Mèdiques Bàsiques, Universitat de Lleida, IRB Lleida)*
A metabolic cycle within the cell division cycle. The role of the Fox transcription factor Hcm1

Redox regulation of cell signaling by superoxide and electrophilic lipids

Chairs: **Consuelo Borrás** *(Department of Physiology, University of Valencia)* and **José Ignacio Ruiz-Sanz** *(Department of Physiology, University of the Basque Country UPV/EHU, Bilbao)*

12:15-12:45 **Antonio Martínez-Ruiz** *(Instituto de Investigación Sanitaria Princesa, Madrid)*
Acute hypoxia signals mediated by reactive oxygen species: From superoxide production to thiol modification

12:45: 13:15 **Dolores Pérez-Sala** *(CIB, CSIC, Madrid)*
Electrophilic lipids in the elucidation of novel mechanisms of redox regulation

Afternoon session

15:15-16:15 **Poster session:**

Chairs: **Dolores Pérez-Sala** *(CIB, CSIC, Madrid):* Posters **P1-P13** **Víctor Víctor** *(FISABIO-Hospital Dr Peset, Valencia)*: Posters **P14-P27** **Raquel Taléns** *(Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Valencia):* Posters **P28-P41**

Oxidative stress and antioxidants in tissue damage (I)

Chairs: **Mª Begoña Ruiz-Larrea** *(Department of Physiology, University of the Basque Country UPV/EHU, Bilbao)* and **Jordi Muntané** *(Department of General Surgery, Virgen del Rocío University Hospital, IBiS, Sevilla)*

16:15-16:45 **Carmen Vázquez** *(Department of Physiology, University of Sevilla)*
The beneficial effect of antioxidants in hypertension-related organ damage

16:45-17:15 **Guillermo Sáez** *(Department of Biochemistry and Molecular Biology, University of Valencia)*
8-Oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) in human gastrointestinal tumors. Beyond oxidative stress

17:15-17:45 **Reinald Pamplona** *(Department of Physiology, University of Lleida)*
Lipidomic and longevity

17:45-18:15 **Manuel Portero Otín** *(Department of Physiology, University of Lleida)*
Dietary modulation of lipoxidative stress in a transgenic model of amyotrophic lateral sclerosis

18:15-18:30 Break

Oxidative stress and antioxidants in tissue damage (II)

Chairs: **Teresa Mitjavila** *(Department of Physiology and Immunology, University of Barcelona)* and **Guillermo Sáez** *(Department of Biochemistry and Molecular Biology, University of Valencia)*

18:30-19:00 **Enrique O'Connor** *(Department of Biochemistry and Molecular Biology, University of Valencia)*
Cytomic analysis of oxidative stress

19:00-19:30 **Susana Cadenas** *(Centro de Biología Molecular "Severo Ochoa" CSIC-UAM, Madrid)*
Uncoupling proteins and the control of mitochondrial ROS production: implications for cardiac ischemia-reperfusion injury

19:30-20:00 **Joan Roselló Catafau** *(IIBB, CSIC, Barcelona)*
Free radicals in liver cold ischemia-reperfusion injury

20:00 **Homage to Luis Alfonso del Río and Pere Puig-Parellada**
Assembly of the Spanish Group for Research on Free Radicals (GEIRLI)

3rd June 2014

Morning session

Chairs: **Susana Cadenas** *(Centro de Biología Molecular "Severo Ochoa" CSIC-UAM, Madrid)* and **Antonio Martínez-Ruiz** *(Instituto de Investigación Sanitaria Princesa, Madrid)*

8:30-9:30 **Oral communications**

O6 **David Alsina** *(Ciències Mèdiques Bàsiques, Facultat de Medicina, Universitat de Lleida/ IRB Lleida)*
ADR1 and CTH2 mediate metabolic remodeling in Frataxin deficient yeast

O7 **Cristina Espinosa-Diez** *(Departamento de Biología Celular e Inmunología, Centro de Biología Molecular "Severo Ochoa," CSIC, Universidad Autónoma de Madrid, Madrid)*
miR-433 targets γ-glutamyl-cysteine-ligase and regulates endothelial function, redox hormesis and fibrogenesis

O8 **Guillermo Zalba** *(Department of Biochemistry and Genetics. University of Navarra, Pamplona)*
NADPH oxidase-mediated oxidative stress promotes telomere shortening in phagocytic cells: involvement in human atherosclerosis

O9 **Gustavo Egea** *(School of Medicine, University of Barcelona; IDIBAPS, Barcelona)*
TGF-β-dependent NADPH oxidase 4 (NOX4) overexpression in Marfan Syndrome aggravates the formation of aortic aneurysm

O10 **Jordi Muntané** *(Department of General Surgery, Virgen del Rocío-Virgen Macarena University Hospitals, IBiS, Sevilla)*
Nitric oxide synthase type III overexpression by gene therapy exerts antitumoral activity in hepatocellular carcinoma

Redox signaling in neurodegenerative diseases and inflammation

Chairs: **Corinne Spickett** *(School of Life & Health Sciences, Aston University, Birmingham, UK)* and **Juan Sastre** *(Department of Physiology, University of Valencia)*

9:30-10:00 **Daniel Ramón** *(Biópolis, Valencia)*
Using *Caenorhabditis elegans* as a model for the evaluation of antioxidant food ingredients

10:00-10:30 **José Viña** *(Department of Physiology, University of Valencia)*
Role of radicals in glutamate excitotoxicity in Alzheimer disease

10:30-11:00 **Lisardo Boscá** *(Instituto de Investigación Biomédica Alberto Sols, CSIC, Madrid*
Interplay between macrophage polarization, glucose metabolism and oxidative stress

11:00-11:30 **Joaquim Ros** *(Deptartment of Basic Medical Sciences, University of Lleida)*
Neurons and cardiomyocytes as cell models to study Friedreich Ataxia

11:30-12:00 Break

Transcriptional regulation of redox signaling

Chairs: **Lisardo Boscá** *(Instituto de Investigación Biomédica Alberto Sols, CSIC, Madrid)* and **José Viña** *(Department of Physiology, University of Valencia)*

12:00-12:30 **Elena Hidalgo** *(Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona)*
Regulation of gene expression programs by the stress-dependent MAP kinase Sty1 of *Schizosaccharomyces pombe*

12:30-13:00 **María Monsalve** *(Instituto de Investigación Biomédica Alberto Sols, CSIC, Madrid)*
Control of endothelial function and angiogenesis by PGC1-α

13:00-13:30 **Santiago Lamas** *(Centro de Biología Molecular "Severo Ochoa", CSIC, Madrid)*
MicroRNAs as regulators of glutathione homeostasis

13:30-14:00 **Giovanni Mann** *(King's College London, UK)*
Nrf2-mediated neurovascular protection against oxidative stress in experimental stroke

Afternoon session

16:30-17:30 **Round table with Nobel Laureate Dr. Randy Schekman**

Redox signaling in aging

Chairs: **Santiago Lamas** *(Centro de Biología Molecular "Severo Ochoa", CSIC, Madrid)* and **Giovanni Mann** *(King's College London, UK)*

17:30-18:00 **Joao Laranjinha** *(Center for Neurosciences and Cell Biology, University of Coimbra, Portugal)*
Dysfunctional nitric oxide-mediated neurovascular coupling in Alzheimer´s disease and aging

18:00-18:30 **Bertrand Friguet** *(Life Science Department, University Pierre et Marie Curie, Paris, France)*
Oxidative proteome alterations target specific cellular pathways during oxidative stress and cellular aging

18:30-19:00 Coffee break

Phospholipid oxidation in cell signaling

Chairs: **Federico Pallardó** *(Department of Physiology, University of Valencia)* and **Joao Laranjinha** *(Center for Neurosciences and Cell Biology, University of Coimbra, Portugal)*

19:00-19:30 **Corinne Spickett** *(School of Life & Health Sciences, Aston University, Birmingham, UK)*
Oxidized phospholipids in the vascular system and their cellular effects

19:30-20:00 **Rosario Domíngues** *(Department of Chemistry, University of Aveiro, Portugal)*
Anionic phospholipid oxidation in cell signalling

4th June 2014

Antioxidants and signaling by ROS and RNS in plants (I)

Chairs: F**rancisca Sevilla** *(CEBAS, CSIC, Murcia)* and **José M. Palma** *(EEZ, CSIC, Granada)*

09:00-09:25 **Francisco Javier Corpas** *(EEZ, CSIC, Granada)*
Nitric oxide under a "green" perspective

09:25-09:50 **Ana Jiménez** *(CEBAS, CSIC, Murcia)*
Plant mitochondrial Trxo1: looking for its transcriptional gene regulation and functions

09:50-10:15 **Juan Carlos Begara-Morales** *(Group of Biochemistry and Cell Signaling in Nitric Oxide. Department of Biochemistry and Molecular Biology, University of Jaén)*
Post-translational modifications mediated by nitric oxide under physiological and stress conditions in plants

10:15-10:40 **Manuel Becana** *(EEAD, CSIC, Zaragoza)*
Interactions of ROS and RNS with plant hemoglobins

10:40-11:10 **Oral communications**

O11 **Marta Rodríguez-Ruiz** *(Group of Antioxidants, Free Radicals and Nitric Oxide in Biotechnology and Agro-food; Dept. Biochemistry, Cell and Molecular Biology of Plants; EEZ, CSIC, Granada)*
Characterization and RNS-modulation of the ascorbate-synthesizing enzyme galactono-γ-lactone dehydrogenase from pepper fruits

O12 **Capilla Mata-Pérez** *(Group of Biochemistry and Cell Signaling in Nitric Oxide. Department of Biochemistry and Molecular Biology, Campus Universitario "Las Lagunillas" University of Jaén, Jaén)*
Presence of endogenous nitro-fatty acids in olive oil as mediators of cell signaling

O13 **Daymi Camejo** *(Departamento de Biología del Estrés y Patología Vegetal, CEBAS-CSIC, Murcia)*
Antioxidant system and carbonylation of proteins in mitochondria from pepper fruits at two maturation stages

11:10-11:40 Break

Antioxidants and signaling by ROS and RNS in plants (II)

Chairs: **Eva Barreno** *(Departamento de Botánica, Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universidad de Valencia)* and **Luisa Mª. Sandalio** *(EEZ, CSIC, Granada)*

11:40-12:05 **Juan B. Arellano** *(IRNASA, CSIC, Salamanca)*
Programmed cell death activated by Rose Bengal in *Arabidopsis thaliana* cell suspension cultures requires functional chloroplasts

12:05-12:30 **Eva Barreno/Myriam Catalá** *(Departamento de Botánica, Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universidad de Valencia; Departamento de Biología y Geología, Física y Química Inorgánica y Analítica. E.S. de Ciencias Experimentales y Tecnología. Universidad Rey Juan Carlos)*
Lichen symbionts strategies to overcome harsh abiotic stress: Diversity and cooperation

12:30-12:55 **María Romero-Puertas** *(EEZ, CSIC, Granada)*
Role of NADPH oxidases in the network regulating cell response to short and long term cadmium exposure

12:55-13:20 **Carmen González Bosch** *(Department of Biochemistry and Molecular Biology, University of Valencia)*
Hexanoic acid protects plants against pathogens by priming defence responses and reducing oxidative stress

13:20-13:50 **Frank van Breussegem** *(Oxidative Stress Signalling Laboratory, Department of Plant Biotechnology and Bioinformatics, Ghent University, Belgium)*
H_2O_2 signal transduction in plants, puzzling the pieces

Chair: **José Viña** *(Department of Physiology, University of Valencia)*

14:00-14:30 **Commemorative Lecture "Ana Navarro"**: **Luis Alfonso del Río** *(EEZ, CSIC, Granada)*
Peroxisomes as a cellular source of ROS and RNS signaling molecules

14:30 **SFRR-E Awards to Young scientists and concluding remarks**

LECTURES

UNLOCKING THE REDOX CONTROL OF THE EPIGENOME

José Luis García Giménez

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Center for Biomedical Network Research on Rare Diseases (CIBERer).
Department of Physiology. School of Medicine and Dentistry School.
University of Valencia

Epigenetics is defined as the mitotically/meiotically heritable changes in gene expression that are not due to changes in the primary DNA sequence. Over recent years, growing evidence has suggested a link between redox metabolism and oxidative stress and the control of epigenetic mechanisms. This link between redox control, oxidative stress, and glutathione (GSH) and epigenetics occurs at different levels affecting DNA and histone methylation, histone acetylation, and creating a new panel of redox post-translational modifications that enrich the histone code. Pioneer works showed how oxidized GSH inhibits the activity of S-adenosyl methionine synthetase, MAT1A, a key enzyme involved in the synthesis of S-adenosyl methionine (SAM), which is used by DNA methyltransferases (DNMTs) and histone methyltransferases (HMTs). Alteration in NAD+/NADH ratio affects the activity of class III histone deacetylases (HDACs) and poly-ADP ribosyltransferases (PARPs). Furthermore, the iron redox state influences the activity of HDACs and the activity of Tet methylcytosine dioxygenases that act as DNA demetylases.

In this communication we will show the intricate mechanisms that participate in the redox control of the epigenome. We specially focus our work in the characterization of new redox-posttranslational modifications in histones, such as histone carbonylation, parsylation, and glutathionylation. Demonstrating how GSH influences the epigenetic mechanisms beyond mere regulation of SAM levels.

The mechanisms described in this communication place GSH and redox control in the landscape of the epigenetic regulation. The results shown underscore the relevant role that oxidative stress and GSH play as key factors in epigenetics, opening a new window for understating the underlying mechanisms that control cell differentiation, proliferation, development, and disease.

DECREASED GLUTATHIONE AND LOW CATALASE ACTIVITY CONTRIBUTE TO INCREASED OXIDATIVE STRESS IN EARLY STAGES OF ALPHA-1 ANTITRYPSIN DEFICIENCY

Amparo Escribano[1,2,3], Mónica Amor[1,2], Sara Pastor[3,4], Silvia Castillo[1,2,3], Francisco Sanz[5], Pilar Codoñer-Franch[1,6], Francisco Dasí[3,4]

E-mail: Francisco.Dasi@uv.es

[1]University of Valencia. School of Medicine. Department of Paediatrics, Obstetrics and Gynecology. Valencia. Spain. [2]Hospital Clínico Universitario Valencia. Paediatrics Pneumology Unit. Valencia. Spain. [3]Fundación Investigación Hospital Clínico Universitario de Valencia/Instituto de Investigación Sanitaria INCLIVA. Valencia. Spain. [4]University of Valencia. School of Medicine. Department of Physiology. Valencia. Spain. [5]Consorcio Hospital General Universitario de Valencia. Pulmonology Unit. Valencia. Spain. [6]Hospital Universitario Dr. Peset Valencia. Paediatrics Unit. Valencia. Spain

Background: Alpha-1 antitrypsin deficiency (AATD) is a hereditary condition that leads to decreased circulating AAT levels, significantly increasing the risk of serious lung and liver disease in children and adults. Recent investigations in animal models have revealed oxidative stress (OS) and oxidative damage in the pathogenesis of AATD.

Rationale and aims: We have previously demonstrated that OS is increased in serum of children with AATD. However, the mechanisms leading to the OS observed in these patients are currently unknown.

Methods: OS parameters and the activity of the main antioxidant enzymes were prospectively measured in serum of fifty-one clinically healthy children diagnosed with AATD and thirty-eight control individuals.

Results: Oxidative stress was increased in the serum of children with intermediate- (MZ; SZ) and high-risk (ZZ) phenotypes for developing AATD-related emphysema and/or liver disease. When compared with the control group, intermediate- and high-risk groups showed significantly lower total glutathione and reduced glutathione levels, decreased catalase activity and increased glutathione peroxidase activity leading to an accumulation of hydrogen peroxide that would explain the significantly increased levels of oxidative stress biomarkers observed in these patients. No differences were observed between the control (MM) and the low-risk (MS; SS) groups.

Conclusions: Increased OS together with reduced antioxidant defence are involved in the pathophysiology of AATD at early stages. An increased chronic oxidative status could contribute, at least in part, to the higher risk of lung and liver damage observed in these patients and reinforces the importance of avoiding cigarette smoking in AATD patients.

HORMONE REPLACEMENT THERAPY: IT IS CRITICAL TO PROVIDE IT ON TIME

Consuelo Borrás

Departamento de Fisiología, Facultad de Medicina, Universidad de Valencia

Aims: The usefulness of estrogen replacement therapy (ERT) in preventing oxidative stress associated with menopause is controversial. We aimed to study if there is a critical time window for effective treatment of the effects of ovariectomy with estrogens at the molecular, metabolic, and cellular level.

Results: Our main finding is that early, but not late onset of ERT prevents an ovariectomy-associated increase in mitochondrial hydrogen peroxide levels, oxidative damage to lipids and proteins, and a decrease in glutathione peroxidase and catalase activity in rats. This may be due to a change in the estrogen receptor (ER) expression profile: Ovariectomy increases the ER a/b ratio and immediate estrogen replacement prevents it. Positron emission tomography analysis shows that ovariectomy decreases the brain glucose uptake *in vivo* and that estrogen administration is beneficial, but only if administered immediately after deprivation. Ovariectomy decreases GLUT-1 and 3 glucose transporters in the brain, and only early onset estrogen administration prevents it. Plasma from rats treated with estrogens immediately after ovariectomy show similar metabolomics profiles as controls. Innovation: We provide molecular basis for the recommendation of early onset ERT and explain its lack of effectiveness if a significant time period elapses after ovariectomy and probably after the onset of menopause.

Conclusion: Only early, but not late onset administration of estrogens after ovariectomy has beneficial effects at molecular levels on oxidative stress, brain glucose uptake, and metabolomic profiles.

REDOX REGULATION OF E3 UBIQUITIN LIGASES IN SKELETAL MUSCLE AND ITS ROLE IN MUSCULAR ATROPHY

Gómez-Cabrera MC, Ferrando B, Viña J.

University of Valencia. Fundación Investigación Hospital Clinico Universitario/INCLIVA. Department of Physiology. Spain

Muscle atrophy is linked to reactive oxygen species (ROS) production during hindlimb-unloading due, at least in part,to the activation of xanthine oxidase (XO). The major aim of our study was to determine the mechanism by which ROS cause muscle atrophy and its possible prevention by allopurinol, a well-known inhibitor of XO widely used in clinical practice, and indomethacin, a nonsteroidal anti-inflammatory drug. We studied the activation of p38 MAP Kinase and NF-κB pathways, and the expression of two E3 ubiquitin ligases involved in proteolysis, the Muscle atrophy F-Box (MAFb) and Muscle RING Finger-1 (MuRF-1).

Male Wistar rats (3 mold) conditioned by 14 days of hindlimb unloading (n=18), with or without the treatment, were compared with freely ambulating controls (n=18). After the experimental intervention, soleus muscles were removed, weighted and analyzed to determine oxidative stress and inflammatory parameters. We found that hindlimb unloading induced a significant increase in XO activity in plasma (39%, p=0.001) and in the protein expression of CuZnSOD and Catalase in skeletal muscle. Inhibitionof XO partially prevented protein carbonylation, both in plasma and in soleus muscle, in the unloaded animals.

The most relevant new fact reported is that allopurinol prevents soleus muscle atrophy by ~20% after hindlimb unloading. Combining allopurinol and indomethacin wefound a further prevention in the atrophy process. This is mediated by the inhibition of the p38 MAPK-MAFbx and NF-κB -MuRF-1 pathways. Our data point out the potential benefit of allopurinol and indomethacin administration for bedridden, astronauts, sarcopenic and cachexic patients.

This work was supported by grants SAF2010-19498, ISCIII2006-RED13-027, PROMETEO2010/074, 35NEURO GentxGent and EU Funded COSTB35 and CM1001. The study has been cofinanced by FEDER funds from the European Union.

ACUTE HYPOXIA SIGNALS MEDIATED BY REACTIVE OXYGEN SPECIES: FROM SUPEROXIDE PRODUCTION TO THIOL MODIFICATION

Pablo Hernansanz-Agustín[a,b], Alicia Izquierdo-Álvarez[a], Elena Ramos[a], Tamara Villa-Piña[a], Antonio Martínez-Ruiz[a]

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[a]Servicio de Inmunología, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IP), C/ Diego de León 62, E-28006 Madrid, Spain.
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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.

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 ROS signals can be initiated by increased superoxide production in mitochondria, which can be translated in the oxidation of sensitive cysteine residues in cells subjected to acute hypoxia, mediating further functional adaptations.

ELECTROPHILIC LIPIDS IN THE ELUCIDATION OF NOVEL MECHANISMS OF REDOX REGULATION

Dolores Pérez-Sala

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Chemical and Physical Biology Department, Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu, 9, 28040 Madrid, Spain

Electrophilic lipids are reactive mediators which are generated in increased amounts under conditions of oxidative stress. Depending on their structure and levels and on the cellular context, electrophilic lipids participate in signaling mechanisms, cytoprotective and defense responses or in pathogenic processes. A key mechanism for the action of electrophilic lipids is the covalent binding to proteins, mainly at cysteine residues. This is at the basis of the interplay between electrophile action and mechanisms of redox signaling. Among electrophilic lipids, prostaglandins with cyclopentenone structure (cyPG) form stable adducts with proteins, and this has been exploited for their use in the identification and characterization of targets by means of tagged derivatives. These strategies have brought to the spotlight proteins or residues previously unnoticed to undergo redox regulation, thus allowing the elucidation of novel electrophile- and redox-responsive processes. Moreover, modification of cysteine residues by cyPG may coexist or compete with other cysteine-directed modifications, according to defined spatio-temporal patterns in the cell. This results in a complex network of interactions that may influence protein association, localization and activity. In recent years, our work has focused on the modifications of cysteine residues of proteins involved in cell defense and/or chemoresistance, such as glutathione transferases and aldo-keto reductases, signaling proteins of the Ras family, and cytoskeletal proteins. These studies will contribute to characterize new roles of the modifications, and in some cases, of the proteins of interest, in the cellular responses to oxidative stress.

Funding: SAF2012-36519, RD12/0013/0008, EU COST CM1001.

UNCOUPLING PROTEINS AND THE CONTROL OF MITOCHONDRIAL ROS PRODUCTION: IMPLICATIONS FOR CARDIAC ISCHEMIA-REPERFUSION INJURY

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[1]Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM) and Departamento de Biología Molecular, Universidad Autónoma de Madrid, Madrid, Spain; Instituto de Investigación Sanitaria Princesa (IP), Madrid, Spain
[2]Bristol Heart Institute, University of Bristol, Bristol BS2 8HW, United Kingdom

Mitochondria are a major source of reactive oxygen species (ROS) in the cell. It is generally thought that the two main sites of mitochondrial ROS production are complexes I and III of the respiratory chain. Uncoupling proteins 2 and 3 (UCP2, UCP3) might be involved in controlling the production of mitochondrial ROS and protecting against oxidative stress, although the mechanism is unclear. UCPs are activated by superoxide and the lipid peroxidation product 4-hydroxy-2-nonenal (4-HNE), to induce proton leak leading to membrane depolarization (mild uncoupling) and the decrease in ROS production. In addition, UCP2 and UCP3 are controlled by covalent modification by glutathione. We recently found that the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2), a master regulator of the cellular antioxidant response, induces UCP3 expression under conditions of oxidative stress. Ischemia-reperfusion (IR) injury in myocardial infarction is caused, in part, by oxidative damage. It has been shown that 4-HNE-induced Nrf2 activation protects against cardiac IR. We aim to determine the potential cardioprotective role of UCP3 against IR damage and its role during ischemic preconditioning (IPC). We studied UCP3 and Nrf2 protein expression in preconditioned and non-preconditioned hearts from UCP3 knockout and wild-type mice subjected to IR using a Langendorff perfusion system. Myocardial infarction was studied by staining with triphenyltetrazolium chloride, and creatine kinase activity was measured in the coronary effluent to determine reperfusion damage. The results obtained so far suggest that the Nrf2/UCP3 signalling pathway can be targeted to protect the heart against the damaging effects of IR.

8-OXO-7,8-DIHYDRO-2'-DEOXYGUANOSINE (8-OXO-DG) IN HUMAN GASTROINTESTINAL TUMORS. BEYOND OXIDATIVE STRESS

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Oxidative stress (OS) plays a critical role in the progression and clinical complications of degenerative diseases. Nucleic acids are preferential targets of oxygen reactive species (ROS). Oxidation induced DNA damage has been implicated in the pathophisiology of cancer. The increase of the mutagenic base 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) observed in human colorectal tumors has been correlated with the accumulation of genetic alterations which underlie their pathogenic progression.

Besides its value as an OS indicator, it has been also suggested that 8-oxo-dG could be used as a potential tumor marker although, so far, this proposal has not been successfully resolved. We have recently showed that OS is enhanced in the tumor and in the circulating mononuclear cells of gastric carcinoma patients (n:60), presenting high levels of lipid peroxidation (MDA) and DNA damage (8-oxo-dG) products compared with a group of healthy volunteers (n:60). The release of 8-oxo-dG by the urine is extraordinary increased in the gastric cancer group as compared with the normal healthy subjects. In the patients' group, tumor resection results in a progressively decrease of urine 8-oxo-dG levels, down to those of the healthy population between 9 and 12 months after surgery.

Statistical ROC curve analysis for 8-oxo-dG in the pre-intervention state gave the following values: AUC: 0.91, Sensitivity: 93%, Specificity: 78%, PPV: 100%, NPV: 96% and a cut-off value of 15.7 (8-oxo-dG nmol/mmol creat.) for the clinical discrimination of gastric tumors.

These results may support the validation of 8-oxo-dG as a useful tumor marker for the diagnosis and monitoring of gastric carcinoma.

LIPIDOMIC AND LONGEVITY

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The role of classical lipids in aging diseases and human longevity has been widely acknowledged. Triglyceride and cholesterol concentrations are clinically assessed to infer the risk of cardiovascular disease while larger lipoprotein particle size and low triglyceride levels have been identified as markers of human longevity.

The rise of lipidomics as a branch of metabolomics has provided an additional layer of accuracy to pinpoint specific lipids and its association with aging diseases and longevity.

The molecular composition and concentration of lipid species determine their cellular localization, metabolism, and consequently, their impact in disease and health. Moreover, the identification of specific lipid species in aging diseases and longevity would aid to clarify how these lipids alter health and influence longevity. Up-to-date, a minor fraction of the human plasma lipidome has been associated to healthy aging and longevity, further research would pinpoint toward specific lipidomic profiles as potential markers of healthy aging and metabolic diseases.

DIETARY MODULATION OF LIPOXIDATIVE STRESS IN A TRANSGENIC MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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The implication of lipid peroxidation in neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) derive from high abundance of peroxidation-prone polyunsaturated fatty acids in central nervous system and its relatively low antioxidant content. In the present work we evaluated the effect of dietary changes aimed to modify fatty acid tissue composition in survival, disease onset, protein and DNA oxidative modifications in the hSODG93A transgenic mice, a model of this motoneuron disease. Both survival and clinical evolution is dependent on dietary fatty acid unsaturation and gender, with high-unsaturated diet leading to loss of the disease sparing effect of feminine gender. This was associated with significant increases of protein carbonyl and glycoxidative modifications as well as non-nuclear 8-oxo-dG, a marker of mitochondrial DNA oxidation. Comparison of these data with γH2AX immunostaining, a marker of DNA damage response, suggests that the highly unsaturated diet blunted mitochondrial-nuclear free-radical dependent crosstalk, since increased 8-oxo-dG was not correlated with increased DNA damage response.

CYTOMIC ANALYSIS OF OXIDATIVE STRESS

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Cytomics is the science of single cell-based analyses linking genomics and proteomics with the dynamics of cell and tissue function, as modulated by external influences. Inherent to cytomics are the use of sensitive, scarcely invasive, multiparametric methods and the event-integrating concept of individual cells to understand the complexity and behaviour of tissues and organisms. Among cytomic technologies, conventional, fluorescence-based FCM and other recent technologies based on non-fluorescent markers and on single-cell bioimaging and bioinformatic tools have become an important tool, because of their both high content and high-throughput. The analysis of oxidative stress has become one of the most frequent applications of cytometry. However, cytometric studies of oxidative stress are complicated by the short half-life of ROS and NOS, the complexity of the oxidative/antioxidant processes, the relative lack of specificity of many reagents and the need to preserve the functional competence of cells during the analysis. This presentation will cover the basic and technological aspects of the cytomic analysis of oxidative stress, as well as proof-of-concept of its relevance in predictive Toxicology.

Sponsoring: All scientific results presented here have been generated in research projects sponsored by European Comission (grants 15348-1999-10FIED ISP ES; LSHB-CT-2004-512051; LSHB-CT-2004-504761), MICINN, Spain (grants BIO2007-6662, DEP2007-73234-CO3-03 and BIO2010-19870) and Generalitat Valenciana (ACOMP2013/102).

THE BENEFICIAL EFFECT OF ANTIOXIDANTS IN HYPERTENSION-RELATED ORGAN DAMAGE

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Oxidative stress is one of the main processes involved in those molecular mechanisms contributing to the pathophysiology of arterial hypertension and its related organ damage. For this reason, antioxidant therapies have been proposed in order to ameliorate the oxidative stress associated with hypertension and hypertension-related organ damage, especially cardiovascular and renal diseases. L-carnitine (LC) is a natural compound whose main physiological function is the transport of long-chain fatty acids inside the mitochondria for ATP production. Exogenous administration of LC is mainly used in those pathologies presenting with a deficit of this substance; however, several studies suggest that a supplementation with LC might be relevant in other disorders, including cardiovascular and kidney diseases. Using different *in vivo* and *in vitro* approaches, we have demonstrated the beneficial antioxidant effect of LC in arterial hypertension and related diseases, and we have studied the underlying signaling pathways involved in LC effect. According to our results, LC inhibits the production of reactive oxygen species (mainly superoxide anions) by reducing the activity of the enzyme NADPH oxidase. In addition, LC treatment also restores the expression of the different subunits and isoforms of this enzyme. Our experiments suggest that LC may be considered as a good candidate for treatment of arterial hypertension and hypertension-related organ diseases.

FREE RADICALS IN LIVER COLD ISCHEMIA-REPERFUSION INJURY

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Ischemia-reperfusion injury (IRI) is inherent to liver transplantation (LT) and is responsible for graft failure usually within the first week after surgery. IRI is initiated during the cold preservation of the liver following the organ recovery from the donor, it continues at re-warming of the graft prior to transplant and proceeds at graft revascularization after LT. In these multifactorial processes, the oxygen free radicals are involved in the subsequent graft viability after LT. The suitable graft conservation in preservation solution and the subsequent washout are determinant conditions for the successful outcome. The presence of PEG35 (high molecular weight polymer, nontoxic, and water soluble) in organ preservation and graft washout solutions reduced hepatic injury and improved liver function after reperfusion. It prevented the oxidative stress, mitochondrial damage and liver autophagy. Also, PEG35 was responsible for the e-NOS activation which was concomitant with the increased expression of cyto-protective heat shock proteins (HSPs) such as HO-1 and HSP70, AMP-activated protein kinase that finally contributed to restore the cytoskeleton integrity following IRI.

USING *CAENORHABDITIS ELEGANS* AS A MODEL FOR THE EVALUATION OF ANTIOXIDANT FOOD INGREDIENTS

Daniel Ramón

Biopolis SL

Functional foods represent an active sector of the agri-food industry. Over the past 10 years its sales have increased significantly with an annual compound growth rate of over 10%. The reasons for this growth are to be found in the growing consumer interest in the health & food paradigm.

From the technical point of view, and following the entry into force of Regulation 1924/2006 on nutrition and health claims made on foods of the European Parliament, in the EU commercialization of functional foods and ingredients must be based on a rigorous evaluation, where scientific knowledge should be the main driver. The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)reviews this assessment. Following the EFSA-NDA decissions, the requirements for any ingredient and/or functional food are: i) to have an unequivocal identification (mainly for probiotics), ii) to have knowledge about the molecular basis of its action, iii) to define biomarkers linked to its effect, and iv) to demonstrate its biological effect in clinical studies with healthy volunteers.

To reduce the time for the evaluation of these foods and lower their costs without losing scientific accuracy is a challenge that requires new approaches. In our company we have decided to do this by combining the use omic technologies (mainly genomics, transcriptomics and metabolomics) with the use of model organisms such as the nematode *Caenorhabditis elegans*. Throughout the conference, several examples of the use of this animal model in the study of functional ingredients that have an effect on oxidative stress, obesity, inflammation, aggregation of amyloid plaque or infection by viruses or bacteria will be present. Finally the strengths and weaknesses of the system will be discussed.

ROLE OF RADICALS IN GLUTAMATE EXCITOTOXICITY IN ALZHEIMER DISEASE

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The anaphase-promoting complex APC/C, an E3 ubiquitin ligase which is activated by the non-phosphorylated form of cdh1, is involved in the regulation of essential mechanisms in neurons. The malfunctioning of APC/C-Cdh1 and the accumulation of its degradation targets has been related with neurodegenerative diseases. We could identify glutaminase (gls) as a relevant degradation target of APC/C-Cdh1 in primary neurons, an enzyme that converts glutamine to glutamate. When cdh1 decreases after the treatment with Aβ, gls accumulates in a similar manner as cyclin B1, a known target of the ubiquitin ligase which has been related with Alzheimer's disease (AD). The same treatment causes a high increase of glutamate levels in the supernatant of neurons in culture, which subsequently leads to an increase of Ca^{2+} inside the cells. The increase of glutamate due to the Aβ treatment can be partially repressed by a glutaminase inhibitor. This result suggests that the APC/C-Cdh1 signaling way is involved in the glutamate increase after the treatment with Aβ. Moreover, high levels of glutamate have been observed to further decrease cdh1 levels what also leads to an accumulation of gls. These results led us to propose that neurons might enter into a positive feedback loop of glutamate production due to a lack of APC/C-Cdh1 signaling. This signaling way reveals a new way to excitotoxicity in neurons, which could be relevant in AD.

INTERPLAY BETWEEN MACROPHAGE POLARIZATION, GLUCOSE METABOLISM AND OXIDATIVE STRESS

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Macrophages have a wide variety of locations and functions that are determined by its origin and the type of activation imposed by the environment. Under an academic point of view macrophage activation can be classified as pro-inflammatory (M1 polarization), anti-inflammatory (M2) or pro-resolution/deactivation (M0), these profiles coexisting in the course of the immune response, and play a relevant functional role in the onset of inflammation. Our group has focused its work on studying the role of macrophages in the pathophysiology of major organs. A line of interest has been the characterization of signaling pathways that determine the polarization and its effect on the release of mediators of inflammation. In addition to this, these mediators affect the function and gene expression in differentiated cells, such as hepatocytes, cardiomyocytes and myofibroblasts. We recently analyzed the metabolic aspects associated with macrophage activation trying to answer the question about what changes in the regulation of energy metabolism and precursors (NADPH, riboses, etc..) accompany the different types of polarization and to what extent these changes are necessary for the activation phenotype. To get an idea of the magnitude of changes involved, for example after M1 activation through TL4 challenge, there is an alteration in the expression of over a thousand of genes. The interest of these studies is to envisage the possibility to regulate macrophage function by altering their metabolic activity as a complementary strategy to regulate their participation in the inflammatory response. We could show that regardless of the stimulus used and the availability of energy substrates, the macrophage is in more than 90% glycolytic, with limited use of other fuels for energy purposes; however, the pathways to generate metabolites from the TCA and glutaminolysis are fully functional and these molecules are used for other purposes. In this context, we have investigated the role of macrophages in the development of atherogenesis, its diagnosis and its contribution to plaque stability and culprit and non-culprit acute coronary events. Indeed, this response has been also evaluated in the context of the infiltration in the heart under pathophysiological situations. Following an ischemic process or in the course of infectious myocarditis the heart elicits an inflammatory response that promotes the infiltration and activation of resident and circulating inflammatory cells, representing up to 4-5% of the heart population. The analysis of the infiltration in prototypical situations such as post-myocardial ischemia and myocarditis (through activation of TLRs or models of autoimmune myocarditis) allows the examination of the nature of the process of macrophage activation in the heart and might help to unravel the role of the recruitment of inflammatory cells to the establishment of the cardiac dysfunction and the contribution of the metabolic profile, including the oxidative stress to this pathological situation.

NEURONS AND CARDIOMYOCYTES AS CELL MODELS TO STUDY FRIEDREICH ATAXIA

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Frataxin is a mitochondrial protein involved in cellular iron homeostasis whose deficiency in humans causes Friedreich ataxia. Levels of frataxin in these patients vary from 5 to 30% of normal levels. To understand the cellular consequences of frataxin deficiency we use primary cultures of cardiac myocytes and dorsal root ganglia neurons as cell models because these tissues are primarily affected in the disease. Using lentiviral particles frataxin levels are reduced -by RNA interference- to those found in the disease. In cardiomyocytes, frataxin depletion triggers mitochondrial rearrangements and disturbs lipid metabolism, suggesting impairment of specific mitochondrial functions. Dorsal root ganglia neurons show, after frataxin depletion, markers of apoptotic cell death, increased levels of intracellular calcium, neurite degeneration and altered mitochondrial membrane potential. These deleterious effects can be reversed by the addition of a cell-penetrant TAT peptide coupled to BH4, the anti-apoptotic domain of Bcl-x_L protein. Other compounds tested in both cardiomyocytes or neurons, also reversed the toxic effects caused by frataxin reduction. As a conclusion, the use of these cell models provide precise clues to understand the events taking place after frataxin depletion and the rationale for new therapies.

REGULATION OF GENE EXPRESSION PROGRAMS BY THE STRESS-DEPENDENT MAP KINASE STY1 OF *SCHIZOSACCHAROMYCES POMBE*

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Microorganisms are invariably exposed to abrupt changes in their environment, and consequently display robust, high plasticity gene programs to respond to stress. In *Schizosaccharomyces pombe,* the Sty1 MAP pathway is activated in response to diverse stress conditions, such as osmotic and oxidative stress, heat shock, or nitrogen deprivation. The MAP kinase Sty1 and its main substrate, the transcription factor Atf1, engage a wide gene expression program aimed to allow cell survival by decreasing and repairing the damage exerted. Searching for mediators of this wide gene expression program, we isolated several chromatin-related mutant strains very sensitive to oxidative stress conditions. Among them, strains lacking SAGA or Elongator components such as the histone acetyl transferases Gcn5 or Elp3 are sensitive to H_2O_2. SAGA contributes to the engagement of the gene expression program by exerting a strong transcriptional control of stress genes, whereas Elongator introduces certain tRNA modifications to ensure efficient translation of the stress-induced, highly expressed mRNAs. Furthermore, Atf1-dependent promoters contain especially broad nucleosome depleted regions (NDRs) prior to stress imposition. We will also describe here how basal binding of Atf1 to these promoters competes with histones to create wider NDRs at stress genes, and how this affects chromatin organization and nucleosome phasing along open reading frames of stress genes.

CONTROL OF ENDOTHELIAL FUNCTION AND ANGIOGENESIS BY PGC1-α

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Rationale: Peroxisome proliferator activated receptor gco-activator 1α(PGC-1α) is a regulator of oxidative metabolism. Endothelial cell migration requires the downregulation of PGC-1α, suggesting that PGC-1α could play a role in angiogeneis.

Objective: This study aims to evaluate the relevance of endothelial PGC-1ain angiogenesis.

Methods and results: Endothelial cells (ECs) from mice deleted for expression of PGC-1α show reduced adhesion to the extracellular matrix, slower spreading, poor formation of cellular junctions, and a disorganized cytoskeleton and random motility, with enhanced tip phenotype and a poor response to Vascular endothelial growth factor-A (VEGF-A). *In vivo*, deletion of PGC-1α results in a reduced retinal vascular density and poor pericyte coverage. PGC-1α deleted mice exposed to hyperoxia during retinal vascular development exhibit exacerbated vascular abnormalities, including extensive hemorrhage, highly unstructured areas and very poor perfusion compared with wild-type mice. Structural analysis showed reduction of endothelial VE-cadherin, suggesting defective inter-cellular junctions. Interenstingly, this phenotype is partially reversed by antioxidant administration, suggesting that elevated production of mitochondrial reactive oxygen species (ROS) in the absence of PGC-1α is functionally important. Finally, *in vitro* studies show that antioxidant treatment improves VEGF-A signaling, suggesting the toxic effect of ROS may be caused by the alteration of the VEGF-A signaling pathway.

Conclusions: Our findings indicate that PGC-1α plays a role in the control of *de novo* angiogenesis, and its absence results in a poor vascular stability.

MicroRNAS AS REGULATORS OF GLUTATHIONE HOMEOSTASIS

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Glutathione (GSH) is the main antioxidant responding against cell damage. Several pathological states course with reduced nucleophilic tone and perturbation of redox homeostasis due to changes in the 2GSH/GSSG ratio. We have investigated the regulation of the rate limiting GSH biosynthetic holoenzyme γ-glutamate cysteine ligase (GCL) by microRNAs (miRNAs). "In silico" analysis of the 3'-UTR regions of both catalytic (GCLc) and regulatory (GCLm) subunits of GCL, allowed to identify miR-433 as a strong candidate for the targeting of GCL. Transitory overexpression of miR-433 in HUVEC showed a downregulation of both GCLc and GCLm in a Nrf2-independent manner. Increases in pro-oxidant stimuli such as exposure to H_2O_2 or GSH depletion in endothelial and hepatic cells caused an expected increase in GCLc and GCLm protein expression and abrogation of miR-433 levels, thus supporting a cross-regulation of these pathways. Treatment of HUVEC with miR-433 resulted in reduced antioxidant and redox potentials, increased S-glutathionylation and reduced eNOS activation. Studies in an in vivo model of hepatic fibrosis showed TGF-β related reduction of GCLc and GCLm levels that were miR-433 dependent. We describe for the first time a miRNA, miR-433, capable of directly targeting GCL and promoting functional consequences in endothelial physiology and fibrotic processes by decreasing GSH levels.

NRF2-MEDIATED NEUROVASCULAR PROTECTION AGAINST OXIDATIVE STRESS IN EXPERIMENTAL STROKE

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Disruption of the blood-brain barrier (BBB) and cerebral edema are the major pathogenic mechanisms leading to neurological dysfunction and death after ischemic stroke. The brain protects itself against infarction via activation of endogenous antioxidant defense mechanisms, and we here report the first evidence that sulforaphane mediated pre-activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and its downstream target heme oxygenase-1 (HO-1) in the cerebral vasculature protects the brain against stroke.[1,2] To induce ischemic stroke, Sprague-Dawley rats were subjected to 70 min middle cerebral artery occlusion (MCAo) followed by 4, 24 or 72 h reperfusion. Nrf2 and HO-1 protein expression was upregulated in cerebral microvessels of peri-infarct regions after 4-24 h, with HO-1 preferentially associated with perivascular astrocytes rather than the cerebrovascular endothelium. In naïve rats, treatment with sulforaphane increased Nrf2 expression in cerebral microvessels after 24h. Upregulation of Nrf2 by sulforaphane treatment prior to transient MCAo (1h) was associated with increased HO-1 expression in peri-vascular astrocytes in peri-infarct regions and cerebral endothelium in the infarct core. BBB disruption, lesion progression, as analyzed by MRI, and neurological deficits were reduced by sulforaphane pretreatment. As sulforaphane pretreatment led to a moderate increase in peroxynitrite generation, we suggest that hormetic preconditioning underlies sulforaphane-mediated protection against stroke. In conclusion, we propose that pharmacological or dietary interventions aimed to precondition the brain via activation of the Nrf2 defense pathway in the cerebral microvasculature provides a novel therapeutic approach to prevent BBB breakdown and neurological dysfunction in stroke.

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DYSFUNCTIONAL NITRIC OXIDE-MEDIATED NEUROVASCULAR COUPLING IN ALZHEIMER´S DISEASE AND AGING

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The coupling between neural activity and cerebral blood flow (CBF) is essential for normal brain function. Using an experimental approach that enabled to appreciate quantitatively and in real-time the dynamics of neuronal-derived nitric oxide ($^{\bullet}$NO), CBF and O_2 upon glutamatergic activation of a volume of hippocampal tissue *in vivo* we have previously shown that neuronal-derived $^{\bullet}$NO directly mediates the neurovascular coupling (NVC) process. Therefore, neurons not only communicate serially across chains of synapses but might also broadcast their signals widely, via diffusible $^{\bullet}$NO, targeting different cell types, including vascular smooth muscle cells, in a particular volume of brain tissue. Brain aging has been associated with hemodynamic and neuronal responses in the dependency of cardiorespiratory system. However, given that the functional coupling between neuronal activity and local blood flow is inherently independent of the cardiac output it is of interest to analyze whether this autoregulation mechanism is affected during aging, thus compromising brain function. Using a triple transgenic mice model of Alzheimer´s disease (AD) and the Fisher 344 rat model of aging it was observed that in both models the neurovascular coupling is impaired but due to cerebrovascular dysfunction, rather than to a dysfunctional signaling from neurons to blood vessels. Moreover, the changes observed in middle-aged AD mice recapitulate those observed in old control mice, pointing to the notion of AD as accelerated aging.

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OXIDATIVE PROTEOME ALTERATIONS TARGET SPECIFIC CELLULAR PATHWAYS DURING OXIDATIVE STRESS AND AGEING

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Senescent cells are causally implicated in generating age-related phenotypes and their removal not only prevent or delay tissue dysfunction, but also extend healthspan. Adult human skeletal muscle stem cells (satellite cells or myoblasts) constitute an interesting cellular model of aging because they divide throughout the life of the individual and experience both chronological and replicative aging. In addition, their replication and differentiation is compromised with age, contributing to the development of sarcopenia. However, the molecular events related to myoblasts dysfunction during ageing are not completely understood. In this study, we provide evidence for the accumulation of oxidatively damaged proteins, as well as proteins modified by glycation and conjugated with lipid peroxidation products during replicative senescence of human myoblasts. These modified proteins are involved in key cellular function such as energy metabolism, cellular assembly and morphology, as well as in lipid metabolism. To provide mechanistic insights into the role of oxidized proteins in the development of the senescent phenotype untargeted metabolomic profiling was performed between young and senescent myoblasts. Major metabolic differences are related to energy and lipid metabolism. Due to the fact that glycolytic enzymes were specifically affected by oxidative damage functional analyses were performed. Glucose oxidation was decreased more than two-fold in senescent myoblasts. Metabolic failure in senescent myoblasts is also supported by a decreased cellular reducing potential. This study establishes a new concept in relation with the impact of oxidative protein modifications on the impairment of cellular metabolism and the development of the senescent phenotype.

OXIDIZED PHOSPHOLIPIDS IN THE VASCULAR SYSTEM AND THEIR CELLULAR EFFECTS

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Oxidized phospholipids (oxPL) 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. This is important in the cardiovascular system, where LDL and other forms of lipid can be oxidized and taken up into the vessel wall, leading to atherosclerosis. Oxidized LDL and its components have been found to have a wide range of biological effects, such as enhancing ROS production, monocyte-endothelial adhesion, and proliferation and differentiation of SMCs, via complex signalling pathways. In order to understand the biological effects of oxPLs, it is necessary to have sensitive and specific methods of measuring them, as LDL contains more than 350 different native lipid species, and oxidation greatly increases the number. Mass spectrometry (MS) is a powerful technique for analysing both native and oxidized PLs, but the large number of different species present in biological and clinical samples presents a substantial challenge. Targetted or semi-targetted MS approaches can facilitate identification of individual oxPLs or groups of oxidation products such as PL hydroperoxides or chlorohydrins, and have been applied to analyse lipid oxidation in diabetic plasma. Some lipid oxidation products are reactive and able to generate adducts with proteins, a process known as lipoxidation. MS is also able to detect these modifications and map them to specific sites on the protein. For example, the formation of oxPL adducts on ApoB-100 has been shown to occur in LDL both from healthy volunteers and patients with chronic kidney disease.

ANIONIC PHOSPHOLIPID OXIDATION IN CELL SIGNALLING

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Phospholipids are components of cell membranes and bio fluids, playing diverse roles in cell signalling. The presence of polyunsaturated fatty acid makes phospholipids prone to oxidation and oxidized phospholipids are known intermediaries of cellular and inflammatory events. Among these oxidized phospholipids, anionic phospholipids, phosphatidylserine (PS) and cardiolipin (CL) and their oxygenated derivatives are key intervenient in programmed cell death and phagocytotic clearance of apoptotic cells. CL is found almost exclusively in mitochondria, playing an important regulatory role in cytochrome c release and apoptosis. Accumulation of CL oxidation products has been associated with loss of mitochondrial function, with the release of pro-apoptotic factors from mitochondria into the cytosol and externalization of PS on the cell surface. PS is preferentially located in the inner leaflet of the membrane bilayer and is particularly abundant in the brain. Externalized non-oxidized and oxidized PS species are the ligand recognized by macrophages receptors for clearance of cells undergoing apoptosis. Oxidation of CL (oxCL) and of PS (oxPS), have been associated with a variety of pathological conditions. In the recent years, mass spectrometry based approaches have been important for the study of the biological role of oxCL and oxPs. Oxidative lipidomics allowed detecting the accumulation of oxidized PS and Cl, which were associated with several inflammatory, cardiovascular and brain diseases, such as depression and neurodegenerative diseases.

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NITRIC OXIDE UNDER A "GREEN" PERSPECTIVE

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In the mid-eighties, it was reported that animal cells had the endogenous capacity to produce the gas nitric oxide (NO) from L-arginine, by a family of enzymes designated as nitric oxide synthases (NOS). Then, it was demonstrated that this free radical molecule participates in a broad spectrum of functions including the cardiovascular, immune, and nervous systems and also in a wide array of human pathologies such as tumours, heart disease, asthma, among others. By comparison, research on NO in plants has advanced more slowly, although at present it is widely admitted that this radical molecule is also endogenously generated in plant cells. NO is involved in many processes including seed germination, primary and lateral root growth, flowering, pollen tube growth regulation, fruit ripening, senescence, defence response and abiotic stresses. Nitric oxide is also a key signaling molecule in different intracellular processes affecting the function of many proteins throughout post-translational modifications (PTMs) such as nitration or S-nitrosylation which are mediated by NO-derived molecules including peroxynitrite or nitrosothiols. Thus, a summary of the main advances obtained in plants will be presented as well as it will be discussed the controversial identification(s) of the enzymatic source(s) of endogenous NO in plant cells and its subcellular localization.

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PLANT MITOCHONDRIAL TRXO1: LOOKING FOR ITS TRANSCRIPTIONAL GENE REGULATION AND FUNCTIONS

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Mitochondrial Trxo is a thioredoxin involved in the reduction of disulfide bonds of mitochondrial target proteins regulating their activities. Its functional role has been scarcely studied during stress conditions rather its gene regulation. In our laboratory, the study of the AtTrxo1 promoter has revealed several motifs as possible regulators of AtTrxo1 expression. Using an arrayed yeast library of *Arabidopsis* transcription factors, AZF2 was identified as possible regulator of the promoter. Besides, AtTrxo1 expression pattern was analysed in the different parts of *Arabidopsis* plants and during seed germination, finding a rise during the silique development and seed germination. In order to study the possible regulation of AtTrxo1 by AZF2, both expression patterns were compared in both processes.

In order to gain insight into the functional role of Trxo1 under oxidative stress provoked by salinity and H_2O_2, we have used KO AtTrxo1 and over-expressed PsTrxo1 TBY-2 cells. The lack of AtTrxo1 in Arabidopsis plants produced no relevant phenotypic changes in control or even under salinity conditions. However, under this stress situation, the germination occurred earlier in time. On the other hand, the treatment with H_2O_2 of over-expressing PsTrxo1 TBY-2 cells did not provoke a change in growth dynamic but increased viability. In this situation, oxidative stress parameters were measured as well as several components of the antioxidant system involved in the metabolism of H_2O_2. Different hallmarks of programmed cell death were also analysed trying to elucidate the possible involvement of Trxo1 in the response of the cells to H_2O_2 used as inductor of cell death.

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POST-TRANSLATIONAL MODIFICATIONS MEDIATED BY NITRIC OXIDE (NO) UNDER PHYSIOLOGICAL AND STRESS CONDITIONS IN PLANTS

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Nitric oxide (NO) is a key signalling molecule that has been associated with different physiological or abiotic and biotic stress conditions in higher plants. In this sense, there are growing evidences that NO and its related species (RNS) can alter the gene-expression profile and mediate post-translational modifications of proteins (PTM) such as S-nitrosylation or tyrosine nitration. Using pea (*Pisum* sativu*m* L.) plants under physiological and abiotic stress conditions, key components of RNS metabolism (NO, S-nitrosothiols and peroxynitrite) and NO-related PTM mechanisms were analyzed. We identified proteins that undergo tyrosine nitration under physiological conditions such as NADP-dependent isocitrate dehydrogenase (NADP-ICDH) or NADH-dependent hydroxypyruvate reductase (NADH-HPR), reaching to identify the NO-target residues and their functional implications. Furthermore, we analysed the effect of NO-related PTMs on the activity of recombinant pea cytosolic ascorbate peroxidase (APX), a key antioxidant of the ascorbate-glutathione cycle. We demonstrated that APX is modulated by both irreversible tyrosine nitration and reversible S-nitrosylation which lead to a decrease or an increase in APX activity, respectively. In addition, we showed that S-nitrosylation of APX contributes in the mechanism of response against the nitro-oxidative stress provoked by salinity in pea plants.

Taking together, we verified that NO-related PTMs occurs under physiological and stress conditions in which the NO metabolism may contribute to the regulation of key processes in plants.

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INTERACTIONS OF ROS AND RNS WITH PLANT HEMOGLOBINS

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Hemoglobins are present in virtually all plant tissues. However, some hemoglobins, called leghemoglobins (Lbs), only occur in the cytoplasm of host cells of legume nodules. These are unique plant organs formed by the interaction between legume root cells and soil bacteria. Nodules reduce atmospheric N_2 into ammonium, and this N_2 fixation process has considerable benefits for agriculture and the environment. The heme of Lbs must be in ferrous form to transport O_2 inside the nodules. However, ferric and ferryl Lbs are nonfunctional forms that may be generated by ROS-mediated oxidation. Ferric Lb may be generated by autoxidation of oxygenated Lb, and ferryl Lb by oxidation with H_2O_2. The oxidative attack of H_2O_2 on Lbs gives rise to protein radicals, which are quenched *via* formation of intramolecular heme-protein cross-links and intermolecular cross-links producing Lb dimers. Lbs react also with RNS. Ferrous Lb binds NO avidly to form the nitrosyl complex, which has been detected *in vivo*, and indeed modulation of NO levels may be an additional function of Lbs. Recently, we reported the presence in nodules of Lb green derivatives having a NO_2 group on the heme (nitri-Lbs), and now we have found, also *in vivo*, Lbs with nitrated Tyr residues. Specifically, Tyr-30 is the major target of nitration. *In vitro* assays revealed that Tyr nitration requires nitrite and H_2O_2 but not metal ions. Nitrated Lb is formed *via* ferryl Lb, which generates nitrogen dioxide and tyrosyl radicals. This mechanism is distinct from that involved in nitri-Lbs. Formation of NO_2-Tyr in Lbs is a consequence of active metabolism in functional nodules, whereas nitri-Lbs are primarily produced during senescence.

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PROGRAMMED CELL DEATH ACTIVATED BY ROSE BENGAL IN *ARABIDOPSIS THALIANA* CELL SUSPENSION CULTURES REQUIRES FUNCTIONAL CHLOROPLASTS

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Light-grown *Arabidopsis thaliana* cell suspension cultures (ACSC) were subjected to mild photooxidative damage with Rose Bengal (RB) with the aim of gaining a better understanding of singlet oxygen-mediated defence responses in plants. Additionally, ACSC were treated with H_2O_2 at concentrations that induced comparable levels of protein oxidation damage. At low-medium light conditions both RB and H_2O_2 treatments activated transcriptional defence responses and inhibited photosynthetic activity, but they differed in that programmed cell death (PCD) was only observed in cells treated with RB. When dark-grown ACSC were subjected to RB in the light, PCD was suppressed, indicating that the singlet oxygen-mediated signalling pathway in ACSC requires functional chloroplasts. Analysis of up-regulated transcripts in light-grown ACSC, treated with RB in the light, showed that both singlet oxygen-responsive transcripts and transcripts with a key role in hormone-activated PCD (i.e. ethylene and jasmonic acid) were present. A co-regulation analysis proved that ACSC treated with RB exhibited higher correlation with the conditional fluorescence (*flu*) mutant than with other singlet oxygen producing mutants or wild-type plants subjected to high light. However, there was no evidence for the up-regulation of *EDS1*, suggesting that activation of PCD was not associated with the EXECUTER- and EDS1-dependent signalling pathway described in the *flu* mutant. Indigo Carmine and Methylene Violet, two photosensitizers unable to enter chloroplasts, did not activate transcriptional defence responses in ACSC; however, whether this was due to their location or to their inherently low singlet oxygen quantum efficiencies was not determined.

LICHEN SYMBIONTS' STRATEGIES TO OVERCOME HARSH ABIOTIC STRESS: DIVERSITY AND COOPERATION

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Lichens are symbiotic associations of very dissimilar organisms as a fungus (mycobiont) and a cyanobacteria or a microalga or both. This intrinsic diversity results in a huge taxonomic and ecophysiological diversity. In the whole holobiont - thallus-, the cooperation between the symbionts generates a synergy that allows the colonization of harsh and extreme habitats. Adapted to anhydrobiosis, their existence is dominated by desiccation – rehydration cycles, since their water content depends on environmental humidity (poikilohydry). Rehydration is accompanied by a ROS burst, contributed greatly by photobionts' photo-machinery. Our group demonstrated that fungus-derived NO is related with the photooxidative protection of microalgae photosystems, reinforcing the hypothesis of a mutual upregulation of antioxidant systems. The role of each symbiont in this cooperative defense remains to be elucidated. We recently reported the presence of several phycobionts (microalgae) with different ecophysiological performances within the same thalli of the model lichen *Ramalina farinacea.* This phenomenon reveals new perspectives on the adaptive mechanisms that provide such biological success. This combination of morphological simplicity and functional complexity turns lichens into a extremely interesting model, not only as bioindicators, biosensors or source of new biotechnological applications, but also to deepen into cell communication mechanisms supporting relevant relations for global ecological balances as plant – mycorrhizae or plant – *Rhyzobium* symbioses. Indeed, anhydrobiosis mechanisms seem intimately related with general mechanisms of tolerance to abiotic stress (hypersaline media, heavy metals). All these processes involve an excellent modulation of oxidative stress, in which, according to our results, NO plays a relevant role.

ROLE OF NADPH OXIDASES IN THE NETWORK REGULATING CELL RESPONSE TO SHORT AND LONG TERM CADMIUM EXPOSURE

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Cadmium is toxic for plants, animals and humans. Different approaches have demonstrated that oxidative stress is one of the final effects of long-term Cd exposure although reactive oxygen species (ROS) produced under short-term Cd treatment could play a central role in the signalling networks that regulate plant response to this metal. In this work, the role of NADPH oxidases as a Cd-dependent source of ROS has been studied by using *Arabidopsis rboh C*, *D* & *F* mutants.

Cadmium (100µM) significantly decreased the germination rate of seeds and leaf biomass production after long-term (5d) Cd treatment in WT plants, although this reduction was not observed in *rbohF* probably due to a less Cd accumulation in *rbohF* leaves. Under these conditions lipid peroxidation and H_2O_2 accumulation increased and no differences were observed between WT and the mutants. Under short-term conditions we have analysed the time lapse of peroxisomal dynamics and it shows changes in the morphology (peroxule formation), number and speed of peroxisomes during Cd treatment and we have found that NADPH oxidases have a role in regulating peroxisomal dynamics and proliferation in response to the metal.

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HEXANOIC ACID PROTECTS PLANTS AGAINST PATHOGENS BY PRIMING DEFENCE RESPONSES AND REDUCING OXIDATIVE STRESS

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Treatment with the resistance priming inducer hexanoic acid (Hx) protects tomato plants from *Botrytis cinerea* by activating defence responses. The microarray analysis at 24 h post-inoculation provided the expression profile of tomato (*Solanum lycopersicum*) plants upon inoculation of the necrotrophic fungi.

This showed the up-regulation of genes encoding proteinase inhibitors, DNA-binding factors belonging to several families of transcription factors, enzymes involved in plant hormone signaling and synthesis, and, remarkably, the genes involved in oxidative stress. The natural inducer Hx mostly primed Botrytis-induced genes. Interestingly, Hx also promoted genes not early induced by the fungus that could shed light on the priming mechanism. Given the relevance of the oxidative burst occurring in plant–pathogen interactions, the effect of Hx on this process was studied in depth. We showed by specific staining that reactive oxygen species (ROS) accumulation in Hx-treated and infected plants was reduced and more restricted around infection sites. In addition, these plants showed higher ratios of reduced to oxidized glutathione and ascorbate, and normal levels of antioxidant activities. The results obtained indicate that Hx protects tomato plants from *B. cinerea* by regulating and priming *Botrytis*-specific and non-specific genes, preventing the harmful effects of oxidative stress produced by infection. An untargeted global metabolomic analysis was aimed at characterizing and understanding the global metabolic effect of *Botrytis* infection in tomato plants as well as of the priming agent Hx. The variations in metabolites observed also evidenced interesting changes in signaling molecules and antioxidant compounds in response to *Botrytis* and to Hx.

HYDROGEN PEROXIDE SIGNAL TRANSDUCTION. GATHERING THE PIECES

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Different biotic and abiotic stresses adversely affect plant growth and development leading to worldwide yield losses. A common theme within these environmental factors is the perturbation of reactive oxygen species (ROS) homeostasis. Despite the great economic importance, the signal transduction mechanisms of the oxidative stress response in plants are poorly understood. We conducted several genetic and chemical screens, together with proteomic approaches in order to obtain a more comprehensive overview on the different components of the network that govern the oxidative stress response. These efforts identified several genes as new members of the oxidative stress gene network in plants, together with small molecules that are able to interfere with specific events during the oxidative stress response in plants. The potential of these genes and molecules for providing abiotic stress tolerance in commercially relevant plants will be assessed in the future.

PEROXISOMES AS A CELLULAR SOURCE OF *ROS* AND *RNS* SIGNALING MOLECULES

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Peroxisomes are subcellular organelles bounded by a single membrane and devoid of DNA, with an essentially oxidative type of metabolism and are probably the major sites of intracellular H_2O_2 production. In recent years it has become increasingly clear that peroxisomes are involved in a range of important cellular functions in most eukaryotic cells [1].

Peroxisomes produce superoxide radicals ($O_2^{\cdot -}$) and also contain a complex battery of antioxidative enzymes. The existence of L-arginine-dependent nitric oxide synthase activity (NOS) and the generation of the reactive nitrogen species (RNS) nitric oxide (NO) have been demonstrated in plant peroxisomes. Besides NO, the presence in these cell organelles of the RNS *S*-nitrosoglutathione (GSNO) and the generation of peroxynitrite ($ONOO^-$) have also been reported. As a result of the presence of NO and GSNO, and the production of the powerful oxidant and nitrating chemical $ONOO^-$, important post-translational modifications can take place in peroxisomes, such as *S*-nitrosylation and nitration of proteins which could have an impact on cellular metabolism.

It is known that plant peroxisomes have a RNS- and ROS-mediated metabolic function in leaf senescence and certain types of abiotic stress but these organelles can also function as a source of the signaling molecules NO, GSNO, $O_2^{\cdot -}$ and H_2O_2. The diverse physiological functions that have been demonstrated for peroxisomes from different origins evidence the importance of these organelles as a cellular source of ROS and RNS signaling molecules.

[1] del Río L.A. (ed.) (2013) *Peroxisomes and their Key Role in Cellular Signaling and Metabolism*. Subcellular Biochemistry 69. Springer

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ORAL COMMUNICATIONS

O1

THE ANTI-HIV DRUGS ABACAVIR AND DIDANOSINE INDUCE OXIDATIVE STRESS AND ENHANCE ACETAMINOPHEN-INDUCED HEPATOTOXICITY

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Background. Liver disease is a leading cause of mortality among HIV-infected patients and has been related in some cases to antiretroviral therapy. Nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) are important components of this treatment, but little is known about their acute side effects. We analysed the mitochondrial effects of clinically relevant concentrations of NRTI and their impact on the viability of hepatic cells. Additionally, we explored potential synergisms with other hepatotoxic drugs commonly prescribed to HIV-infected patients.

Methods. Parameters of mitochondrial function and cellular viability were assessed in cells treated (1-48h) with NRTI. Sub-damaging concentrations of hepatotoxic stimuli (acetaminophen, the antiretroviral drugs ritonavir and nevirapine, and ethanol) were used in key experiments.

Results. Abacavir and didanosine produced an immediate and significant decrease in mitochondrial function, evidenced by an inhibition of O_2 consumption, increased ROS production, and a reduction in $\Delta\psi m$ and intracellular ATP levels, but they did not compromise cell survival after 24h-treatment. However, co-administration of these drugs with acetaminophen concentrations below those considered toxic in hepatic cellular models exacerbated the deleterious effects of both treatments on mitochondrial function and cellular viability, thus decreasing GSH concentrations. Interestingly, a significant positive correlation was detected between GSH levels and cell viability.

Conclusions. The combination of abacavir or didanosine with low concentrations of acetaminophen significantly affects GSH concentrations in a way that increases the risk of acetaminophen-mediated liver injury. Our findings are relevant given that acetaminophen is prescribed to patients taking NRTI and that HIV infection itself has been reported to undermine GSH levels.

O2

MULTIPLE TARGETS INVOLVED IN ALZHEIMER'S DISEASE ARE AFFECTED BY MORIN AND ISOQUERCITRIN

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Pharmacological studies on flavonoids as phytochemical constituents from herbal medicinal products should be performed to identify their therapeutically relevant activities on Alzheimer's disease (AD) targets. With this view, two flavonols, morin and isoquercitrin, were analyzed and compared in their modes of action, as single-compound drugs, in different AD targets by biochemical cell-free systems and cell-based assays (APPswe cell cultures) in order to identify and characterize their potential multi-target neuroprotective properties which could be further translated to the AD therapy.

Our results show that morin and isoquercitrin possess significant free radical scavenger ability, inhibitory capacity of beta and gamma-secretase activities, and capacity to inhibit amyloid aggregation. Moreover, isoquercitrin has a relevant disaggregating effect comparing with morin. Both flavonoids are also able to prevent the proteasome activity modification caused by hydrogen peroxide, as well as partially avoid the activation of caspases 3 and 9 in the APPswe cell line. Consequently, morin and isoquercitrin potentially could be key molecules for the development of therapeutics for AD.

O3

OXIDATIVE AND ENDOPLASMIC RETICULUM STRESS ARE INFLUENCED BY GLYCAEMIC CONTROL IN LEUKOCYTES OF TYPE 2 DIABETIC PATIENTS

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Objective. Oxidative and endoplasmic reticulum (ER) stress are related to type 2 diabetes (T2D), but the influence of glycaemic control on these parameters and its relationship with leukocyte-endothelial interactions is not known.

Research Design and Methods. Oxidative stress, ER stress and interactions with the endothelium were assessed in leukocytes from 164 T2D patients (102 with HbA1c <7% and 62 with HbA1c >7%) and 84 non-diabetic controls. Anthropometric and metabolic parameters were also evaluated. Correlations among these parameters and the influence of glycaemic control on said parameters were studied.

Results. Weight, BMI, waist circumference, blood pressure, glucose, insulin and HOMA-IR were higher in diabetic patients than in controls, while total cholesterol, HDL and LDL levels were reduced and triglycerides were increased in the former group. hsCRP, TNFα and E-selectin levels were enhanced in the HbA1c >7% group with respect to controls. O_2 consumption and mitochondrial membrane potential were lower in diabetic patients than in controls. Mitochondrial and total ROS production were enhanced in diabetic patients than in controls and positively correlated with HbA1c levels. GRP78 levels were higher in both diabetic groups. However, whereas HbA1c <7% patients displayed higher levels of sXBP1, HbA1c >7% patients exhibited preferentially enhanced levels of CHOP and ATF6. Reduced leukocyte rolling velocity and increased rolling flux and adhesion were observed in diabetic patients.

Conclusions. Loss of glycaemic control in T2D enhances oxidative stress, thus promoting chronic ER stress in leukocytes and endothelial dysfunction, which in turn poses a risk of vascular complications for these patients.

O4

ACUTE HYPOXIA PRODUCES A SUPEROXIDE BURST IN CELLS

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Oxygen is a key molecule for cell metabolism. Eukaryotic cells sense the reduction in oxygen availability (hypoxia) and trigger a series of cellular and systemic responses in order to adapt to hypoxia, including the optimization of oxygen consumption. Many of these responses are mediated by a genetic programme induced by the hypoxia-inducible transcription factors (HIFs), regulated by a family of prolyl hydroxylases (PHD or EGLN) that use oxygen as a substrate producing HIF hydroxylation. In parallel to these oxygen sensors modulating gene expression within hours, acute modulation of protein function in response to hypoxia is known to occur within minutes. Free radicals acting as second messengers and oxidative post-translational modifications have been implied in both groups of responses. Localization and speciation of the paradoxical increase in reactive oxygen species (ROS) production in hypoxia remains debatable.

We have developed a series of techniques for measuring superoxide in short times of hypoxia in cells. We have observed that several cell types respond to acute hypoxia with a transient increase in superoxide production for about 10 minutes, probably originated in the mitochondria. This may explain in part the apparently divergent results found by different groups that have not taken into account the time frame of hypoxic ROS production.

We propose that cells subjected to hypoxia produce an initial burst of superoxide anion which may be translated in later times into oxidative signals contributing to hypoxic adaptation and preconditioning.

O5

A METABOLIC CYCLE WITHIN THE CELL DIVISION CYCLE. THE ROLE OF THE FOX TRANSCRIPTION FACTOR HCM1

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It has been known for decades that diploid yeast strains exhibit robust metabolic cycles during continuous growth under nutrient-limiting conditions. These metabolic cycles are characterized by a respiratory phase (oxidative), alternating with a fermentative phase (reductive), with oscillations in oxygen consumption. Previous results from our group suggest that this metabolic cycle may occur in any dividing cell whatever the culture condition or genetic load. Using genomic, proteomic and metabolomic approaches we hope to demonstrate the existence of this metabolic cycle along the cell division cycle and the regulatory role of the Hcm1, a forkhead transcription factor with homology to mammalian FoxOs.

Hcm1 is expressed periodically during cell cycle (peaks at late G1-early S) and regulates chromosome segregation genes and budding. We demonstrated that this factor positively regulates mitochondrial mass, activity, and mtDNA copy number. In addition, Hcm1 favors oxidative metabolism over glucose fermentation. This metabolic shift is accompanied by an increase in cellular stress resistance. Under oxidative or nutrient stress treatments Hcm1 shifts to the nucleus and its transcriptional activity is activated. We have also demonstrated that Sir2, and the AMPK (Snf1) and TOR/Sch9 pathways regulate Hcm1 under oxidative and nutrient stress, respectively.

Taken together, our results indicate that Hcm1 is not only a cell cycle regulator, as it is also involved in the oxidative stress response and metabolism. Thus, Hcm1 may be one of the links between the yeast metabolic cycle and the cell cycle.

O6

ADR1 AND CTH2 MEDIATE METABOLIC REMODELING IN FRATAXIN DEFICIENT YEAST

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Frataxin is a small mitochondrial protein whose deficiency in humans leads to the neurodegenerative disease Friedreich Ataxia. Frataxin is a highly conserved protein; however its precise function is not known but is thought to be related to iron-sulfur cluster biogenesis. Oxidative stress and activation of low iron response are hallmarks of Frataxin deficient cells.

In this work we used yeast conditional mutants to identify the effects that take place after Frataxin depletion. We identified two metabolic networks affected by the lack of this protein that finally lead to respiratory failure. The first network, affected the glucose-repressed genes and is governed by the transcription factor ADR1. This transcription factor displayed a nucleus-to-cytosol re-localization upon frataxin repression without affecting its protein levels. The same response was observed when we treated cells with the oxidizing agent H_2O_2, pointing to a regulation of this transcription factor by oxidative stress. The second metabolic network affected the iron-sulfur proteins. We identified a huge induction of the mRNA binding protein CTH2, a gene member of the iron regulon, which was responsible for the down-regulation of these proteins. Iron-sulfur protein levels and activities, like Aconitase and Succinate Dehydrogenase, were preserved in a double mutant for Frataxin and CTH2. To summarize, we can conclude that lack of Frataxin involves drastic metabolic remodelling governed by ADR1 and CTH2 that finally leads to respiratory failure and growth arrest.

O7

miR-433 TARGETS γ-GLUTAMYL-CYSTEINE-LIGASE AND REGULATES ENDOTHELIAL FUNCTION, REDOX HORMESIS AND FIBROGENESIS

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Glutathione (GSH) is a key regulator in redox signaling and the main antioxidant in mammalian cells. It controls key cellular processes such as proliferation, apoptosis, immune response and fibrogenesis. A dysregulation of GSH synthesis has been found in several pathological conditions including diabetes, fibrosis, renal damage and cholestatic liver injury. GSH is synthesized in a two-step reaction where the rate limiting enzyme is governed by the heterodimeric enzyme γ-glutamate cysteine ligase (GCL). One level of post-transcriptional regulation where there exists very limited information is related to microRNAs and this may be particularly important in vascular pathologies associated with endothelial dysfunction. "In silico study" of 3'-UTR regions of both subunits allowed to identify miR-433 as a strong candidate for the targeting of GCL. To confirm the relevance of these findings we transiently overexpressed miR-433 in HUVEC for 48 hours. The analysis of GCLc and GCLm showed a significant 50% decrement in their expression in a Nrf2-independent manner. Functionally, these observations correlated with decreased nucleophilic tone and increased GSSG/GSH ratio. Increases in pro-oxidant stimuli such as exposure to H_2O_2 or GSH depletion in endothelial and hepatic cells caused an expected increase in GCLc and GCLm protein expression and abrogation of miR-433 levels, thus supporting cross-regulation in these pathways. Transfection of HUVEC with miR-433 resulted in reduced antioxidant and redox potentials, increased S-glutathionylation and reduced eNOS activation. *In vivo* models of renal and hepatic fibrosis were associated with TGF-β related reduction of GCLc and GCLm levels that were miR-433-dependent. These data sustain that miR-433 is capable of directly targeting GCL and promoting functional consequences in endothelial physiology, redox hormesis and fibrotic processes by decreasing GSH levels.

O8

NADPH OXIDASE-MEDIATED OXIDATIVE STRESS PROMOTES TELOMERE SHORTENING IN PHAGOCYTIC CELLS: INVOLVEMENT IN HUMAN ATHEROSCLEROSIS

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Oxidative stress may induce telomere shortening. The aim of this study was to analyze the relationship of the NADPH oxidase-mediated oxidative stress with telomere shortening in phagocytic cells and to study its impact in human atherosclerosis.

The study was performed in 505 healthy subjects without apparent atherosclerotic disease, and in 30 patients with coronary artery disease. Telomere length was determined by PCR in genomic DNA isolated from blood samples. Superoxide production was evaluated by luminescence in peripheral blood mononuclear cells. Serum concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured by ELISA. Intima-media thickness (IMT) and the presence of atheroma plaques were determined by ultrasonography in carotid arteries. In order to elucidate the *in vivo* findings, we performed *in vitro* experiments in the macrophagic cell line RAW264.7.

In the asymptomatic population, telomere length associated inversely with phagocytic NADPH oxidase activity, with serum 8-OHdG levels, and with carotid IMT. In this population, individuals presenting atheroma plaques exhibited higher IMT and NADPH oxidase activity, and lower telomere length than those subjects without plaques.

In patients with coronary artery disease, NADPH oxidase activity and serum 8-OHdG levels were maximum and associated with maximum telomere shortening.

In vitro studies showed that enhanced NADPH oxidase activity promoted oxidation of genomic DNA, and induced telomere shortening in cultured macrophages; these effects were prevented when NADPH oxidase was silenced in knocked-down cells by lentiviral vectors.

Our findings suggest that an exaggerated NADPH oxidase-mediated superoxide production promotes telomere shortening in phagocytic cells, and that this process should participate relevantly in atherosclerosis.

O9

TGF-β-DEPENDENT NADPH OXIDASE 4 (NOX4) OVEREXPRESSION IN MARFAN SYNDROME AGGRAVATES THE FORMATION OF AORTIC ANEURYSM

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Marfan syndrome (MFS) is characterized by the formation of ascending aortic aneurysms resulting from altered assembly of extracellular matrix microfibrills and chronic activation of TGF-β signaling. TGF-β is a potent regulator of ROS production and expression of some cardiovascular relevant NADPH oxidases (NOXes), particularly NOX4. We hypothesized that as a result of the chronic TGF-β signaling, VSMCs from human Marfan aortic tunica media overproduce ROS mediated by some NOX family members, which altogether could facilitate the formation of aneurysms. Immunohistochemical analysis of aortas from Marfan patients showed increased levels of NOX4 and protein nitrotyrosination. Vascular smooth mucle cells (VSMCs) explanted from Marfan aortic aneurysms confirmed NOX4 overexpression at transcriptomic level and showed increased ROS and nitrotyrosine levels. All these changes were abolished by the TGF-β receptor I inhibitor LY364947. To examine the functional significance of NOX4 overexpression in MFS, we have generated the murine Marfan model (FBN1+/C1039G) NOX4 KO and evaluated the formation of aortic aneurysm. Marfan-NOX4 KO animals showed a significantly lesser amount of elastic fibers fragmentation in comparison with Marfan littermates. In addition, aortic wall architecture revealed by the organization of elastic fibers was also much less impaired in Marfan-NOX4 KO animals. Ecocardiographic analysis also revealed that unlike Marfan mice, Marfan-NOX4 KO animals presented aortic root diameter values undistinguishable from wild-type and NOX4 KO littermates. We conclude that in MFS, VSMCs undergo a TGF-β dependent upregulation of NOX4 and an increased intracellular oxidant status, which aggravates the formation of aortic aneurysm. Results suggest NOX4 as a potential new pharmacological target to fight against aortic aneurysms.

O10

NITRIC OXIDE SYNTHASE TYPE III OVEREXPRESSION BY GENE THERAPY EXERTS ANTITUMORAL ACTIVITY IN HEPATOCELLULAR CARCINOMA

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Background: The overexpression of nitric oxide synthase type III (NOS-3) in HepG2 cells or intratumoral NO donor administration reduce tumor cell growth and increased the expression of p53 and cell death receptors in tumors developed in a xenograft mouse model.
Objectives: The aim of the study was the development of a gene therapy strategy to overexpress NOS-3 in tumor cells.

Methods: The first generation adenovirus included NOS-3 and luciferase cDNA under regulation of murine alpha-fetoprotein and RSV promoter, respectively (ViraQuest, Inc, Iowa, USA). Liver fibrosis was induced by CCl_4 (2 ml/kg bw, ip) administered three times a week for 12 weeks in nude mice. Hepa 1-6 (5000 cells) were implanted in the right hepatic lobule. AFP-NOS-3/Luciferase and AFP-GFP/Luciferase adenovirus were administered through tail vein (200 µl, $2x10^{11}$ pfu/ml) two weeks after cell implantation. PET/TAC analysis and bioluminescence signaling were recorded. NOS-3, DNA damage, p53, cell death receptor, caspase-8 and posttranslational protein modifications were assessed in tumors.

Results: AFP-NOS-3/Luciferase increased NOS-3 expression, oxidative/nitrosative-related posttranslational modifications, TUNEL staining, CD95 and caspase-8 activity, as well as reduced PCNA expression and tumor size. AFP-NOS-3/Luciferase (MOI 50-200) increased oxidative DNA damage, p53, CD95/CD95L expression and caspase-8 activity in cultured Hepa 1-6 cells. The increased expression of CD95/CD95L was abolished by L-NAME or p53 siRNA.

Conclusions: 1) The overexpression of NOS-3 by gene therapy reduced tumor size developed in fibrotic livers. 2) The increase of oxidative/nitrosative stress induced by NOS-3 overexpression was associated with DNA damage, p53, CD95/CD95L expression and cell death in cultured Hepa 1-6 cells.

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O11

CHARACTERIZATION AND RNS-MODULATION OF THE ASCORBATE-SYNTHESIZING ENZYME GALACTONO-γ-LACTONE DEHYDROGENASE FROM PEPPER FRUITS

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Nowadays pepper (*Capsicum annuum L.*) is the second most consumed vegetable worldwide, used as raw, canned food, condiment, food colorant and others. Pepper fruits are one of the main plant sources of vitamin C (ascorbate), what make them greatly appropriate for human diet. In fact, 100 g fruits provide about 2.5-fold the RDA of vitamin C.

The synthesis of ascorbate in plants occurs through several pathways, with the most important one involving the oxidation of galactono-γ-lactone (GL) to ascorbate by the galactono-γ-lactone dehydrogenase (GalLDH) in a coenzyme-independent manner. GalLDH has been reported to be located close to the oxidase complex of the respiratory chain at the inner mitochondrial membrane, where it may transfer the electron from the GL directly to the cytochrome c.

Due to its importance in nutrition and plant metabolism, we have accomplished the molecular and immunological characterization of GalLDH and its involvement in the pepper physiology. On the other hand, in recent works performed in our laboratory, we found that nitric oxide plays a relevant role in the ripening of fruits. Accordingly, the investigation of the modulation by reactive nitrogen species (RNS) of the GalLDH has been addressed. The full cDNA sequence of pepper GalLDH was determined. The analysis of the predicted sequence assigns, at least, one transmembrane helix to the mature polypeptide. GalLDH seems to be post-transcriptionally regulated during ripening of pepper fruits, and this possibly takes place through nitration/nytrosylation processes.

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O12

PRESENCE OF ENDOGENOUS NITRO-FATTY ACIDS IN OLIVE OIL AS MEDIATORS OF CELL SIGNALING

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Extra virgin olive oil (EVOO) and olives provide health benefits to humans. Nitric oxide (NO) and nitrite (NO_2)-dependent reactions of unsaturated fatty acids yield electrophilic nitroalkene derivatives (NO_2-FA) that manifest salutary pleiotropic cell signaling responses in mammals [1-3]. Herein, the endogenous presence of NO_2-FA in both EVOO and fresh olives was demonstrated by mass spectrometry. The electrophilic nature of these species was affirmed by the detection of significant levels of protein cysteine adducts of nitro-oleic acid (NO_2-OA-cysteine) in fresh olives, especially in the peel. Further nitration of EVOO by NO2 under acidic gastric digestive conditions revealed that human consumption of olive lipids will produce additional nitro-conjugated linoleic acid (NO_2-cLA) and nitro-oleic acid (NO_2-OA). Since NO_2-FAs instigate adaptive anti-inflammatory gene expression and metabolic responses, these redox-derived metabolites may contribute to the cardiovascular benefits associated with the Mediterranean diet [4].

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O13

ANTIOXIDANT SYSTEM AND CARBONYLATION OF PROTEINS IN MITOCHONDRIA FROM PEPPER FRUITS AT TWO MATURATION STAGES

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Maturation of fruits is a complex phenomenon unique to plant, in which different physical and biochemical changes take place. During this process the production of reactive oxygen species (ROS) plays an important role, particularly in the biosynthesis of carotenoids and in the transformations of chloroplasts to chromoplasts. Pepper fruits, in both green and red stages, were selected to study at mitochondrial level, changes in the enzymes related with the ascorbate–glutathione cycle. The activity of the reductive enzymes monodehydroascorbate reductase, glutathione reductase, and dehydroascorbate reductase, was higher in mitochondria isolated from green than from red fruits. Ascorbate peroxidase and Mn-superoxide dismutase were higher in the red fruits which might play a role in avoiding the higher accumulation of any ROS generated in these organelles. Pattern of proteins modified by oxidation indicated an increased accumulation of carbonylated proteins in mitochondria from red fruit providing a spatial component to the localization of oxidative stress at sub-cellular level during maturation. Analysis of the peptides by MALDI-TOF-TOF revealed that some mitochondrial proteins were targets of oxidation in both green and red fruits, such as formate dehydrogenase, NAD-dependent isocitrate dehydrogenase, porin, nucleoside disphosphate kinase and defensin J1-2 pointing out a common regulation by oxidation of these proteins independently of the fruit maturation stage. Sucrose synthase, mannnitol dehydrogenase, ADP-glucose pyrophosphorylase, glycine dehydrogenase, muturase K, mitochondrial phosphate transporter were identified as targets of oxidation specifically in green fruit, whereas in mature red fruits, aconitase, cysteine synthase, ATPase beta subunit, basic beta-1,3-glucanase, orfB protein and cytochrome c oxidase, were found carbonylated. Results suggest that the carbonylation of proteins is a posttranslational modification that drivers the ripening process and it probably establishes the accumulation and functionality of some mitochondrial proteins in pepper fruit.

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POSTERS

P1

A HUMAN-CELL BASED ASSAY OF OXIDATIVE VERSUS NON-OXIDATIVE CYTOTOXICITY USING ACOUSTIC FLOW CYTOMETRY AND VIOLET LASER

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Reactive oxygen species (ROS) and ROS-mediated toxicity (ROS-TOX) are involved in carcinogenesis and degenerative diseases. Expression of certain proteins induced by ROS protects human dopaminergic neuroblastoma SH-SY5Y cells. We have developed an *in vitro* ROS-TOX assay based on flow cytometry (FCM) in which the ROS-TOX induced in SH-SY5Y cells by a series of catechols is compared with that induced in the human hepatoma HepG2 cell line. Different catechols and positive controls were selected on the basis of their toxicity to E. coli OxyR- cells, a ROS-TOX biosensor. Cytotoxicity and ROS-TOX were monitored by FCM analysis of cell death and intracellular glutathione (GSH) using the Attune™ Acoustic Focusing Flow Cytometer (Applied Biosystems) and Monochlorobimane (Invitrogen), a cell membrane-permeant dye for GSH excited by violet laser. The five catechols studied induced acute cytotoxicity in SH-SY5Y cells and were also toxic toward HepG2 cells, although with higher IC50 values. The cytotoxicity profiles for the two simple catechols were similar to those observed for the three catecholamines as well as for menadione, whereas toxicity by m-cresol, a non-catechol compound, was observed only at high doses. SH-SY5Y cells exhibited higher sensitivity to catechol-induced cell death. The cytotoxicity profiles show that SH-SY5Y/HepG2 and E. coli OxyR-/OxyR+ are comparable systems, as SH-SY5Y cells, similarly to OxyR- cells, exhibit a high ROS-TOX sensitivity. In this study, toxic compounds as CAT and 3MCAT were well differentiated, and the ability of the non-toxic compound m-cresol to induce apoptosis was demonstrated.

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P2

IN VITRO CYTOMIC SCREENING OF OXIDATIVE TOXICITY USING HEPG2 AND HEPA-RG CELL LINES

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Prediction of human toxicity of xenobiotics requires validated *in vitro*. Hepatoma cell lines, such as HepG2 cells, are used with limitations in studies of *in vitro* toxicity. The hepatocyte-like cells Hepa-RG are a promising alternative, since they express hepatocyte markers, and perform various secretory, metabolic and drug detoxification functions. We have assessed the predictive value of HepaRG cells and HepG2 hepatoma by applying a cytomic assay panel for screening oxidative stress to a wide range of test substances. We have screened by flow cytometry the cytotoxicity of 60 molecules, including pharmaceuticals, industrial compounds and biocides. The fluorochrome panel included dihydrodichlorofluorescein diacetate for detection of intracellular peroxidative activity, Mitosox Red Dye for detection of superoxide anion and diamino fluorescein-FM diacetate for detection of intracellular nitric oxide. The effects on each endpoint of oxidative stress (EC50) were determined and compared with previously reported data of *in vitro*, rodent and *in vivo* human acute toxicity. Both cell models present a better correlation with human toxicity than the reference test of Neutral Red Uptake in 3T3 cells, with superoxide anion endpoint showing the highest correlation with *in vivo* human toxicity. However, not all the compounds in our list known to form toxic metabolites were more toxic to HepaRG cells than to HepG2 cells. Combination of both cell lines improves *in vitro* predictivity of oxidative stress markers and confirms the low toxicity of some compounds on these cell lines.

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P3

CYTOMETRIC SCREENING OF DRUG CANDIDATES USING BACTERIAL BIOSENSORS OF OXIDATIVE STRESS

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Tideglusib, also known as NP031112, is a potent inhibitor of glycogen synthase kinase-3, currently involved in clinical trials for neurodegenerative diseases. In previous studies, we found that Tideglusib induced oxidative stress in different cellular models. In the present study, the prooxidant activity of Tideglusib and related drug-candidates has been determined by flow cytometry on a series of *Escherichia coli* WP2 strains deficient in key genes of antioxidant defence. We have determined the effect of test compounds plus positive controls for cytotoxicity (2-propanol) and oxidative stress (cumene hydroperoxyde, CHP). A panel of flow cytometric assays was used to quantify cytotoxicity, as well as the induction of peroxidative activity and superoxide production, using an Accuri C6-HyperCyte HTS system. Cytostatic effects were quantified by a disk assay of cell growth inhibition on agar. Cytotoxicity of compounds NPE100928 and NPE110901 was about twice stronger than that of CHP, but superoxide generation induced by NPE100928 and NPE110901 was about half stronger than that of CHP. All compounds except Tideglusib scored positive in growth inhibition assay, and compounds NP031111 and NP111404 could be classified specifically as cytostatic. None of the compounds enhanced peroxidative activity. Only compounds NPE100928 and NPE110901 can be classified as prooxidants with superoxide-related cytotoxicity, NPE100928 being slightly stronger than NPE110901. This combined assay of cytostatic and oxidative stress based on *E. coli* WP2 strains deficient in antioxidant defence is useful to reveal cytotoxic effects of chemical compounds and to investigate the basic mechanisms of their toxicity.

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P4

SACCHAROMYCES CEREVISIAE SOD1 MUTANTS, A TOOL TO EVALUATE ANTIOXIDANT CAPACITY *IN VIVO*

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Oxidative stress is a condition of living beings generated by a redox unbalance produced by a rise of free radicals and low level of antioxidants. Oxidative stress is associated with aging and several diseases (i.e. atherosclerosis, Alzheimer, Parkinson, encephalopathy, myalgia, multiple chemical sensitivity, and periodontitis). An intake rich in antioxidants would have positive effects in the prevention of these diseases. For this reason, there is a growing interest in the evaluation of antioxidant skill of different products, both natural and synthetic.

There are many and varied trials to evaluate the antioxidant capacity, including chemical *in vitro* reactions, or tests with cell cultures or animals. They provide useful information, but extrapolation of results to humans is limited and hazardous.

With this aim, we propose the use of *Saccharomyces cerevisiae* sod1 mutants. These mutants, which cannot eliminate free radicals (ROS: reactive oxygen species) produced by Fenton reactions, are unable to grow in media with obligatory oxidative metabolism. Growth recovery in media with glycerol/ethanol as carbon source allows evaluating the antioxidant capacity of different products on ROS generated inside of the cell.

As an example of the application of this methodology using synthetic molecules, we have studied the superoxide dismutase like activity of copper or manganese complexes, comparing their activities *in vivo* with *in vitro* results. We have also used this technique to analyze the capacity of different wines from the DO Utiel-Requena area to restore the growth of *sod1* mutants in glycerol/ethanol media.

P5

A CYTOMIC ASSAY OF MITOCHONDRIAL OXIDATIVE STRESS USING A HUMAN CELL LINE EXPRESSING MITOCHONDRIAL GFP

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Mitochondrial alterations and oxidative stress are often associated to cytotoxicity. Green fluorescent protein (GFP) has been shown to lose its fluorescence when exposed to reactive oxygen species (ROS) in cell-free assays. The aim of our work is to design a cell-based assay of mitochondria-associated oxidative stress with a human liposarcoma cell line expressing GFP at the mitochondrial level. SW872/GFP cell line was obtained by transfection with a retroviral vector containing the fusion cDNA for human wild type LON protease and eGFP. Cells were exposed to different xenobiotics inducing general or mitochondria-associated production of ROS, as well as to membrane- and soluble antioxidants. Mitochondrial co-localization, topology and intensity of GFP expression was studied by multispectral image-in-flow cytometry (MsIFC) using the ImageStream100, that combines the power of flow cytometry and the high content information of fluorescence microscopy. End-point and kinetic measurements of GFP fluorescence were performed by conventional flow cytometry (FCM) using an Accuri C6 instrument, that allows real-time dynamic analysis of ROS generation and GFP-fluorescence variation. Our results showed that GFP fluorescence was not affected by mitochondrial uncouplers but decreased with prooxidants causing oxidative stress at mitochondrial level, which was prevented by antioxidants. In addition, by measuring the fluorescence-associated morphometric parameters of mitochondrial GFP, variations in mitochondrial surface and distribution were detected and related to the early changes in cell dysfunction. Thus, SW872/GFP cell line is a suitable model for the study of oxidative stress in mitochondria both by MsIFC and FCM.

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P6

AOH EXPOSURE INDUCED OXIDATIVE STRESS IN CACO-2 CELLS

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Alternariol (AOH) is a mycotoxin produced by *Alternaria sp*. often found in fruits and vegetables. In order to determine if the oxidative pathway could be implicated in AOH's toxicity, the cytotoxicity of AOH, the generation of reactive oxygen species (ROS) and lipid peroxidation (LPO) were investigated in Caco-2 cells. Subsequently, the induction of oxidative stress by the antioxidant defense imbalance related to glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) were also evaluated.

Cytotoxicity of AOH (from 3.125 to 100 µM) was determined during 24, 48 and 72 h by the MTT assay. Decreasing cell viability was observed, but no IC50 values were obtained. To determine oxidative stress, AOH at sub-cytotoxic concentrations (15, 30 and 60 µM) was assayed. Early ROS production (1.2-folds of control) by H_2-DCFDA fluorescent probe after 120 min was observed. LPO generation ranged from 50% to 145% compared to the control by TBARS method after 24 h of AOH exposure. AOH oxidative stress was corroborated by alteration of GSH levels and the antioxidant activities. The GPx and CAT activities and totally GSH levels decreased at 60 µM of AOH exposure. However, significant increase in SOD was observed at all concentrations assayed.

P7

THE OXIDATIVE STRESS IMBALANCE IN MICE WITH TYROSINE HYDROXYLASE HAPLOINSUFFICIENCY IS MODULATED BY SOCIAL ENVIRONMENT

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Catecholamines (CAs), products of the sympathetic-adrenal-medullary axis, are involved in anxiety-like behaviour. Furthermore, anxiety is related to an increased oxidative stress as well as an immune impairment. However, it is still unclear if altered levels of CAs may affect the oxidative stress balance in immune cells. Besides, social environment may affect immune responses. The aim of this work was to study if altered levels of CAs could affect the oxidative stress of immune cells and to determine the possible modulatory effects of the social environment.

Adult female ICR-CD1 mice with deletion of a single allele (hemi-zygotic: HZ) of the tyrosine hydroxylase (TH-HZ), the first enzyme of catecholamine synthesis, and wild-type (WT) mice were used. Animals were housed in four groups: WT>50% (in the cage, the proportion of WT mice was higher than TH-HZ), WT<50% (WT mice lower than 50%), TH-HZ<50% (TH-HZ mice lower than 50%), TH-HZ>50% (TH-HZ mice higher than 50%). Peritoneal cells were collected and catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and xanthine oxidase (XO) activities, reduced glutathione (GSH), oxidized glutathione (GSSG) levels and GSSG/GSH ratio, were evaluated.

The results showed that TH-HZ exhibit elevated GPx and XO activities with respect to WT. Moreover, TH-HZ>50% show lower GSH levels and CAT activity, as well as higher GPx and XO activities than WT>50%. Additionally, TH-HZ<50% showed similar values in these parameters to WT<50%.

In conclusion, altered levels of CAs may induce oxidative stress imbalance, which seems to be determined by social environment.

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P8

OXIDATIVE STRESS IN A MODEL OF PREMATURE AGING AND ANXIETY IN MICE

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Anxiety and aging are associated with an oxidative stress situation. In a model of prematurely aging mice (PAM), these animals show higher emotionality and anxiety-like behaviour, a premature immunosenescence, as well as a decreased lifespan in relation to non-prematurely aging mice (NPAM) of the same sex and chronological age. The aim of this work was to study the oxidative redox state of several tissues and immune cells from NPAM and PAM. Adult female mice were classified as NPAM and PAM according to their performance in a T-maze behavioural test. Several oxidant (intracellular superoxide anion levels, intracellular ROS generation, xanthine oxidase activity and expression) and antioxidant parameters (total glutathione levels, total antioxidant capacity, as well as catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase activities) were analyzed in different tissues (liver, kidney, spleen, thymus, heart, cerebral cortex, brainstem, cerebellum, etc.) and in immune cells (splenic and peritoneal leukocytes) from NPAM and PAM. In general, PAM show higher levels of parameters of oxidation and decreased antioxidant defences in most of their tissues and immune cells, especially in peritoneal leukocytes, in comparison to NPAM. Thus, adult PAM show an oxidative stress state, which is characteristic of chronologically older mice. This may have a crucial role in the state of anxiety and the shorter lifespan of these animals. In addition, the assessment of oxidative stress parameters in peritoneal leukocytes, without sacrificing the animals, is useful and representative of the redox state of the organism.

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P9

PROTECTION AGAINST OXIDATIVE STRESS IN SUCCESFUL AGING

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Introduction. Human longevity is a complex and multifactorial phenomenon in which genetic and environmental factors influence. Although the free radical theory of aging has been criticized in recent years, oxidative stress still plays an important role in understanding aging.

Aim. Our aim is to better understand the effect of oxidative stress on human longevity.

Material and methods. We compared oxidative stress parameters in human plasma of three groups; young people (20 to 40 years old), septuagenarians (70 to 80 years old) and centenarians (older than 97 years). We studied the lipid peroxidation levels determined as malondialdehyde (MDA) measured by HPLC and protein oxidation determined as carbonyls groups by Western blotting.

Results. Regarding the levels of MDA in plasma, young people and centenarians have similar levels (1.50 ± 0.52 µM and 1.44 ± 0.45 µM, respectively) and significantly lower than the septuagenarians (1.84 ± 0.59 µM).

Protein carbonylation in plasma of centenarians (67.17 ± 17.50%) is significantly lower than the young people (75.55 ± 12.58%) and septuagenarians (77.15 ± 13.07%).

Conclusions. Centenarians, the best example of successful aging, have less oxidative stress than septuagenarians; and in some cases, even less than the young individuals.

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P10

ROLE OF SEDENTARY AGING AND LIFELONG PHYSICAL ACTIVITY ON EXCHANGE OF GLUTATHIONE ACROSS EXERCISING HUMAN SKELETAL MUSCLE

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Reactive oxygen species (ROS) can be deleterious to cells when not sufficiently counterbalanced by the antioxidant system. Aging is associated with accumulation of oxidative damage to macromolecules. Therefore, skeletal muscle formation of ROS in response to exercise could be excessive, which potentially causes cellular damage in the aged state.

The purpose of the present study was to examine the effect of acute exercise on exchange of GSH and GSSG across the leg in young or older sedentary or older lifelong physically active humans.

We evaluated the effect of acute exercise on changes in blood redox state across the leg of young (23±1 years) and older (66±2 years) sedentary humans by measuring the whole blood concentration of GSH and GSSG. To assess the role of physical activity, lifelong physically active older subjects (62±2 years) were included.

Exercise increased the venous concentration of GSSG in an intensity-dependent manner in young sedentary subjects, suggesting an exercise-induced increase in ROS formation. In contrast, venous GSSG levels remained unaltered during exercise in the older sedentary and active groups despite a higher skeletal muscle expression of the superoxide generating enzyme NADPH oxidase. Arterial concentration of GSH and expression of antioxidant enzymes in skeletal muscle of older active subjects was found to be increased. The potential impairment in exercise-induced ROS formation may be an important mechanism underlying skeletal muscle and vascular dysfunction with sedentary aging.

Lifelong physical activity up-regulates antioxidant systems which may be one of the mechanisms underlying the lack of exercise-induced increase in GSSG.

P11

ROLE OF E3 UBIQUITIN LIGASES IN THE MUSCLE ATROPHY INDUCED BY HINDLIMB UNLOADING. BENEFITS OF PHARMACOLOGICAL TREATMENT

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Muscle atrophy is linked to reactive oxygen species (ROS) during hindlimb-unloading due, at least in part, to the activation of xanthine oxidase (XO). The ubiquitin proteasome system has been recognized as a contributor to a catabolic state leading to loss of skeletal muscle mass and appears to be strongly influenced by oxidative stress.

The major aim of our study was to determine the mechanism by which ROS cause muscle atrophy and its possible prevention by allopurinol and indomethacin.

We studied the main redox sensitive signaling p38MAPK and the inflammatory cascade of NF-κB involved in skeletal muscle atrophy, and the expression of two E3 ubiquitin ligases involved in proteolysis, MAFbx and MuRF-1. In addition, we analysed Akt, a protein kinase that plays a key role in multiple cellular processes, by inhibition of the E3 ubiquitin-ligase Cbl-b.

We used male C57BL/6J mice randomly assigned to the control or hindlimb unloading groups, with or without, indomethacin, allopurinol or indomethacin plus allopurinol treatments.

Our data suggest that XO-induced oxidative stress is involved in the loss of muscle mass through the activation of the p38MAPK-MAFbx and NF-κB-MuRF1-MHC pathways. The most relevant new fact reported is the potential benefit of allopurinol administration for casts subjects, that prevents soleus muscle atrophy by ~5%. Our data point out the potential benefit of allopurinol and indomethacin administration for bedridden, astronauts, sarcopenic and cachexic patients.

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P12

STUDY OF SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED TO CENTENARIANS AS A POSSIBLE CAUSE OF THEIR EXTREME LONGEVITY

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The study of human populations with extreme longevity (centenarians) is one of the major challenges facing scientists. Single nucleotide variations in the genetic sequence between individuals of the same species, which are called single nucleotide polymorphisms (SNPs), may affect both the structure and regulation of the genes. 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 SNPs in centenarians as a possible cause of their extreme longevity.

Were recruited 28 centenarians (case), 30 young and 30 elderly individuals (control) from Hospital de la Ribera Alzira, Valencia, collecting whole blood for DNA isolation. Sequencing was performed using the Axiom Genotyping Array® Exome technology, analyzing 295,988 SNPs. After applying a series of filters and genetic quality controls, the association study was performed by logistic regression analysis for 37,564 SNPs that remained after filtering.

The results show a group of 12 SNPs with a p value less than 0.001. Four of the identified SNPs are in the protein coding region, four in the non-coding region, and the other four are not known.

The presence of SNPs in centenarians can be helpful to find genes involved in longevity.

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P13

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS WITHIN DIFERENT PATHWAYS, AS A POSSIBLE CAUSE OF EXTREME LONGEVITY

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Single nucleotide polymorphisms (SNPs), are nucleotide changes in the DNA sequence, with a minor allele frequency of 1% or greater, that may affect both the structure and the regulation of proteins. Through the analysis of SNPs, pathways that converge on age-associated biological processes may be identified. The aim of this study was the identification of SNPs grouped by signaling pathways in centenarians as a possible cause of their extreme longevity.

For this purpose, we recruited 28 centenarians (cases) and 60 young and elderly individuals (controls) from Hospital de la Ribera, Alzira, Valencia. Whole blood was collected for DNA extraction. Sequencing was performed using the Axiom Genotyping Array® Exome technology. We analyzed 295,988 SNPs among 117 signalling pathways, which were provided by three database: KEGG (42 pathways, 3485 genes), Biocarta (24 pathways, 517 genes), Reactome (51 pathways, 3071 genes).

The results show a total of seventeen SNPs with a p value less than 0.05, located in fourteen different genes among ten signaling pathways. Five SNPs were located in genes involved in immune and inflammatory response processes, six in cell differentiation and apoptosis processes, and three in the metabolism of fatty acids.

The identification of polymorphisms in centenarians could be a new tool to find out signaling pathways involved in longevity.

This work was supported by grants ISCIII2012-RED-43-029 from the “Red Tematica de investigacion cooperativa en envejecimiento y fragilidad” (RETICEF); RS2012-609 Intramural Grant from INCLIVA 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.

P14

STUDY OF THE INFLUENCE OF DISINFECTION BY-PRODUCTS IN INDOOR POOLS ON OXIDATIVE STRESS AND LUNG DAMAGE IN SWIMMERS

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Introduction: The formation of disinfection by-products (DBPs) produces health risks in swimmers. The levels of these DBPs depend on the swimming pool characteristics (disinfection system, ceiling height, and ventilation system).

Objective: The aim of this study was to assess the effect of DBPs present in indoor swimming pools in oxidative stress and lung hyperpermeability parameters after a moderate 40-minute swimming session in three indoor pools with different characteristics.

Methods: Twenty male participants swam aerobically for 40 minutes in different days in three swimming pools studied. Biological samples were collected to measure lung damage (serum surfactant associated proteins A), oxidative stress parameters (plasma protein carbonylation and malondialdehyde, and whole blood glutathione oxidation), and swimming exertion values (blood lactate) before and after exercise. Free chlorine and combined chlorine in water, and chlorine in air samples were determined in all the swimming pools.

Results: We found that chlorination as disinfection treatment leads to the formation of chloramines in bathing water samples, mainly mono- and dichloramine. However, free chlorine is the predominate specie in UV-treated swimming pool. Level of total chlorine increased as function of the swimming activity in swimming pools with chlorination treatment. The concentration level of chlorinated DBPs does not result in significant variation in serum surfactant associated proteins A and oxidative stress parameters in swimmers.

Conclusions: Our results show that a short exposition to DBPs did not induce an increase in oxidative stress and lung damage. More research is needed to evaluate the effect of long term DBPs exposure.

P15

INCREASED OXIDATIVE STRESS AND DYSREGULATION OF THE ANTIOXIDANT ENZYME SYSTEM IN PATIENTS WITH OSTEONECROSIS OF FEMORAL HEAD

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Background: Non-traumatic osteonecrosis of femoral head (ONFH) results from the interruption of blood flow to the bone, which leads to cell death of bone components. Chondral collapse occurs in 75-80% of untreated-cases resulting in bone destruction, pain, and loss of joint function. Recent animal studies suggest that oxidative stress may be involved in the development of the disease. However, there are no studies in humans that examined the correlation between oxidative stress and ONFH development.

Aim: To evaluate the role of oxidative stress in the pathogenesis of the human ONFH.

Materials & methods: The oxidative stress status was determined in blood and urine of 54 ONFH patients and 52 healthy subjects. The ratio of oxidized vs reduced glutathione (GSSG/GSH), 15 F2T-isoprostane, 8-oxo-7, 8-dihydro-2-deoxyguanosine (8OHdG) and oxidized proteins (OP) were measured. Moreover, the enzymatic activities of the main antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) were also assessed.

Results: All the oxidative stress markers (GSSG/GSH ratio, 15-isoprostane, 8OHdG and OP) are increased in patients with ONFH compared to controls. A significant increment in SOD and GR activities and a significant decrease in GPx activity were observed. No significant differences were detected in CAT activity.

Conclusions: Parameters of oxidative stress are augmented in ONFH patients compared to controls, suggesting that oxidative stress may be involved in the pathogenesis of the disease.

P16

PERIODONTAL DISEASE SEVERITY IS ASSOCIATED WITH OXIDATIVE STRESS STATUS

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Background: Chronic periodontitis is a high prevalence disease affecting 10%-15% of the world population. Several evidences have shown an association between oxidative stress (OS) and periodontal disease.

Rationale and aims: It has been suggested that the composition of the biota in periodontal pockets is related to severity of periodontal disease. The aim of this study was to determine the association between OS and the number of different periodontal bacteria with periodontitis status

Methods: OS parameters and the activity of the main antioxidant enzymes were determined in saliva of fifty-three patients with periodontal disease and thirty-three control individuals. PCR was used to determine the presence of the 6 fimA genotypes of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia* and *Treponema denticola*.

Results: Periodontal disease severity was found to be associated with increased OS levels. Moreover, OS levels were rising according to the number and type of different periodontal bacteria found in the periodontal pockets.

Discussion: Determination of OS levels together with the number of periodontal bacteria could a potent tool to control periodontal disease development.

P17

CHARACTERIZATION OF THE ANTIOXIDANT SYSTEMS IN DIFFERENT COMPLEMENTATION GROUPS OF DYSKERATOSIS CONGENITA

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The telomerase complex and telosome regulate, maintain, and repair telomeres. The telomerase complex is formed by complex of protein (TERT, Dyskerin, GAR, NHP2, NOP10) and nucleic acid (TERC) that work together as a reverse transcriptase. The telosome comprises a network of proteins (TRF2, TRF1, TIN2, RAP1, TPP1 and POT1). The rare disease dyskeratosis congenita (DC) (ORPHA1775) shows features that resemble premature aging. DC is a genetically heterogeneous disease caused by mutations in the genes described above.

Recently a correlation between telomerase activity and catalase activity was established, therefore suggesting an antioxidant role for the extranuclear telomerase. However, it is not completely clear whether there is any relationship or connection between molecular telomerase activity and cellular antioxidant defense.

We wonder if there is a cellular effect related to the production of oxidative stress or alteration of antioxidant systems after silencing these components involved in telomere maintenance. In this communication, we show by silencing of DKC1, NOP10 (genes of telomerase complex) and TINF2 (component of telosome) in HeLa cells using the technology of RNA interference (siRNA), the effect on the cellular antioxidant capacity. For this purpose, we have evaluated the levels of DKC1, NOP10, TINF2 after siRNA treatments and the levels of antioxidant enzymes (CuZnSOD, MnSOD, Catalase, Gpx1, Grx1 and Trx1) by RT-qPCR and Western blotting. We also analyzed the production of reactive oxygen species by fluorimetry and also assessed the activity of the telomerase complex by Sybr Green RT-QTrap. Our results point out that telomerase activity and the different telosome components contribute to the regulation of the antioxidant enzymes.

P18

BIOMARKERS RESEARCH IN NEUROMUSCULAR DISEASE CHARCOT-MARIE-TOOTH

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Charcot-Marie-Tooth disease (CMT) (ORPHA166) is the most frequent hereditary neuropathy. CMT is a heterogeneous group of disorders which, despite some variability in their clinical features, share the same general phenotype, usually characterized by wasting and weakness of distal limb muscles, decreased to absent deep tendon reflexes, distal sensory loss, and frequent skeletal deformities. Despite the clinical and molecular description of this disease in the last 20 years, there is no effective drug or advanced therapy available. Here we have pretend the identification of metabolic and oxidative stress biomarkers in plasmas from patients with duplication at PMP22 gene, the most frequent mutation causing CMT, and clinically characterized as CMT1A. The samples were collected in the neuropathy units from “La Fe” Hospital of Valencia, “Bellvitge” Hospital of Barcelona, “La Paz” Hospital of Madrid, and “Virgen del Rocío” Hospital of Sevilla. The metabolic biomarkers research was performed using 2D-DIGE analysis and DeCyder software (GE). Protein identification was made by mass spectrometry by MALDI-TOF-TOF and liquid Chromatography analysis (ABSciex). The oxidative stress biomarkers research consisted in carbonylated proteins analysis by reaction with DNPH and Dot-blot. Total antioxidant capacity and GSSG/GSH ratio were analyzed with Antioxidant Assay kit and Glutathione Fluorescent detection Kit, respectively. Finally now we are performing the MDA levels by HPLC-UV.

We found 8, 13 and 36 proteins with differential expression in mild, moderate and severely affected patients, respectively compared with their own matched controls. Also we found differences on oxidative stress parameters between de different groups analyzed. Our results suggest differences in the oxidative stress profile between the studied phenotypes in CMT1A patients.

P19

ANTIOXIDANT EFFECT OF NATALIZUMAB IN AN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MODEL

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Experimental autoimmune encephalomyelitis (EAE) is a relevant and important model for studying pathophysiology and for designing new therapeutic strategies in multiple sclerosis (MS). Natalizumab, a monoclonal antibody used in the treatment of MS, inhibits the migration of systemic immune cells to the central nervous system (CNS). The aim of the study was to evaluate the neuroprotector effect of dimethyl-fumarate (DMF) and Natalizumab, evaluating their effects on oxidative profile biomarkers in EAE model and clinical score. To achieve our objectives, we performed an EAE model, in Dark agouti rat, induced by myelin oligodendrocyte glycoprotein (MOG). The process was characterized by an increase in the clinical score, together with an enhancement in oxidative stress biomarkers (lipid peroxidation products and carbonylated proteins) and reduction in GSH/GSSG ratio. In addition, Natalizumab and DMF reversed towards normality the changes caused by MOG. In conclusion, our data support the idea that: i) oxidative damage plays an important role in pathophysiology of EAE and MS; and ii) The neuroprotective effects of natalizumab as DMF may be due, at least in part, to an antioxidant effect.

P20

LIVER OXIDATION IN RATS FED HYDROXYTYROSOL, SILICON AND CHIA OIL ENRICHED DIETS

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Diet intervention is recommended as first-line therapy for the high blood cholesterol levels management. Meat pork is an essential component in developed countries diets. In order to achieve healthier and functional meat derivatives, it is necessary to avoid undesired substances (natural or otherwise) or reduce them to appropriate limits; and to increase levels (naturally or by programmed additions) of substances with beneficial properties. In the present study, we hypothesize that enrichment of restructured pork (RP) with active substances combinations (chia oil, hydroxytyrosol and silicon) reduces the hypercholesterolemic effect, helping to maintain the antioxidant status in rats fed RP diets added with hypercholesterolemic inductors. We have studied the antioxidant activity of chia oil, hydroxytyrosol, and silicon combination on liver hypercholesteremic rats and the possible involved mechanisms. We found that the presence of silicon and hydroxytyrosol decreased oxidative stress levels determined by GSSG/GSH ratio, and malondialdehyde. The antioxidant response after determination of protein expression and enzymatic activity of SOD, catalase, GPx and GR was modified by the action of silicon in all groups when compared to controls and groups fed with hydroxytyrosol. Chia oil produced lower oxidative stability, this was improved when combined with silicon and hydroxytyrosol that act against oxidation of unsaturated fatty acids. Results proved that silicon enriched diets induced higher antioxidant stability; this effect is improved by addition of hydroxytyrosol. In conclusion, effects of RP containing chia oil, hydroxytyrosol and silicon have shown to ameliorate hepatic oxidative stress and lipid oxidation when compared to control.

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P20B

ASCORBATE PEROXIDASE-INDUCED DROUGHT TOLERANCE IN PLUM

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Water deficit, is the most serious environmental factors that limit agricultural production. Genetic engineering has been widely used to improve tolerance against the oxidative stress and finally to maintain the productivity of plants under stress conditions.

In a previous work, we described that transgenic tobacco plants overexpressing both cytosolic Cu/Zn-SOD (cytsod) and/or cytosolic APX (cytapx) showed an increased tolerance to water-stress and to Pseudomonas syringae (Faize et al., 2011, 2012). Recently, we have generated transgenic plum plants over-expressing cytsod and/or cytapx genes, and interestingly, these plants showed higher regeneration efficiency and enhanced vigour when compared to the wild type plum plants (Faize et al., 2013). Furthermore, over-expression of these two transgenes enhanced tolerance to salt stress of in vitro plum plants (Diaz-Vivancos et al., 2013).

The aim of this work was to test, from a physiological and biochemical point of view, if increasing the antioxidant ability of commercial plum trees could achieve durable drought tolerance. To achieve this goal we have acclimatised to ex vitro conditions transgenic plum plants (J8-1), overexpressing cytapx, and we study the effect of water stress on photosynthesis, water potential, antioxidative metabolism and protein and gene differential expression in plum plants. Our results showed that the overexpression of cytapx gene induced tolerance to water stress in plum plants.

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P21

CHARACTERIZATION OF ANTIOXIDANT ENZYMES OF OLIVE (*OLEA EUROPAEA L.*) FRUITS

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The commercialization of olive oil and the cultivation of olive trees have a tremendous impact on the economy of Mediterranean countries like Spain, Italy and Greece. To protect plants from the toxic action of ROS, cells possess a battery of enzymatic and non-enzymatic antioxidants.

The olive fruit is a good source of non-enzymatic antioxidants (also named molecular antioxidants), including many different types of phenolic compounds, which some of them are transferred to the olive oil during the extraction process. However, the presence of enzymatic antioxidants has not been investigated in this tissue. In this work we report the presence of different antioxidant enzymes in olive fruits, including catalase, four superoxide dismutase (SOD) isozymes (mainly an Fe-SOD, two Cu,Zn-SODs plus a Mn-SOD), all the enzymes of the ascorbate-glutathione cycle, and four NADPH-recycling dehydrogenases. We also report the presence of reduced and oxidized glutathione, as molecular antioxidant.

The knowledge of the full composition of antioxidants (enzymatic and non-enzymatic) of olive fruits is crucial to understand the processes regulating the antioxidant composition of olive oil.

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P22

INVOLVEMENT OF SOME COMPONENTS OF EXTRA VIRGIN OLIVE OIL ON NITRIC OXIDE PRODUCTION BY ENDOTHELIAL CELLS TO EXPLAIN ITS EFFECT ON BLOOD PRESSURE

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Clinical trials show that the traditional Mediterranean diet (TMD) reduces blood pressure. However, the mechanisms involved are not elucidated. Nitric oxide (NO) preserves vascular function, whereas circulating non-esterified fatty acids reduce NO-dependent vascular-relaxation, contributing to hypertension.

We compared the 1-year effect of two behavioural interventions to implement the TMD, one supplemented with extra virgin olive oil (EVOO) and the other with nuts vs a control group (advice on a low-fat diet) on hypertension and in vasculature NO production, among non-smoking hypertensive women. To study the role of key components of the EVOO we performed an in vitro experimental approach by using endothelial cells, which were incubated with oleic acid in the absence or presence of additional extra virgin olive oil minor compounds.

We found a reduction of systolic and diastolic blood pressure after both TMD and an inverse association between changes in systolic blood pressure and serum NO metabolites concentration after the TMD supplemented with EVOO. The *in vitro* experimental approach showed that linoleic acid reduced (57%) intracellular NO in human umbilical vein endothelial cell (ECV304) cultures more than oleic acid (33%). Hydroxytyrosol and β-sitosterol or an EVOO polyphenol extract reverted the reduction of NO concentrations in the presence of oleic acid.

The biological actions of major and minor components of EVOO on vasculature NO levels can be an underlying mechanism to explain, at least in part, the effect of a dietary intervention with TMD+EVOO on blood pressure lowering among hypertensive women with metabolic syndrome.

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P23

DIFFERENTIAL EFFECTS OF LOSARTAN METABOLITES, EXP3174 AND EXP3179, ON CARDIAC OXIDATIVE STRESS AND REMODELING IN L-NAME HYPERTENSIVE RATS

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Cardiac hypertrophy is a marker of mortality in hypertension. Losartan was the first available orally administered antagonist of the angiotensin II type 1 (AT1) receptor developed for the treatment of hypertension. Two active metabolites of losartan have been described previously, EXP3174 and EXP3179. Whereas EXP3174 is the main antihypertensive AT1 receptor-blocking metabolite, the role of EXP3179 is widely unknown. We investigated the effects of metabolites EXP3174 and EXP3179 on cardiac remodeling and oxidative stress in L-NAME induced hypertension.

The study was carried out during 10 weeks in six groups of 10-week-old Wistar rats. Three groups were treated with vehicle, with EXP3179 (5 mg/kg/day), or with EXP3174 (5 mg/kg/day). The other 3 groups were exposed to L-NAME (30 mg/kg/day) and treated with vehicle, with EXP3179 (5 mg/kg/day), or with EXP3174 (5 mg/kg/day). Blood pressure and cardiac morphology was recorded by telemetry and echocardiography, respectively. Fibrosis and oxidative stress were evaluated by determining collagen volume fraction (CVF), cross-linking degree and expression of NADPH oxidase components.

L-NAME group developed hypertension and cardiac remodeling. The group of EXP3174-treated L-NAME rats was normotensive, although EXP3174 did not prevent the cardiac remodeling. The group of EXP3179-treated L-NAME rats developed also hypertension, although EXP3179 was able to prevent the cardiac fibrosis. In addition, the up-regulation of NADPH oxidase observed in L-NAME treated rats was equally normalized by both metabolites.

Thus, the differential effects of losartan metabolites, EXP3174 and EXP3179, on cardiac remodeling in the experimental hypertension associated with a chronic depletion of nitric oxide levels are independent of NADPH oxidase system.

P24

DETERMINATION OF BIOMARKERS IN PLASMA OF PATIENTS SUFFERING FROM MILD COGNITIVE IMPAIRMENT OR ALZHEIMER'S DISEASE

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Objectives: Alzheimer's disease (AD) is the most common form of dementia, and causes elevated social and economic burden. Finding new early biomarker of the disease has turned into a priority for the scientific community. The aim of this study is to identify bio-markers of mild cognitive impairment (MCI) and AD in blood samples and cerebrospinal fluid (CSF).

Methods: Recently, determination of Aβ-42, total tau and p-tau in CSF have been included as criteria for diagnosis of AD. Samples of 10 healthy patients (controls), 10 patients with MCI and 10 patients which were diagnosed for AD were analysed. CSF was extracted by lumbar puncture, and blood samples were taken to obtain plasma and serum. Levels of Aβ-42, total tau and p-tau, clusterin, RCAN, PKR, calcineurin, RAGE. Furthermore the ApoE genotype of each patient was determined.

Results: Patients diagnosed for MCI or AD showed lower levels of Aβ-42 in the CSF and increased levels of tau total and p-tau. AD patients had lower protein levels of clusterin and increased levels of RAGE. In contrast, patients with MCI displayed increased levels of RCAN. Both, patients of AD and MCI showed decreased levels of PKR.

Conclusions: Determination of Aβ-42, total tau and p-tau correlates with the clinical diagnosis of patients. Clusterin and RAGE could be used as biomarkers for AD, while RCAN seems to be an early biomarker of AD, since it is only elevated in MCI. The correlation of the identified proteins with Aβ-42, tau total, p-tau and the ApoE genotype might be useful for the early diagnosis of AD.

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P25

THE ROLE OF LIVER IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disorder that causes progressive loss of cognitive functions, and is associated with aging and oxidative stress. The liver serves to detoxify pathological substances from bloodstream. One of them is amyloid-β peptide (Aβ). It can reach the liver because the action of lipoprotein receptor-associated protein 1 (LRP1), which mediates the transport from brain to blood, and from blood to liver. Receptor of advanced glycation products (RAGE), mediates Aβ transport from blood to brain. Therefore, hepatic care is fundamental for an integral AD treatment.

We used the double transgenic mice for AD APPswe/PS1dE9, which develops Aβ plaques with aging. We worked with both wild type and transgenic males of different ages, 3-5, 10-13 and more than 20 months (n=5), to follow age-associated changes. We analyzed oxidative stress parameters in liver, such as the rate of mitochondrial H_2O_2 production, protein oxidation (carbonylation) and lipid peroxidation (malondialdehyde). We have also determined blood concentration of Aβ40, LRP1 in brain and liver, and RAGE in brain.

The results show how in transgenic mice, the different oxidative stress parameters decrease with aging, Aβ40 increases with aging, LRP1 decreases with aging in brain and liver, and RAGE in brain has no changes.

The decrease in LRP1 with age may explain the accumulation of Aβ40 in blood (it cannot get into the liver) and the lower oxidative stress in liver.

We can conclude that hepatic LRP1 is the main responsible for hepatic oxidative stress changes in AD.

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P26

EXERCISE TRAINING NEUROPROTECTS OVARIECTOMIZED ALZHEIMER´S DISEASE MOUSE MODEL THROUGH BDNF SIGNALING

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Postmenopausal women may be more vulnerable to cognitive loss and Alzheimer's disease (AD) than premenopausal women because of their deficiency in estrogens, in addition to their usually older age. Aerobic physical exercise has been proposed as a therapeutic approach for maintaining health and well-being in postmenopausal women, and for improving brain health and plasticity in populations at high risk for AD. To study the neuroprotective mechanisms of physical exercise in a postmenopausal animal model, we submitted previously ovariectomized, six-month old non-transgenic or 3xTg-AD mice to three months of voluntary exercise in a running wheel. At nine months of age, we observed lower grip strength and some exacerbation of the behavioral and psychological symptoms of dementia-like involving active exploratory activities. A similar major cognitive impairment was observed of ovariectomized 3xTg-AD mice in comparison with sham-operated 3xTg-AD mice. A reduction of bodily fitness and lack of retention of memory were observed in the ovariectomized non-transgenic mice. Physical exercise protected against all deleterious behaviors and normalized learning and memory. It also protected against body frailty, as expected. Analyses of hippocampal key markers of antioxidant and neuroplasticity signaling pathways showed that ovariectomy impairs the activation of CREB through physical exercise. Furthermore, molecular and behavioral correlates suggested a central role of BDNF in the neuroprotection mediated by physical exercise therapy against apathy and memory loss induced by ovariectomy and the AD-genotype.

P27

THE INVOLVEMENT OF APC/C-CDH1 IN ALZHEIMER'S DISEASE

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BACKGROUND. Excitotoxicity is a pathological process which injures nerve cells by excessive stimulation of L-glutamate and it has a major role in neurodegenerative diseases like Alzheimer's disease (AD), but the molecular mechanisms of this phenomenon are still not fully understood. Here, we describe a new signaling pathway in which Aβ causes enhanced generation of glutamate in neurons. This mechanism involves the inactivation of cdh1, which is the activator protein of the anaphase-promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase.

METHODS. Primary neurons were isolated from fetal Wistar rats (14 dpf). Neurons in culture were used by day 7 for treatments with Aβ (5 μM for 20 h), glutamate (100 μM for 20 h), APC/C-Cdh1 inhibition (12μM for 20 h), glutaminase inhibition (100μM for 30 h) or cdh1-silencing. The samples were analysed by immunoblotting and confocal microscopy.

RESULTS. Our results show that Aβ treatment decreases the protein level of cdh1 in cultured neurons. This leads to a subsequent accumulation of glutaminase, an enzyme that converts glutamine to glutamate, and it is a degradation target of APC/C-Cdh1. We showed that Aβ enhances glutamate generation and that this is mediated by deactivation of APC/C-Cdh1. The increase of glutamate caused by Aβ can be attenuated using a glutaminase inhibitor and it is completely abolished when using medium without glutamine.

CONCLUSIONS. These results indicate that APC/C-Cdh1 plays a central role in the generation of excitotoxic environments in AD. This pathway could be an interesting target against neurodegeneration.

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P28

ACTIVATION OF P38, P21 AND NRF2 MEDIATES DECREASED PROLIFERATION OF DENTAL PULP STEM CELLS CULTURED UNDER 21% O_2

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INTRODUCTION. 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.

OBJECTIVE. 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.

RESULTS. 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.

DISCUSSION. 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.

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P29

OXIDATIVE STRESS PROTECTION OF A GRAPE POMACE FUNCTIONAL INGREDIENT IN COLON CANCER CELLS. ROLE OF GSH AND REDOX REGULATION OF GSH METABOLISM GENES

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Oxidative stress is implicated in the pathogenesis of colon cancer being important sources of free radicals both intracellular ROS and those derived from the digestive process. Therefore, dietary antioxidants might exert beneficial effects in colon cancer prevention due to their direct antioxidant effects and their implication in the redox control of gene transcription that modulates the cells response to oxidative stress [1,2].

Using HT-29 cells, we have evaluated the protection against oxidative damage of a grape pomace powder ingredient, comparing the effects, bioavailability and antioxidant environment generated by the product (Sk) and the fractions obtained after a simulated gastrointestinal digestion (dSk) and colonic fermentation (d+fSk). Cells were incubated with non-cytotoxic concentrations of each treatment, followed by an oxidative stress induction with t-BOOH.

The oxidative insult caused a decrease in cell viability (MTT assay), increased the cytoplasmic membrane permeability (LDH assay), induced proteins and lipids oxidation (CG and MDA levels), and reduced the GSH/GSSG ratio. All treatments improved these oxidative stress parameters. In most cases, dSk and d+fSk retained or increased the protective effect of Sk. However, we observed a different trend when the GSH/GSSG ratio was evaluated as a biomarker of the cells redox status, being dSk the less protective fraction. As this ratio reflects a steady state resulting of enzymatic use and regeneration of GSH [3], we assessed the transcriptional regulation of several enzymes involved in GSH metabolism (GPxs, GR, GSTs) and synthesis (GS, γ-GCL) by RT-PCR. Gene expression patterns and possible relationships with GSH/GSSG ratios will be discussed.

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P30

CYTOPROTECTIVE EFFECT OF BIOACCESSIBLE FRACTIONS OF MANDARIN JUICES AGAINST H202-INDUCED OXIDATIVE STRESS IN CACO-2 CELLS

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INTRODUCTION: Mandarin juices are rich in antioxidant bioactive compounds (vitamins, polyphenols and carotenoids), with a potential effect in reducing reactive oxygen species (ROS) involved in the aetiology of many diseases.

MATERIALS AND METHODS: Mandarin juices non-treated (NT) or subjected to high pressure processing (HPP) (400 MPa), as a non-thermal processing technology, were used to evaluate the cytoprotective effect of their bioaccessible fractions (BF) obtained after simulated gastrointestinal digestion against H_2O_2-induced oxidative stress in Caco-2 cells. These were pre-incubated with BF diluted 1:5 v/v with DMEM and then treated or not with 5mM H_2O_2 for 2h, and viability (MTT test), lipid peroxidation (TBARS), intracellular ROS levels, changes in mitochondrial membrane potential (MMP) and cell cycle distribution were measured.

RESULTS: BF of samples preserved viability (68-70%) vs. H_2O_2 treated cells (53%), and reduced the rise in ROS levels and modifications in MMP (better in HPP sample) evoked by H_2O_2, but not reaching control levels. Lipid peroxidation was decreased with BF to control basal levels. Regarding cell cycle, a significant reduction in cell proportions in G1 phase accompanied by an increase in sub-G1 population (apoptosis) was shown in H_2O_2 treated cells. Incubation with BF allowed a recovery close to control levels. In any case, treatment with BF without H_2O_2 showed differences with controls.

CONCLUSION: BF of mandarin juices protected Caco-2 cells from oxidative stress by preserving cell viability, changes in MMP and correct cell cycle progression, and diminishing lipid peroxidation and ROS. In addition, HPP treatment can exert similar protection than NT juices.

P31

ROS MEDIATE THE CYTOTOXIC ACTIONS OF *VISMIA BACCIFERA* AGAINST HEPG2 HEPATOCARCINOMA CELLS

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Nowadays there is an increasing interest in finding bioactive compounds which can be used as chemopreventive agents against cancer. In this work, we have studied the cytotoxic actions of aqueous extracts from leaves of the Colombian Amazonian *Vismia baccifera* plant on the human hepatocarcinoma HepG2 cell line. Our results showed that *V. baccifera* induced cytotoxicity to HepG2 hepatoma cells, producing cell cycle arrest at G2/M and an increase of caspase-3 activity. The extract also markedly increased the intracellular ROS levels and mitochondrial superoxide anions, as measured by flow cytometry using the dichlorofluorescein and mitoSOX probes. Significant changes in the specific activities of antioxidant enzymes were also found in the presence of the *V. baccifera* leaf extract. Thus, superoxide dismutase activity increased at 24 h, while glutathione peroxidase activity was reduced at this time. The addition of catalase concomitantly with the extract significantly decreased the intracellular ROS levels and prevented the HepG2 viability loss. These results suggest that hydrogen peroxide mediates the HepG2 cytotoxic response to the *V. baccifera* aqueous leaf extract and support evidence to study more deeply these anti-cancer actions.

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RUTIN IMPROVES ANTIOXIDANT RESPONSE IN THE BRAIN, LIVER, GILLS, KIDNEY AND MUSCLE OF CATFISH

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The catfish, *Rhamdia quelen* (*Heptapteridae* family), is one of the most cultivated species in the southern Brazil. Products designed to improve the cultivation and production of silver catfish are required due to the importance of this species for aquaculture. The oxidative stress is one of the main challenges in aquaculture, and the use of compounds with antioxidant capacity can be useful. Thus, the aim of this study was to evaluate the oxidative biomarkers in brain, liver, gills, kidney and muscle of silver catfish fed diets containing the flavonoid rutin. Three diets (in triplicate) were tested: standard 0 (control) and in the other added different concentrations of rutin: 0.15 and 0.30 % rutin in the diet. After 21 days of feed, the animals were euthanized and tissues were removed for analysis as follows: lipid peroxidation measured by the thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide (LOOH) levels; enzymatic activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST); non-enzymatic antioxidants by determining content of non-protein thiols (NPSH), ascorbic acid (AA) and total reactive antioxidant potential (TRAP). The results demonstrated that the animals fed diet containing rutin presented a decrease in LOOH and TBARS levels, an increase in CAT, SOD, GPx and GST activities and an increase in NPSH, AA content and TRAP when compared to the control. In conclusion, these results suggest that supplementation containing rutin to the diet of silver catfish is recommended because increases the tissue antioxidant response.

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CYTOPROTECTIVE AND ANTIOXIDANT EFFECT OF RESVERATROL IN CHO-K1 CELL EXPOSED TO BEAUVERICIN

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Beauvericin (BEA) is a secondary metabolite of *Fusarium fungus*. BEA is an ionophore, forming a complex with essential cations (Ca^{2+}, Na^{+} and K^{+}), that increases ion permeability in biological membranes. BEA produces cytotoxicity, lipid peroxidation (LPO), apoptotic activity, DNA fragmentation and it is a potent specific inhibitor of cholesterol acyltransferase (Acyl-CoA), in mammalian cells. Moreover, resveratrol (3, 5, 4'-trihydroxystilbene; RSV) is a non-flavonoid polyphenolic compound abundant in grapes and derivate, peanuts, berries, and dark chocolate. RSV has been studied for its biological properties. RSV exits as *trans* and *cis* isomer. The aim of this study was to evaluate the cytoprotective and the antioxidant effect of the isomers of RSV in Chinese Hamster Ovary (CHO-K1) cells exposed to BEA. For this, the CHO-K1 cells were exposed to a pre-treatment with *trans*-RSV and *trans*-RSV/*cis*-RSV (70:30) at 2.5 and 5 µM for 24 h and, then exposed to BEA at 1 and 5 µM for 24 h. The cytotoxicity and LPO were determined by MTT assay and thiobarbituric acid reactive substances (TBARS) assay. The cytoprotective effect of *trans*-RSV was 57% respect to control. Whereas, cytoprotection of *trans*-RSV/*cis*-RSV (70:30) ranged from 25% to 42%. A decrease of LPO of 28% with *trans*-RSV and 37% with *trans*-RSV/*cis*-RSV (70:30), respect to the control, was observed. Therefore, it can be concluded that foods containing *trans*-RSV or isomer mixtures of RSV help to reduce the toxicological risk produced by BEA in food.

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NUTRITIONALLY RELEVANT CONCENTRATIONS OF RESVERATROL HAVE ANTIOXIDANT EFFECTS ON MCF-7 CELLS VIA PTEN/AKT SIGNALING PATHWAY

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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.

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P35

THE PROTECTIVE EFFECT OF N-ACETYLCYSTEINE ON ASPARTAME-INDUCED OXIDATIVE STRESS IN THE LIVER OF RATS

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Long-term intake of aspartame at the acceptable daily ingestion dose causes oxidative stress in the rat liver through dysregulation of glutathione homeostasis. N-acetylcysteine provides the cysteine required for the production of GSH, being effective in treating disorders associated with oxidative stress. The aim of this research was to investigate the effects of N-acetylcysteine on the aspartame-induced oxidative stress in the rat liver. The animals received aspartame by gavage for six weeks (40 mg/kg). From the 5th week, N-acetylcysteine (150 mg/kg, intraperitoneal) was injected for two weeks. Then, they were anaesthetized for obtaining blood samples and euthanized for the liver collection. The blood was centrifuged at 1800 g for 15 min and the serum was separated for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) measurements. The tissue was homogenized in 1.15% KCl buffer and centrifuged at 700 g for 10 min at 4ºC. The supernatant fraction obtained was used for the measurements of oxidative stress biomarkers. N-acetylcysteine led to a reduction in the ALT, AST and ALP activities in the serum as well as the thiobarbituric acid reactive substances, lipid hydroperoxides, and hydrogen peroxide levels, which were increased in the rat liver after aspartame exposure. Additionally, N-acetylcysteine caused an elevation in the glutathione peroxidase, glutathione reductase, and glutathione S-transferase activities and non-protein thiols, ascorbic acid, and total antioxidant capacity levels, which were decreased in the rat liver after aspartame exposure. In conclusion, N-acetylcysteine may be useful for the protection of the rat liver against aspartame-induced oxidative stress.

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INFLUENCE OF OXIDATIVE STRESS IN THE CONTROL OF CYTOKINESIS IN PRIMARY HEPATOCYTES

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ROS signaling can activate or suppress cell cycle progression depending on the activation stimulus. Low concentrations of H_2O_2 are generally growth stimulatory, but higher concentrations of superoxide and H_2O_2 may inhibit cell cycle or even have deleterious effects (1). The aim of this work was to assess the role of oxidative stress during mitosis progression studied in isolated murine primary hepatocytes as in vitro model.

Our results showed that isolation of hepatocytes with collagenase in presence of 5mM N-acetyl cysteine (NAC) diminished by 10% the percentage of tetraploid cells and by 5% the percentage of binucleated cells. It also caused an increase in phospho-histone 3, a mitotic marker. Levels of cyclins, key regulators of cell cycle, were also affected. Moreover, 5mM NAC addition to the isolation medium reduced the levels of oxidative stress.

Therefore, the oxidative stress generated during hepatocyte isolation seems to cause cytokinesis failure, triggering high binucleation rate in primary hepatocytes isolated without NAC. This work could be useful for clinical applications as isolated hepatocytes perfusion is an alternative for patients who cannot receive liver transplantation. In this case, it is necessary to obtain NAC isolated hepatocytes with higher proliferation potential which respond better in the host patient.

(1) Tormos AM, Taléns-Visconti R, Nebreda AR, Sastre J. p38 MAPK: a dual role in hepatocyte proliferation through reactive oxygen species. Free Radic Res. 2013 Nov;47(11):905-16.

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EXTRACELULAR HEMOGLOBIN ENHANCES THE SYSTEMIC RESPONSE IN ACUTE PANCREATITIS

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Extracellular hemoglobin may cause oxidative stress and cytotoxicity due to its peroxidase activity. It may also release free hemin which increases vascular permeability, leukocyte recruitment, and adhesion molecule expression. Pancreatitis-associated ascitic fluid is reddish and may contain cell-free hemoglobin. Our aim has been to determine the role of extracellular hemoglobin (EHb) in the local and systemic inflammatory response during severe acute pancreatitis in rats. To this end taurocholate-induced necrotizing pancreatitis in rats was used. Firstly, EHb was quantified in ascites and plasma and the hemolytic action of ascitic fluid was tested. Secondly, hemoglobin was purified from rat erythrocytes and administered i.p. to assess the local and systemic effects of ascitic-associated EHb during acute pancreatitis. EHb markedly increased in ascitic fluid and plasma during necrotizing pancreatitis. Peroxidase activity was very high in ascites. The administration of EHb enhanced ascites, dramatically increased abdominal fat necrosis, up-regulated the gene expression of *tumor necrosis factor-*α, *interleukin 1β* and *interleukin 6* and decreased expression of *interleukin 10* in abdominal adipose tissue during pancreatitis. EHb enhanced the gene expression and protein levels of VEGF and other hypoxia inducible factor-related genes in the lung. EHb also increased myeloperoxidase activity in the lung. In conclusion, EHb contributes to the inflammatory response in severe acute pancreatitis through abdominal fat necrosis and inflammation and increasing VEGF and leukocyte infiltration in the lung.

P38

MITOCHONDRIAL SUPERCOMPLEXES ASSEMBLY EXPLAINS DIFFERENCES IN REACTIVE OXYGEN SPECIES PRODUCTION BETWEEN 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 each of the four different neural cell types to ROS formation. Here, the abilities of mitochondria isolated form rodent neurons and astrocytes, the most abundant brain cell types, to spontaneously form ROS were characterized. We found that ROS production is about one order of magnitude higher in astrocytes than in neurons. This observation is intriguing in view of the widely held notion that astrocytes, which express high levels of antioxidants, are efficient ROS detoxifiers. We also found that the main source of ROS in both cell types is the mitochondrial electron transport chain, but the difference is ROS production cannot be accounted by the relative abundance of total complex I, a major superoxide anion source within mitochondria. Furthermore, the high ROS production by astrocytes is conserved amongst several rodents, including Wistar rats and C57BL6 mice. In view that the mitochondrial supercomplex assembly factor, SCAFI, is not functional in C57BL6 mice, we conclude that the high ROS production in astrocytes is not due to a putative differential expression of this assembly protein. Interestingly, we found that in astrocytes a large proportion of complex I protein occurs free, whereas in neurons most complex I is part of supercomplexes. We conclude that this differential complex I assembly into supercomplexes explains the high mitochondrial ROS production by astrocytes. These results suggest cell-specific physiological roles for mitochondrial ROS in the brain.

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ANALYSIS OF THE REDOX STATE FROM CYSTEINE PROTEIN RESIDUES IN FRATAXIN-DEFICIENT CARDIOMYOCYTES

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Friedreich ataxia (FRDA), is a neurodegenerative disease accompanied by hypertrophic cardiomyopathy. This hereditary disease is caused by deficient frataxin expression, a mitochondrial protein that has been related to iron homeostasis, energy metabolism, and oxidative stress. We have recently set up a cellular model of the disease based on neonatal rat cardiac myocytes (NRVM) and RNA interference. In this model, frataxin-deficient cells show signs of oxidative stress, with the presence of carbonylated proteins and increased sensitivity to the oxidant agent tert-butyl hydroperoxide. Frataxin-depleted myocytes also present altered mitochondrial morphology and impaired lipid catabolism. We decided to explore the potential contribution of thiol modifications on these phenotypes. With this purpose the presence of reversible oxidized cysteine residues in control and frataxin-deficient NRVM was investigated using the fluorescent probe Bodipy-iodoacetamide and the reducing agent DTT. No increased content of oxidized thiol groups could be observed by analysis in one-dimesional SDS-PAGE. Instead, some bands presented a decreased content, suggesting the presence of thiol modifications not reversible by DTT. We also investigated the thiol redox state of VLCAD and HADHA, two enzymes involved in lipid catabolism, as lipid metabolism is compromised in frataxin-deficient myocytes. This was investigated using two thiol-reactive agents (iodoacetamide and AMS) and western blot. Again, we did not found significant differences between control and frataxin-deficient cells. These results indicate that although frataxin-deficient NRVM do not show a general increase in oxidized protein thiols, several proteins may experience thiol modifications not reduced by DTT.

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PROTEOMICS AND METABOLOMICS FOR MECHANISTIC INSIGHTS AND BIOMARKER DISCOVERY IN A CARDIOMYOCYTE MODEL OF FRIEDREICH ATAXIA

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Friedreich ataxia (FRDA) is a neurodegenerative rare disease caused by deficient frataxin expression. Frataxin is a mitochondrial protein that has been related to iron homeostasis, energy metabolism, and oxidative stress. Patients with FRDA suffer from cardiomyopathy, which is the leading cause of death. Using primary cultures of neonatal rat ventricular myocytes (NRVM) and RNA interference we have described that oxidative stress, mitochondria and lipid metabolism are primary targets of frataxin deficiency, with no signs of an alteration in iron metabolism. In an attempt to discover novel biomarkers and investigate the mechanism underlying these alterations, proteomic and metabolomics analysis were performed. Two-dimensional electrophoresis and nLC-MS/MS were used to analyze different protein expression in frataxin deficient NRVM. The metabolomic approach was carried out in a LC-QTOF. The preliminary results show that frataxin deficiency in NRVM induces a metabolic shift, with altered glucose and lipid metabolism together with an alteration of proteins involved in contraction, stress protection and signaling. Combining proteomics and metabolomics to quantify changes in metabolites and their corresponding enzymes will advance our understanding of pathophysiological mechanisms. Moreover, the identification of novel biomarkers could be favorable for diagnosis, prognosis and treatment of such a severe disease.

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NUCLEAR TRX1 AND TRXR1 ARE GLUTATHIONYLATED IN MOUSE FIBROBLAST CELLS

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Background: Glutathionylation has been considered as a cellular response to oxidative stress conditions. The importance of thioredoxin (Trx) and thioredoxin reductase (TrxR) to many aspects of cell function including protection against oxidant injury, and cell growth has been demonstrated.

Rationale and aims: Recent studies have shown that protein glutathionylation occurs under basal conditions, supporting its involvement in cell signaling and redox regulation of protein function. Our aim was to investigate whether Trx and TrxR1 modify their glutathionylation pattern in 3T3 mouse fibroblasts during the different phases of cell growth and its regulation by glutathione levels.

Methods: 3T3 cells were grown in the presence of 10 μM buthionine sulphoximine (BSO) or 100 μM diethyl maleate (DEM). Control cells were left untreated. For glutathionylation experiments, 6, 48 and 120 hr after seeding, cells were incubated with 40 mM N-ethylmaleimide (NEM) for 5 min; scraped off the plate and nuclear proteins isolated. Glutathionylated proteins were alkylated with NEM-biotin, purified by avidin-agarose columns and analysed by Western blot.

Results: DEM-treated cells showed a significantly lower growth than control or BSO-treated cells. Basal glutathionylation levels of TrxR1 were observed, supporting the importance of this post-translational modification in cell´s physiology. On the other hand, treatment of cells with 100
Trx1 glutathionylation above basal levels. No significant differences were observed in BSO-treated cells.

Conclusion: Since it is generally assumed that glutathionylation leads to inhibition of enzyme activity; DEM-induced TrxR1 and Trx1 glutathionylation could explain the differences observed in DEM-treated cell growth.

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SIR2 IS REGULATED BY GLUTATHIONYLATION AND REQUIRES GRX3 AND GRX4 TO PRESERVE ITS REDOX STATE

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Sir2, a NAD+-dependent histone deacetylase, plays an important role in DNA recombination, genomic stability, oxidative stress and aging in *S. cerevisiae*. Our work is focussed on Sir2 redox regulation and the regulatory role of nuclear Grx3 and Grx4. *In vitro* studies demonstrated that upon oxidative stress the deacetylase activity of Sir2 decreased, and was recovered with reducing agents. By western blot, we showed that Sir2 became S-glutathionylated upon diamide stress. Using site-directed mutagenesis we proved that Cys363 (next to the catalytic His) and Cys 372 (involved in Zn binding) are essential for its deacetylase activity. Moreover, Cys513, located at the entrance to the active site pocket is the most reactive cysteine and involved in conjugation with glutathione upon stress.
In vivo, Grx3 and Grx4 (but not cytosolic Grx2 or mitochondrial Grx5) are the physiological enzymes involved in protecting and restoring the reduced state of Sir2 after oxidative stress. Supporting these results, a physical interaction between Sir2 and Grx3 and Grx4, was observed by co-immunoprecipitation experiments. Currently we are working on the *in vivo* Sir2 activity under oxidative stress conditions and the effect of Grx3 and Grx4. In addition, we are studying how the redox modifications of the protein can affect its interaction with the silencing complex Sir2/3/4 and its consequences on cell metabolism.