Oxygen Club of California World Congress 2015

"*Oxidants and Antioxidants in Biology*"

June 24-26, 2015, Valencia, Spain

Joint Meeting with

The Spanish Group for Research on Free Radicals,

The Portuguese Group of Free Radicals,

Le Société Française de Recherches sur les Radicaux Libres.

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http://www.rosvalencia.eu/

http://www.oxyclubcalifornia.org/

Meeting Notes by **John D. Furber**

Introduction: This meeting was organized by

Juan Sastre , Professeur Josiane Cillard, and **João Laranjinha .**

There were nearly 200 posters presented during the poster sessions. Posters remained up all 3 days in the coffee-break area, so that there was ample opportunity for contemplation and discussion.

The **OCC** was founded in 1994, and has been organizing meetings in California and Europe.

The Advisory Board for the meeting comprises Lester Packer, Enrique Cadenas, and Helmut Sies.

Abstracts of all talks and posters are in the **296 page conference book**, which is available as a **PDF download** on the OCC website. In addition, many photographs from the meeting are available for viewing and download on the OCC website. **http://www.oxyclubcalifornia.org/**

More information on the work of any presenter can be found by searching PubMed or Google Scholar or Scirus, and reading their papers. Consequently, my notes here are not a comprehensive chronicle, but rather a sketchy introduction to people and ideas that you might wish to investigate further.

For related upcoming meetings, please see http://www.oxyclubcalifornia.org
Oxygen Club of California World Congress on "***Redox Medicine and Nutrition***",
May 4-6, **2016** at the University of California Davis
http://www.oxyclubcalifornia.org/OCC2016/

Highlights of the Meeting

(More details on these excerpts are found further down in the extended notes of each talk, and in the PDF Program/Abstracts book, which can be downloaded from www.oxyclubcalifornia.org/*)*

Bharat Aggarwal: Prooxidant and anticancer potential of spice-derived nutraceuticals. Cancer incidence is less in spice-consuming countries. Chemotherapies increase ROS to kill cancer cells, but they also increase NFκB. However NFκB increases cancer. Curcumin increases ROS without increasing NFκB.

Stress ==> inflammation ==> NFκB ==> cancer.

NFκB is blocked by black cumin, fenugreek, ginger, fennel, black pepper, long pepper (piper longumine. Nature 2011), cardamom (cardamonin), turmeric (curcumin). However, antioxidants N-acetylcysteine and GSH partially abrogate the suppression of NFκB by curcumin. Curcumin is antibacterial, antiviral, antifungal. Curcumin is even effective against HIV. Turmeric and curcumin are good for osteoarthritis, Uveitis, psoraisis, heart hypertrophy.

Fawaz G. Haj is testing a new kind of drug that might be helpful in treating type-2 diabetes.

Mulchand S. Patel, **Benefits of alpha-lipoic acid and caloric restriction in obesity.** Caloric restriction and dietary supplementation with alpha-Lipoic acid can protect against obesity and type-2 diabetes.

Balz Frei: **Lipoic acid supplementation promotes weight loss, improves body composition, and decreases biomarkers of inflammation and lipid peroxidation in obese adults.** R-α-Lipoic-acid may reduce the risk of CVD in overweight adults.

Irfan Rahman: We have recently shown that defective mitophagy (autophagy of mitochondria) and impaired mitochondrial biogenesis are associated with stress-induced cellular senescence. Mitochondrial elongation is associated with cellular senescence. This affects nuclear genes. Inhibiting mitophagy can cause cellular senescence. A mitochondria-targeted antioxidant (Mito-Tempo) restored impaired mitophagy, decreased mitochondrial mass accumulation, and delayed cellular senescence in Parkin-overexpressing cells.

José Viña: Genistein (found in soy) clears amyloidβ from cells and lowers plaque number in brain of rodent Alzheimer's model. We found that treatment of an Alzheimer's mouse model with genistein results in a remarkable and rapid improvement in various parameters of cognition, such as hippocampal learning, recognition memory, implicit memory, and odor discrimination. This is associated with a lowering of Aβ levels in brain, in the number and the area of amyloid plaques as well as in microglial reactivity. Genestein crosses the BBB.

Corinne Spickett: Targeted Mass Spectroscopy also allows identification of chain-shortened fragment patterns that would be hidden in the noise of non-targeted MS. They are using this technique to study oxidized phospholipids.

Leocadio Rodríguez-Mañas: There is decrease in muscle mass with age. But also change in the energetics of the remaining muscle. The muscle mitochondria produce less energy. For older people, 3-10 days of bed rest in hospital loses a dangerously large fraction of muscle mass. This is very difficult to regain, if it is regained at all. Hormones are involved in frailty: IGF1, Cortisol, Vitamin D. An exercise program with resistance training is very effective in reversing frailty. Good nutrition can help, along with exercise, but the resistance, strength-training exercise is essential. At least 80% of older people can do resistance training, but only about 20% can do aerobic exercise. Resistance strength training, twice/week, shows results in 6-10 weeks in elderly frail people. Many elderly frail have weakness in quadriceps, so they are unsteady on their feet. Also, it is important to work with balance, and if possible, also aerobic exercise.

Michael Murphy: Origin of mitochondrial ROS in ischemia reperfusion injury: Mitochondrial ROS have long been known to contribute to damage in conditions such as ischaemia-reperfusion (IR) injury in heart attack and stroke. We have developed a mitochondria-targeted S-nitrosating agent, called **MitoSNO**, that we showed was effective in preventing ROS formation in IR injury with therapeutic implications/

Catarina Oliveira, **Amyloid-ß disrupts calcium and redox homeostasis in brain endothelial cells.** Amyloid β-protein (Aβ) has been shown to accumulate in the brain of Alzheimer's patients, in senile plaques. But Aβ levels alone are not a reliable predictor of cognitive decline. Evidence exists showing that cerebrovascular function is altered in AD, often preceding the onset of cognitive impairment, contributing to neurodegeneration, and playing a major role in AD pathogenesis. Endothelial cell damage occurring in AD is mediated through the induction of ER-UPR stress. This led to a decrease in proteasome activity, promoted the accumulation of ubquitinated proteins and the impairment of the autophagic flux, culminating in endothelial cells apoptosis.

Giuseppe Poli: Hypercholesterolemia is a major risk factor for AD. Obesity is a PRIMARY risk factor for Alzheimer's.

Kelvin Davies: Lon protease selectively degrades oxidized proteins in the mitochondria. We see the damage accumulate in the last 1/3 of life. Why don't damage repair systems work then as well as they do in the first 2/3? Is it a cause or effect of aging? Lon levels decreases in mouse muscle in old age. In cell culture, Lon levels decline with senescence. If you turn Lon expression down by 70%, you see significantly reduced mouse lifespan. However, overexpression of Lon does not appear to extend lifespan. What are the effects of age dependent loss of Lon? Can we model this? All 4 ETC complexes get decreased in activity.

Posters

Almost 200 posters were on display during the 3 days of the conference. All poster abstracts are available for PDF download in the Program/Abstracts book on www.oxyclubcalifornia.org/
A few of the posters can be seen in photographs on the web site. Below are a couple of interesting posters:

Poster 60: J147 attenuates the metabolic profile of aging and Alzheimer's disease-related pathology in senescence-accelerated SAMP8 mice

Currais, Antonio[1]; Goldberg, Joshua[1]; Farrokhi, Catherine[1]; Prior, Marguerite[1]; Dargusch, Richard[1]; Quehenberger, Oswald[2]; Maher, Pamela[1]; and Schubert, David[1].
[1]The Salk Institute for Biological Studies, Cellular Neurobiology (CNB-S), U.S.A.; and [2]University of

California San Diego, Department of Medicine, U.S.A.

Phenotypic screens based upon old age-associated brain toxicities were used to develop the potent AD drug candidate J147. J147 not only reduced the cognitive deficits and associated metabolic changes observed in old SAMP8 mice, it restored the levels of multiple markers of AD, vascular pathology, synaptic function, and inflammation to those approaching the young phenotype. Our data show that a drug candidate selected upon the basis of preventing old age-related brain toxicities also reduces AD-associated pathology.
[They are preparing for IND to start human clinical trials soon. It is orally bioavailable, crosses the BBB well, and has minimal side-effects.]

Poster 42: Novel inflammatory biomarkers revealed from proteomic analysis of TGRL lipolysis product-induced exosomes from human brain microvascular endothelial cells

Nyunt, Tun[1]; Aung, Hnin H.[1]; Herren, Anthony[2]; Phinney, Brett S.[2]; Wilson, Dennis W.[3]; and Rutledge, John C.[1]. *[1]University of California at Davis, Internal Medicine, USA; [2]University of California at Davis (UC Davis Genome Center); and [3]University of California at Davis, Pathology, Microbiology and Immunology.*

The traditional methodology for clinical diagnostics is the measurement of soluble markers in plasma/serum. Many target proteins in clinical diagnostics may not be truly soluble, but may instead be released on exosomes. Therefore, we employed LC-MS/MS quantitative proteomics to identify the novel biomarkers of neurovascular inflammation from isolated exosomes. Thus, exosomal proteomes may represent novel diagnostic tools to determine the state of neurovascular diseases.
[Dr. Nyunt was formerly a Postdoc with Lester Packer.]

Poster 40: Lipid-Induced oxidative stress and inflammation in brain microvascular endothelial cells

Aung, Hnin H.1; Nyunt, Tun1; Budamagunta, Madhu2; and Wilson, Dennis W.3.
1University of California at Davis, Internal Medicine; 2University of California at Davis, Biochemistry and Molecular Medicine, USA; and 3University of California at Davis, Pathology, Microbiology and Immunology.

Elevation of blood triglycerides, primarily triglyceride-rich lipoproteins (TGRL), is an independent risk factor for atherosclerotic cardiovascular disease. The accumulating evidence indicates that the development of atherosclerosis and vascular dementia are linked to vascular inflammation.
[A diet containing cheese, or high in fats, causes leaks in the BBB, dementia, and pro-inflammatory signaling!]

Conversations, Comments, & Discussion

Prof Michael J. Davies, *Past President SFRR, Editor of Free Radical Research. Dept of Biomed Sci, U of Copenhagen.*
He studies **ECM protein aging**. We discussed my interest in accelerating the turnover and repair of damaged ECM proteins and structure. I suggested that perhaps fibroblasts could be engineered to more rapidly do this job.

Joan Cook-Mills, *Northwestern University School of Medicine, Allergy/Immunology Division, USA.*
Vitamin E benefits or harms depend upon the isoform. She tested this in allergic mouse mothers (See poster P21). Supplementation with purified alpha-tocopherol is beneficial, but gamma-tocopherol is harmful. Unfortunately, many Vitamin E supplements that are labeled as alpha-T are encapsulated in soy oil, which contains enough gamma-T to cause harmful effects. This is the reason for some papers, which erroneously reported that alpha-T is harmful. The best oil for food or cooking is olive oil. The worst oils are soy, corn, and

canola, because they have high levels of gamma-T.

Panel Discussion on Aging and Longevity

Discussion Leader: **Michael Murphy**, *MRC, Mitochondrial Biology Unit, Cambridge, UK*
Panel Members: (Speakers of the Aging and Longevity session) **Tilman Grune, Bertrand Friguet, Gaetano Serviddio, Leocadio Rodríguez-Mañas, Malcolm Jackson**

John Furber: A few recent studies have shown that consumption of antioxidant supplements can abrogate the muscle hypertrophy effects of muscle strength training. I wonder... since ROS generated during exercise may last for only a fraction of a second to send its beneficial signals... but the antioxidant supplements raise the blood level for hours... could there be an optimal timing of consuming the antioxidants? Could it be more beneficial to take anti oxidants after exercise, when they could not quench the ROS signals?
A: Some of those studies were small samples, and of variable quality. There can be many benefits of exercise, in addition to muscle hypertrophy. For example, cardiovascular health and balance improve with exercise.
Lester Packer: Race horses, in order perform well for 2 minutes in a race, they have to do a lot of endurance training.
Dean Jones: Exposure memory. Adaptability.
Antonio Currais: Should we consider aging to be a disease?
Leocadio Rodríguez-Mañas: The only way to avoid aging is to die. When you age, your body performs in a different way. I do NOT consider age-related frailty to be a "disease". Menopause is not a "disease". Pregnancy is not a "disease".
Antonio Currais: How do you prevent age-associated diseases if you don't treat "aging" in advance?
Malcolm Jackson: In many ways, aging IS a disease. Aspects of aging are diseases. Or at least in order to develop treatments financially, we must call those aspects "diseases" in order to get the treatments approved.
Kelvin Davies: Aging is NOT a disease. You can change longevity by changing signaling gene expression, at least in short-lived models. We don't know about people yet.
Morshen Patel: Healthy aging depends on lifestyle and early developmental processes.
Tilman Grune: It seems healthiest to keep dietary protein to < 22%.
Leocadio Rodríguez-Mañas: The "secret" of the Mediterranean diet is not the protein level; it is the pleasure.
Tilman Grune: Also important is the timing of eating, as well as how much is eaten.
Michael Murphy: Dietary advice? a) younger people for healthy old age? b) diets for people already old?
Q: alpha tocopherol is an antagonist of PKC, while gamma tocopherol is a protagonist of PKC. So there is more to the situation than simply antioxidants.
Dean Jones: Protein intake and GSH. The relationship to the sulphur amino acid intake. Methionine (Met) restriction is very interesting. Most Met comes from animal protein. This might be fundamental. We need to look at this carefully.
Jarrow Rogovin: There are premature aging conditions. We say they are diseases. So we are uncomfortable with calling normal aging to be a disease. There are many compounds that are not readily available in normal foods, eg. pterostilbene, CoEnzyme Q10. So the statement, "Just get it from food" is not possible. DSEA is a modern "Folk medicine".
Tilman Grune: The question is misleading whether aging is a "disease". Definitional. We don't call high blood pressure a "disease" but it can lead to "disease". Some of this is related to health care systems and payment processes.

Leocadio Rodríguez-Mañas: Sedentaryness is not the same as not exercising. eg if you run 2 hours per day (lots of exercise), but then you sit for 12 hours in front of a computer or TV (lots of sedentary time), then you don't have such benefit from the exercise.

Extended Notes of the Talks

(More details on these talks are found in the PDF Program/Abstracts book, which can be downloaded from www.oxyclubcalifornia.org/*)*

Opening Ceremony with the Rector of the University of Valencia

Opening Keynote Lecture
Chair: **Helmut Sies**, *Heinrich-Heine-Universitaet Duesseldorf, Germany*
Dr. Sies introduces the first speaker.

(L1) **Physiology and pathophysiology of nitric oxide and mitochondrial interactions**
Salvador Moncada, *Director of Cancer Sciences, Wolfson Institute for Biomedical Research, University College London, UK*

Abstract: Nitric oxide (NO) inhibits cell respiration reversibly and in competition with O_2 through the inhibition of the mitochondrial cytochrome c oxidase (Complex IV). At concentrations lower than those required to inhibit respiration, endogenous NO enhances the reduction of the electron transport chain, thus enabling cells to maintain their O_2 consumption. This facilitates the release of superoxide anion, which initiates the transcriptional activation of NF-kB as an early signal of a stress response.

Notes: A Historical perspective of Nitric Oxide and Oxygen interactions.
Endothelium derived vascular relaxing factor released from endothelial cells (1986 Nature 320: 454-456 Gryglewski, Palmer, Moncada).
Relaxation of rabbit aortae by EDRF and NO. (Palmer. Nature 1987 327:524-526)
They identified EDRF is the same as NO.
Cytochrome c oxidase (= complex IV in mitochondrial ETC) is the site of interaction of O2 and NO. Because this reaction is reversible, it could be a regulatory mechanism. (Cleeter 1994 FEBS Lett 345:50-54)
Working with cells at 21% oxygen does not give normal physiological regulation.
They had to develop their own specialized equipment for visible light spectroscopy at their desired oxygen/redox conditions.
At 3% O2, NO signaling releases superoxide free radicals (and probably H2O2), which activate NF**κ**B. (This does not occur at 21% O2.)
This represents an early warning system of cell stress. 3% is normal physiological O2 concentration; it is not hypoxic.
HEK293 cells treated w tetracycline and L-Arg, they release NO.
HIF-1α accumulates in low NO conditions. (Hagen)
The inhibitory effect of NO on mitochondrial respiration is initially reversible by hemoglobin (First hour). But after 5 hrs, the cells cannot breathe anymore, and must have antioxidants, such as glutathione. Persistent
S-nitrosylation is a result of oxidative stress.
Complex I dynamically changes between active and de-active forms.
Reversible S-nitrosylation of a cysteine switch on mitochondrial complex I is cardioprotective in reperfusion of cardiac tissue.

HIF-1α
Very high concentration of NO can prevent cancer by cytotoxicity and cytostasis, whereas low concentrations of NO promote cancer via angiogenesis.
Kelvin Davies: A lot of your experiments are at 3% O2, which is physiological, so it appears that we are constantly on the border of stress/non-stress.
Moncada: We should culture the cells at 3%. Then we can reduce below 3% in order to see physiological hypoxia.

10:15 – 11:00 h. Coffee break and Poster Session I

11:00 - 13:00 h. Session I. Nutrients, Oxygen Biology, and Medicine
Chairs: **Leopold Flohé**, *University of Padova, Molecular Medicine, Italy*
Guillermo Sáez, *University of Valencia, Spain*

(L2) **Prooxidant and anticancer potential of spice-derived nutraceuticals and their role in cancer**
Bharat Aggarwal, *Cytokine Research Laboratory, Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, USA*

Abstract: Chronic infections, obesity, alcohol, tobacco, radiation, environmental pollutants, and high-calorie diet have been recognized as major risk factors for the most common types of cancers. All these risk factors are linked to cancer through inflammation. While acute inflammation that persists for short-term mediates host defense against infections, chronic inflammation that lasts for long-term can predispose the host to various chronic illnesses, including cancer. Extensive research within the past half-century has indicated that curcumin (diferuloylmethane), a yellow pigment in curry powder, exhibits antioxidant, anti-inflammatory, and proapoptotic activities. Also, the direct proapoptotic effects of curcumin were inhibited by glutathione and potentiated by depletion of intracellular glutathione. Further, N-acetylcysteine partially reversed the effect of curcumin.

Notes: Cancer incidence is less in spice-consuming countries. Inflammation, when under control, is beneficial. But when out of control, inflammation is harmful. A major factor is activation of NFκB. There are > 500 genes regulated by NFκB, which play roles in inflammation. TNF is regulated by NFκB.
When someone has cancer, NFκB is already active. Chemotherapies increase ROS to kill cancer cells, but they also increase NFκB. One the other hand, curcumin increases ROS without increasing NFκB.
Most cancers begin at age ~20, but manifest at age ~40~50.
There is a lot of crosstalk between NFκB and other transcription factors, so single target pharmaceuticals are circumvented. But phytonutrients have multiple targets.
Cigarette smoke raises NFκB for 3 days.
Stress ==> inflammation ==> NFκB ==> cancer.
The beneficial, anti-inflammatory lifestyle is enhanced by: spices, ayurvedic medicine, Chinese medicine.
NFκB is blocked by black cumin, fenugreek, ginger, fennel, black pepper, long pepper (piper longumine Nature 2011), cardamom (cardamonin), turmeric (curcumin) (JBC Aggarwal).
Curcumin is also a chemo sensitizer. Curcumin is also found in saffron, as well as turmeric. Curcumin has many targets. Curcumin is 140x more potent than vitamin E.
GSH abrogates suppression of NFκB by curcumin.

More than 65 clinical trials have been completed and more than 35 clinical trials are under way for curcumin for many disease conditions. (One of the first clinical trials was published in Lancet, March 1937).
1.5 g of turmeric orally/day for 30 days reduced mutagens in the urine of smokers.
Turmeric and curcumin are good for osteoarthritis, Uveitis, psoraisis, heart hypertrophy.
Q: How does piperlongumine interact with cysteine
Q: Is there data on curcumin on infections?
Aggarwal: It is antibacterial, antiviral, antifungal. Curcumin is even effective against HIV.
Q: What is the effect of curcumin on NFκB in immune cell and its effect on leukocyte function.
Aggarwal: Curcumin enhances the effectiveness.

(L3) **High fat diet-induced obesity and insulin resistance in C57BL/6J mice**
Fawaz G. Haj, *Professor, Dept of Nutrition, University of California Davis, USA*

Abstract: Diabetic mellitus (DM) nephropathy is a serious problem that causes renal failure. Soluble epoxide hydrolase (sEH; encoded by *Ephx2*) deficiency and pharmacological inhibition have beneficial effects in kidney function.

Notes: They are treating diabetic nephropathy.
The human population increase in obesity correlates with increase in type 2 diabetes.
podocyte-specific sEH deletion. sEH = soluble Epoxide Hydrolase
Kidneys can clear glucose from the blood via the urine. This can be helpful in reducing blood glucose levels. Some drugs enhance this effect.
They see increased sEH expression in podocytes with high fat feeding and hyperglycemia-- a mouse model of diabetes.
They engineered pod-sEH-KO mice. They had improved insulin sensitivity and enhanced glucose tolerance. Also improved blood pressure and attenuation of high glucose induced renal injury. They conclude that sEH inhibition might be a viable therapy to reduce kidney damage from type 2 diabetes and obesity.
Q: Which inhibitors of sEH are being used?
Haj: GSK is trying sEH inhibitors for COPD.
Q: What is the contribution of autophagy in these mice. Did you try to inhibit autophagy, such as with chloroquine?
Haj: We are starting some studies with autophagy enhancers.

(L4) **Procyanidins can interact with Caco-2 cell membrane lipid rafts: involvement of cholesterol**
Patricia Oteiza, *Dept of Nutrition, University of California at Davis, USA*

Abstract: Intestinal epithelial cells (IEC) membrane lipid rafts can be the target of bioactives that, although not absoved by cells could exert biological actions through their interactions with the cell membrane. Large procyanidins (PAC), oligomers of the flavan-3-ols (-)-epicatechin and ( )-catechin, are not absorbed by IEC but interact with lipid rafts and through these interactions promote beneficial biological effects.

Notes: (-)-Epicatechin and related procyanidins are found in cocoa and other plant products. Polymers of epicatechin can be 2-12 monomers long. These large procyanidins are not very absorbable, so, do they have a beneficial effect in the human GI tract?
They used Caco-2 cells for their tests. Procyanidins protect them from bile acid- and oxidant- induced damage. (FRBM 2006) (DOC = deoxycholic acid)

These polymers interact with membranes and lipid rafts, but without entering the cells.
The procyanidin hexamers inhibit EGF activation of EGFR, which is floating in lipid rafts.
Possibly also the hexamers might promote ubiquitinylation and degradation of EGFR.
Diet rich in procyanidins may be beneficial in diseases of intestinal permeabilization.
Q: Do you think that mitochondria are involved in these effects?
Oteiza: Many of these plant substances are metabolized in the GI tract to smaller compounds before they can reach the cells. So even the monomers are unlikely to reach the mitochondria.
Q: Do these compounds reach the dendritic cells in the intestine?
Oteiza: We have not looked.

(L5) **Benefits of alpha-lipoic acid and caloric restriction in obesity**
Mulchand S. Patel, *University of Buffalo, Buffalo, New York, USA*

Abstract: The findings show the importance of nutrition throughout the life course in programming early and adult-onset obesity and highlight the application of caloric restriction and LA supplementation as effective means to protect against obesity. In another experiment with adult Zucker male rats, dietary alpha-lipoic acid supplementation (LA, 0.25% w/w) protected against high fat diet-induced weight gain, aberrant responses in plasma lipid and lipoprotein profile, and hepatic triglyceride infiltration by altering gene expression that regulate fatty acid synthesis and oxidation.

Notes: Obesity has complications: type 2 diabetes, insulin resistance, etc.
Mal-programming of the pancreatic beta cell and hypothalamic neurons in early life contribute to development of adult onset obesity.
CR helps to normalize the body weight of the obese Zucker rats.
α-Lipoic-acid provides many of the same benefits as CR.
Put Zucker rats on 0.5% or 0.25% α-Lipoic-acid in their chow. This resulted in reduced particle size of VLDL and HDL. Reduced HMG-CoA reductase mRNA.
Increased LDL recptor protein for enhanced cholesterol clearance.
Lowered PCSK9 mRNA in liver.
Q: Did you use the R- or R,S- isomers of α-Lipoic-acid?
Patel: They work with the R- isomer of α-Lipoic-acid. They did not use the racemic R,S-
John Furber: What human dose would be appropriate?
Patel: No suggestions.

Session II. Redox Signaling in Inflammation
Chairs: **César Fraga**, *University of Buenos Aires-CONICET, Argentina*
José María Mato, *CIC bioGUNE, Bizkaia, Spain*

(L6) **Lipoic acid supplementation promotes weight loss, improves body composition, and decreases biomarkers of inflammation and lipid peroxidation in obese adults**
Balz Frei, *Linus Pauling Institute, Oregon State University*

Abstract: We have shown previously that α-lipoic acid inhibits atherosclerosis, body weight gain, and vascular inflammation in apoE$^{-/-}$ and apoE$^{-/-}$ LDLR$^{-/-}$ mice. Further, α-lipoic acid induces Nrf2-mediated gene expression of antioxidant enzymes and inhibits the age-related increase in lipid peroxidation in rats.

Notes: Tory Hagen was a postdoc in Bruce Ames' lab. Rats on α-Lipoic-acid increased their physical activity, which might contribute to weight loss in obesity.
α-Lipoic-acid accentuates circadian rhythm expression profiles. α-Lipoic-acid decreases

hepatic lipogenesis. α-Lipoic-acid inhibits formation of atherosclerosis in mice with genes to cause atherosclerosis.
In a Clinical trial, double blinded 600 mg/day R-α-Lipoic-acid vs placebo for overweight or obese humans with plasma triglycerides >100 mg/dL, 18-60 yrs old, non smoking, CRP<10 mg/dL.
There was a significant decrease in body weight, and fat mass, especially for women.
Lipid peroxidation was decreased by R-LA treatment, as assessed by urinary concentrations of total F2-isoprostanes
Some subjects had heartburn. They took the α-Lipoic-acid in the morning on an empty stomach. Next time, they will advise subjects to take α-Lipoic-acid with a meal. Seven subjects noted a strong smelling urine, due to the sulfur compounds from α-Lipoic-acid.
They conclude that R-α-Lipoic-acid may reduce the risk of CVD in overweight adults.
Q: Did they exercise more?
Frei: The α-Lipoic-acid group did not exercise more. They consumed 200 Cal less per day.
Lester Packer: α-Lipoic-acid is rapidly metabolized (turned over). 600 mg is a lot of metabolites.
Frei: We did not look at metabolites.
Lester Packer: Some of the metabolites have effects of their own.
Frei: But even the metabolites don't stay around very long.

(L7) **GSH in cholestatic liver injury**
Shelly Lu, MD, *Div of Gastroenterology, Cedars-Sinai Medical Center, Los Angeles, USA*

Ursodeoxycholic acid (UDCA) is the only FDA approved medication for the treatment of primary biliary cirrhosis, but it doesn't work for 40% of patients.
Expression of GSH-synthetic enzymes declines in CLI.
UDCA can increase the production of GSH-synthetic enzymes: GCL and GS. GCL is the rate limiting enzyme.
Regulators of ARE-mediated gene expression: Nrf2 and Jun and small Mafs bind to the ARE (Antioxidant Response Element, a region of DNA).
What are the therapeutic options for CLI? They looked at UDCA with or without SAMe. Each is protective, but together, they are much better.
PHB1 pos regs Nrf2 and GCL subunits.
Therapeutically, both S-adenosylmethionine (SAMe) and UDCA are protective but work better when combined and target all of the key mechanisms.

(L8) **Sirt1 protects against cigarette smoke-induced lung oxidative stress via a FoxO3-dependent mechanism**
Irfan Rahman, PhD, *Professor, Dept of Environmental Medicine, University of Rochester Medical Center, New York, USA*

There are 2 types of cellular senescence:
1) telomere shortening
2) Campisi premature senescence. Cells secrete inflammatory mediator signal chemicals.
SASP = senescence associated secretory phenotype.
Smoking induces SASP in the cells lining the airways. This leads to COPD and inflammation.
SIRT1-FOXO3-mediated genes. SIRT1 regulates cellular senescence via regulating FOXO3, p53, and NF-κB, oxidative stress (antioxidant genes) as well as various proteins/coactivators involved in DNA damage and repair.

The Mitophagy pathway (autophagy of mitochondria) becomes activated when the mitochondrial membrane depolarizes. (Hattori J B Cell Biol 2014).
We have recently shown that defective mitophagy and impaired mitochondrial biogenesis are associated with stress-induced cellular senescence. Impaired mitophagy and perinuclear accumulation of damaged mitochondria associated with cellular senescence occur in both human lung fibroblasts and small airway epithelial cells (SAECs) by cigarette smoke.
Mitochondrial elongation is associated with cellular senescence. This affects nuclear genes.
Inhibiting mitophagy can cause cellular senescence.
Airway epithelial cells from COPD patients exhibit reduced Parkin translocation to mitochondria.
A Mitochondria-targeted antioxidant (Mito-Tempo) restored impaired mitophagy, decreased mitochondrial mass accumulation, and delayed cellular senescence in Parkin-overexpressing cells.
In conclusion, defective mitophagy leads to cigarette smoke stress-induced lung cellular senescence, and restoring mitophagy delays cellular senescence, which provides a promising therapeutic intervention in chronic airway diseases.
Q: If you think that mitochondrial elongation caused by smoking is responsible for SASP, could you try to induce mitochondrial fission to fix it?
Rahman: We see PINK1, so we believe that fission is happening.
We use MDIB

(L9) **Redox regulation of FOXO transcription factors**
Tobias B. Dansen, *University Medical Center Utrecht, Molecular Cancer Research, The Netherlands*

Long-lived daf2 mutant C. elegans have greater resistance to oxidative stress.
FOXO regulates resistance to oxidative damage. The forkheadbox O (FOXO) family of transcription factors regulates a variety of cellular programs, including cell cycle arrest, reactive oxygen species (ROS) scavenging, and apoptosis, and are of key importance in the decision over cell fate.
H2O2 treatment of cells can sometimes cause FOXO to move from cytoplasm to nucleus.
Cysteines in FOXO transcription factors become oxidized in response to redox signaling and this leads to the formation of highly specific intermolecular disulfide bondsROS (R'-cys-SS-cys-R)
Does this regulate FOXO? ROS-induced Lysine acetylation on FOXO depends on the formation of an intermolecular disulfide with the p300 or CBP acetyltransferases. Furthermore, nuclear shuttling of FOXO is triggered by a shift in the cellular redox state towards more oxidizing conditions and this depends on intermolecular disulfide formation between FOXO and nuclear import receptors
FOXO3 and FOXO4 have different cysteines, so maybe they have different binding partners. P300 is an important binding partner for FOXO.

(L10) **Oxidized phospholipids and cellular signaling in inflammation**
Corinne Spickett, *(School of Life and Health Sciences), Aston University, Birmingham, UK*

Abstract: Oxidized phospholipids (oxPLs) can be produced by attack of free radicals and reactive oxidizing compounds on unsaturated lipids, for example in inflammatory processes where phagocytic cells are activated. Esterified oxPLs, as well as small reactive breakdown products derived from them, have a variety of biological effects, many of which are considered detrimental. These include enhancing ROS production, monocyte-endothelial adhesion, and proliferation and differentiation of SMCs. There has been much interest in defining the receptors and cell signalling mechanisms that contribute to these effects. oxPLs can be considered as "damage-associated

molecular patterns" and interact with several immune receptors, including toll-like receptors. The outcomes can be inflammatory or anti-inflammatory, depending on the oxidized phospholipid, cell type and concentration. Therefore to understand the contributions of oxPLs, it is necessary to have sensitive and specific methods of identifying different oxidized species. Mass spectrometry is a powerful technique for analysing both native and oxidized phospholipids, but the large number of different species present in biological and clinical samples presents a substantial challenge. LDL contains more than 350 different native lipid species, and cell membranes are similarly complex; oxidation further increases the complexity. Targetted or semi-targetted MS approaches can facilitate identification of individual oxPLs or groups of oxidation products and have been applied to analyse phospholipid oxidation in chronic conditions such as diabetes and obesity. Products commonly observed included the reactive products POVPC and PONPC, which are able to generate adducts with proteins by lipoxidation. This is thought to be an additional mechanism by which oxPLs can influence cell signalling in pathophysiology.

Notes: Targeted MS also allows identification of chain-shortened fragment patterns that would be hidden in the noise of non-targeted MS.
Q: Esterified vs non-esterified FA, which dominate?
Spickett: There are higher levels of the free non- esterified FA, but the situation is variable.
We take samples with BHT and EDTA to keep iron from oxidizing the sample, and then freeze as soon as possible.

(L11) Ca^{2+}-independent biosynthesis of pro- and anti-inflammatory lipid mediators from mitochondrial cardiolipins

Valerian E. Kagan, *Center for Antioxidant Health, University of Pittsburgh, USA*

Abstract: The central role of mitochondria in metabolic bioenergetics, metabolic reactions and cell-death mechanisms requires sophisticated and diversified signaling. Essential in this signalling process is an array of lipid mediators derived from polyunsaturated fatty acids. However, the molecular machinery for the production of oxygenated polyunsaturated fatty acids is localized in the cytosol.

Notes: The Metabalome comprises 240,000 compounds, of which 140,000 are the lipidome. Cardiolipin forms curvature in membranes, such as mitochondrial membranes. It is a switch. Cardiolipin (CL) can end up in lysosomes, as a result of phagocytosis. Externalization of CL triggers phagocytosis. CL gets externalized when the molecule is damaged (eg oxidized).
Cytochrome-c (cyt-c) molecule sticks to CL in the membrane, and can oxidize the CL.
Q: Are other CL-interacting proteins taking a role?
Kagan: That's an important question. There are probably 60 or 70 proteins that have some affinity for CL.

(L12) Biochemical mechanisms of mitochondrial SOD nitration and inactivation: pathological relevance

Rafael Radi, *Department of Biochemistry and Center for Free Radical and Biomedical Research, Facultad de Medicina Universidad de la República, Montevideo, Uruguay*

Mn-containing superoxide dismutase (MnSOD) is an essential antioxidant enzyme of the mitochondrial matrix in mammalian cells. Under inflammatory conditions that enhance nitric oxide (ŷNO) formation, MnSOD is tyrosine nitrated and inactivated .
Peroxynitrite formation in mitochondria.

hMnSOD has 9 Tyr residues per monomer. The inactivation of MnSOD *via* tyrosine nitration requires the exclusive modification of only one out of nine tyrosine residues present in mammalian MnSOD, namely Tyr34. This tyrosine is located in the entrance channel to the active site, only 5 A away from the Mn atom. These studies may provide clues for protein engineering of MnSOD variants resistant to peroxynitrite.
Q: Can you eliminate another explanation?
Radi: That is a pending possibility to solve. I need to think how to solve this experimentally.

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

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

Alternative pathways to produce nitric oxide. NO* is rapidly oxidized to nitrate and nitrite (NO_3^- and NO_2^-). NO formation by the cells was found to be pH, nitrite, and dioxygendependent

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

Glutamine synthetase (GS) is a key metabolic enzyme that catalyzes the ATP-dependent synthesis of glutamine from glutamate and ammonia. In the nervous system, it is mainly located in the cytosol of astrocytes, playing an important role in ammonia detoxification and prevention of glutamate-dependent excitotoxicity. Hu GS is inactivated by peroxynitrite (ONOO-). They also found covalent crosslinking, probably di-Tyrosine.

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

The optimized omega-3 formulation EPA:DHA 6:1 has been shown to be a potent inducer of endothelium-dependent nitric oxide (NO)-mediated relaxations in isolated arteries. This study was done in rats. Omega 3 PUFAs protect the cv system. Patients after cardiac infarct are 33% less likely to have repeat events if they consume omega-3. EPA and DHA are the two important active PUFAs. EPA:DHA in 6:1 ratio seems to be the most effective.

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

Beate; and Roob, Johannes M. *University of Graz, Austria.*

To identify and characterize robust biomarkers predictive of healthy metabolic phenotype during aging. To assess health claims made for food. Subjects completed a 240 item food questionnaire. Blood plasma was tested for vitamins, phyto-compounds, and markers of oxidative stress. They found correlation between blood levels of healthy compounds, and consumption of healthy plants.

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

This study was done with rat islet cells in cell culture.

19:30 h. General Assembly of the Spanish Group for Research on Free Radicals (GEIRLI)

25th June 2015

Session III. Aging and Longevity
Chairs: **Regina Brigelius**, *University of Potsdam, Germany*
Enrique Cadenas, *University of Southern California, Los Angeles, USA*
Regina: The OCC has created a network of friends around the planet.

(L13) **Redox biology: Where exposome meets genome**
Dean P. Jones, *School of Medicine, Emory University, Atlanta, USA*

Exposome vs Genome:
Cumulative lifelong environmental exposures are defined as the exposome. NAD and NADP systems function at near equilibrium with energy precursors to maintain bioenergetics, metabolic organization and defense. NAD and NADP are used to generate and metabolize H_2O_2, maintaining a kinetically controlled structural organization system linked to bioenergetics and metabolism through redox switches in the proteome. Activation/deactivation cycles of H_2O_2 generation support spatiotemporal sensing and signaling in molecular function, tissue differentiation and response to exposures. Importantly, metazoans evolved genetically encoded exposure memory systems to enhance survival and reproductive potential in the O_2-rich atmosphere by providing mechanisms for an individual to adjust during lifespan to environmental resources and challenges. Activation of differentiation and exposure memory systems with lasting impact on structure or function decreases subsequent adaptability. Thus, an organism decreases flexibility from conception onward due to differentiation and cumulative responses to environmental exposures. Based upon this, I recently concluded that aging is a decline in plasticity of genome-exposome interaction that occurs as a consequence of execution of differentiation and exposure memory systems. I term this the Redox Theory of Aging because it accounts for major hallmarks of aging and provides a foundation for new approaches to improve healthspan and longevity.
(2012) 15% of lifetime risk is due to the genome; 85% is due to exposures to risks, including diet and infections.
Redox biology is the mechanistic interface (Go and Jones. Redox Biology 2:358-360)

The memory system:
Exposure memory systems (Go and Jones. Redox Biology 5:71-79) We have genetically encoded memory systems. Our immune systems can learn from previous exposures. Also

there are other memory systems: Lipome, Proteome, epigenetics.

The Redox Code

Helmut Sies and Dean Jones recently presented The Redox Code as a set of principles to complement the epigenetic code in using the genome for organization, differentiation and adaptation to environment. NAD and NADP systems operating at near equilibrium with central metabolic fuels.

Kinetically controlled sulfur switches in the redox proteome. Protein-protein interactions.

The NADPH system acts as a capacitor, able to cycle back and forth. Activation/deactivation cycles of H_2O_2 generation support spatiotemporal sensing and signaling in molecular function, tissue differentiation and response to exposures. eg. tadpole tail regeneration demonstrates a spatio-temporal wave of H2O2.

(Go and Jones FRBM 2015) The cysteine proteome network.

Integrated network responses to environmental resources and challenges

Redox proteome <==> redox meabalome <==> metabalome <==> Proteome, epiproteome <==> transcriptome

(Go 2014 Tox)

Transcriptome, Genome, metabalome

Q: How would you use this to study what is going on?

Jones: We did a study with cadmium in mice. We looked at the top pathways in the metabolome. We saw top pathways in oxidation of lipids. We can see hierarchical structure and hubs.

(L14) **Impact of mitochondrial fusion proteins on ROS handling**

Antonio Zorzano, *Universitat de Barcelona, Spain*

Mitochondrial filaments are dynamic. They move, sometimes getting longer, and sometimes getting divided. Mitochondrial dynamics modulates mitochondrial metabolism, and signaling, and participates in the maintenance of mitochondrial DNA.

Fusion proteins: Mfn1, Mfn2, OPA1

Fission proteins: Drp1, Fis1

Mfn2 = mitofusin2 is an OMM protein. Mfn2 participates in the fusion of the outer mitochondrial membrane. In addition, Mfn2 participates in the tethering of contact sites between mitochondria and endoplasmic reticulum (ER). Mfn2 integrates external signals with mitochondrial behaviors. Mfn2 is key in the UPR. Mfn2 deficiency causes alterations in mitochondrial and ER morphologies. In addition, Mfn2 loss-of-function causes mitochondrial dysfunction, and a chronic activation of the unfolded protein response (UPR) in cells and in tissues.

The antioxidant compound, N-acetylcysteine (NAC), ameliorated glucose tolerance and insulin signaling in liver-specific Mfn2 KO mice. This indicates that oxidative stress is responsible for, at least, some of the alterations linked to Mfn2 deficiency.

Does Mfn2 participate in aging? Mfn2 deficiency in skeletal muscle accelerates age-related metabolism disease in mice. Increased body wt and adiposity. Reduced thermogenesis. Mfn2 KO old mice show muscle atrophy and impaired muscle performance.

(Song Cell Metabolism 2015) Mitochondrial fission and fusion factors reciprocally orchestrate mitochondrial culling by mitophagy.

Q: Interesting that Mfn2 is also outside the OMM in the ER. Is it also other places?

Zorzano: It probably depends upon cell types.

Carlos: Have you tried to overexpress Mfn2?

Zorzano: It causes mito perinuclear clustering. This is not healthy for the cells.

Lester Packer: Curious whether Rapamycin and the mTOR system are involved in fission and fusion system because of relation to longevity?
Zorzano: I don't think so.

[Tom Prolla was on the program, but could not attend.**]**

A conserved transcriptional signal of delayed aging and reduced disease vulnerability
Jamie L . Barger, PhD, *University of Wisconsin, Madison, USA*

Mice live longer, with increased healthspan on a lifelong calorie-restricted (CR) diet than on an ad lib diet. CR protected against cancer, diabetes, cataracts, sarcopenia.
Two studies have been done in Rhesus monkeys: One at U of Wisc. The other at NIH.
They looked at gene expression in CR animals: monkeys, flies, mice.
Is the pattern of effects on gene expression pathways evolutionarily conserved?
There is a small correlation.
The CR gene expression signature is similar between CR mice an long-lived dwarf mice, such as Ames, Snell, little, GHRKO. But difference in the ribosomal pathways.
In contrast, mice on high fat diet show inverse gene expression signature.
Treatment with thiazolamine diamine drugs help to reverse the effect of obesity and high fat diet.
Is Sirt3 involved? They have Sirt3-KO mouse. Sirt2 is required for induction of mitochondrial and inflammatory responses to CR.
It might be helpful in treating age-related disease to develop treatments that would be activators of mitochondrial metabolism or anti-inflammation agents.
Enrique Cadenas: Some children are born with an obesity metabolic program.
Q: Some people die of communicable diseases. Have you seen any resistance to infection in CR animals? Has anyone tested for this?
Barger: I'm not an expert in immunology. There could be a fine line when CR decreases immune response. Experimental CR monkeys and mice are raised in pathogen-free facilities, so we would not have seen whether there would be a difference in immunity.

(L16) **Metabolic regulation of stem cell maintenance and aging**
Danica Chen, *Program in Metabolic Biology, Nutritional Sciences & Toxicology, University of California, Berkeley, USA*

SIRT3 protein localizes in the mitochondria. It is a mitochondrial deacetylase enzyme, which regulates the global acetylation landscape of mitochondrial proteins and reduces oxidative stress.
Sirt3 is required for the reduction in oxidative stress caused by CR.
The protective programs mediated by sirtuins are suppressed in HSCs at an older age, underlying aspects of aging-associated HSC deterioration. We demonstrate that sirtuins can be targeted to reverse HSC aging and improve tissue homeostasis. Sirt3 is highly expressed in stem cells, but not very high in other cell types. Old mice (2 yrs) Sirt3-KO have fewer HSCs.

SIRT7 is nuclear-localized. It is a H3K18 deacetylase, functions at chromatin and controls a regulatory branch of mitochondrial unfolded protein response(mtUPR). (Mohrin. Science 2015)
Sirt7 represses Nrf1 to promote survival in low nutrient conditions.
The mtUPR is different than the ER-UPR. There is a mtUPR-mediated metabolic checkpoint.
Sirt7 is required to maintain HSC quiescence.
Overexpression of Sirt3 and Sirt7 improves the function of aged HSCs.
Sirt3 and Sirt7 regulate the transition between HSC quiescence and proliferation.

Kelvin Davies: Have you looked whether LON protease increased during the mtUPR? I expect that LON would be useful for degrading unfolded proteins.
Chen: Is that conserved? Good idea. We will look.
Chen: Sirt3 is a tumor suppressor, by suppressing oxidative stress.
Helmut Sies: What is the molecular basis of the regulation of oxidative stress by Sirt3?
Chen: Sirt3 increases the enzymatic activity of Sart2. Sirt3 might also reduce the generation of ROS. It deacetylates all mitochondrial proteins that could be acetylated.
Q: Have you tested this system on other stem cell types?
Chen: I welcome collaborations to do this.

11:00 – 12:00 h. Coffee break and Poster session (II)

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

(O6) **Redox dependent selective targeting of mutant K-Ras expressing cancer cells**
Iskandar, Kartini; Rezlan, Majidah; Bellot, Gregory; Yadav, Sanjiv; Foo, Jonathan; and Pervaiz, Shazib. *1Department of Physiology, National University of Singapore.*

Ras is amongst the most commonly mutated oncogenes in a variety of human cancers. They recently described the death inducing activity of a small molecule compound, C1, which triggered ROS dependent autophagy-associated apoptosis in cancer cells. The compound specifically targets human colorectal and pancreatic cancer cells harboring mutant K-RAS. It has little activity against wild type RAS expressing cells.
(*Wong CH, Iskandar KB, Yadav SK, Hirpara JL, Loh T, Pervaiz S. Simultaneous induction of non-canonical autophagy and apoptosis in cancer cells by ROS-dependent ERK and JNK activation. PLoS One. 2;5(4):e9996, April 2010.)*

(O7) **Repair of peroxidized membrane phospholipids: critical role of peroxiredoxin 6**
Fisher, Aron; and LI, Haito. *Institute for Environmental Medicine, Physiology, University of Pennsylvania, USA.*

Phospholipids are a major structural component of all cell membranes; their peroxidation represents a severe threat to cellular integrity and their repair is important to prevent cell death. Peroxiredoxin 6 (Prdx6), a protein with both GSH peroxidase and phospholipase A_2 (PLA_2) activities, plays a critical role in antioxidant defense of the lung and other organs.
Protection against lipid peroxidation might occur at 3 levels: 1. scavenging ROS.
2. Chain-breaking antioxidants (eg Vit E) stop the chain reaction of lipid peroxidation.
3. "Repair" of oxidized lipids.

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

Telomeres are bound to a multiprotein complex that protects the chromosome, which has a role in the regulation of the telomeres length, called shelterin. This complex is composed of six core proteins: telomeric repeat binding factor 1 and factor 2 (TRF1/2), the TRF1- and TRF2-Interacting Nuclear protein 2, Protection Of Telomeres 1, TPP1 (also known as TINT1, PToP and PIP1) and

the human ortholog of the yeast Repressor/Activator Protein 1. It is possible that this complex is altered by oxidative stress during stress-induced premature senescence, which may be the cause of telomere attrition.
Cigarette smoke, as a form of oxidative/carbonyl stress, disrupts the shelterin complex, associated with telomere attrition, a hallmark of chronic airway diseases, such as COPD and Idiopathic Pulmonary Fibrosis (IPF), associated with accelerated aging.

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

ROS production is higher (from 1.5- to 10-fold) in astrocytes than in neurons (rats and mice). Furthermore, higher ROS production was found to take place in mitochondria isolated from cultured astrocytes when compared with those isolated from neurons. They found that, in astrocytes, a large proportion of complex I occurs free, whereas in neurons, most complex I is embedded into supercomplexes.

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

Specific induction of reverse electron transport (RET) through respiratory complex I produces a ROS signal that extends *Drosophila* lifespan. We show that induction of RET rescues pathogenesis induced by severe oxidative stress, confirming the importance of origin site in ROS signaling. Finally, we show that RET is instrumental for appropriate mitochondrial turnover and fine-tune of Target of rapamycin (Tor) signaling under stress.

Lunch with the Publisher of Elsevier, and with Prof Michael J. Davies, Past President SFRR, Editor of Free Radical Research. Dept of Biomed Sci, U of Copenhagen. He studies ECM protein aging.

Chairs: **Nesrin Kartal-Özer**, *Marmara University, Istanbul, Turkey*
John McGuire,

(L17) **Aggregated lipids and proteins in aging, Alzheimer's disease, and diabetes**
Tilman Grune, *Department of Molecular Toxicology, German Institute of Human Nutrition, Nuthetal, Germany*

They are studying proteasome; the core is the 20S proteasome. Proteins which are oxidatively modified are degraded by the 20S proteasome in an ATP- and ubiquitin-independent pathway. If the proteasomal system is overwhelmed, oxidized proteins aggregate and form a hydrophobic yellow-brownish material that accumulates predominantly in lysosomes by macroautophagy. Some unfolded proteins form aggregates inside the cell, unless they are first digested by the

proteasome. Many aggregates are fluorescent. (Grune FRBM 2005) These aggregates also inhibit the proteasome activity. One of the dominant effects of cross-linked proteins is the inhibition of the proteasomal system, regardless whether the cross-linking of proteins was achieved directly by protein-protein crosslinking or via lipid peroxidation mediated cross-linking agents. Originally it was thought, that these protein aggregates are merely waste products of cellular metabolism, but mounting evidence demonstrates an active participation of such protein aggregates in several physiological and pathophysiological cellular responses, relevant for aging and diseases. So we could demonstrate that aggregate-mediated proteasomal inhibition plays a role in Alzheimer's disease, in the accelerated senescence of ß-cells, and in the aging process itself.
Lester Packer: Bernard Strahler's book in 1950's showed 50% of hearts of older people are filled with lipofuscin. Is there any evidence that lipofuscin would cause heart attack?
Grune: No evidence of that.

(L18) **Differential expression and glycative damage affect specific mitochondrial proteins with aging**

Bertrand Friguet, *Université Pierre et Marie Curie, Sarbonne Universities, Paris, France*
Damaged proteins can be removed by proteasome and autophagy, or repaired by systems such as thioredoxin. With increasing age, there is age-related accumulation of oxidized proteins (Levine & Stadtman 2001 Exp Gerontol 36:1495-1502).
Proteins are increasingly carbonylated in senescent fibroblasts. They have more HNE, more AGEs, more carbonylation. Many of these modified proteins are involved in energy metabolism, protein maintenance, and cytoskeleton. About half are from the mitochondria.
Skeletal muscle aging. 2-Dimensional oxi-proteome analysis of young and old human skeletal muscle. (Lourenco do Santos 2015 Redox Biology) We see increasingly carbonylated proteins in old muscle.
Satellite cells are key players in muscle regeneration. They lose proliferative capacity after exposure to oxidative stress. They lose differentiation capacity with age.
Senescent human myoblasts are modified with aging by carbonylation, HNE, and AGEs. Some of these proteins are involved in carbohydrate metabolism.
Damaged proteins ==> Reduced carbohydrate metabolism ==> Increased turnover of storage and membrane lipids ==> altered lipid signaling
Lester Packer: A problem with past measurements in cultured cells is that they were cultured in air (21% O2 is very toxic). Would you expect different outcome with optimum 6-8% O2?
Friguet: I agree. We used air as our model. Maybe this helps to show age-related pathologies.
Q: Another study killed all of the satellite cells, and those animals aged normally, so maybe you (and the Conboys) were looking at muscle repair, rather than aging.
Friguet: Perhaps yes.
Q: Are there specific moieties that make some proteins more susceptible to oxidative attack?
Friguet: Well, attack is one thing. But also there may be differences in how quickly some proteins are degraded after oxidation, so that what we measure is a combination of both processes.

(L19) **Clock Genes Regulate Neuronal redox homeostasis and Aging in Brain**
Gaetano Serviddio, MD, *Associate Professor of Internal Medicine*, *Director of Graduate School of Geriatrics & Gerontology, University of Foggia, Italy*

Managing Editor of the Journal of Gerontology and Geriatrics

The circadian clock is in the SCN of the brain. The circadian clock controls physiological processes such as metabolism, hormone secretion and cardiac function, all of which exhibit daily oscillation. A dysfunctional circadian clock can in turn contribute to ageing and pathologies associated with old age.
It has been demonstrated that reduction of the expression of Bmal1, one of the clock-regulating genes, promotes neuronal death in primary cultures and in mice treated with a chemical inducer of oxidative injury. This indicates that clock genes regulate cerebral redox homeostasis and suggests that impaired clock gene function is connected to neurodegeneration. (Genes & Dev 2006) Bmal1 regulates redox gene expression in brain.
Sirt1 mediates central circadian control in the SCN by mechanisms that decay with aging (Guarante. Cell 2013). (J Biol Rhythms Apr 2011 Cermakian)

(L20) **Frailty and sarcopenia**
Leocadio Rodríguez-Mañas, *Service of Geriatrics, Hospital Universitario de Getafe, Madrid, Spain*

We need to detect pre-disability early, in order to intervene *[JDF: but what intervention?]*
Frailty has become the most accepted pre-disability condition, gaining progressive relevance in the last decade. This status, that is the final outcome of the joint action of the aging process plus some chronic diseases and conditions, can now be detected and, for the first time, we have strong evidence about the effectiveness of interventions (mainly based on **physical exercise**).
Frailty contributes to falls, which cause injuries and death.
There is decrease in muscle mass with age. But also change in the energetics of the remaining muscle. The muscle mitochondria produce less energy. For older people, 3-10 days of bed rest in hospital loses a dangerously large fraction of muscle mass. This is very difficult to regain, if it is regained at all.
Hormones are involved in frailty: IGF1, Cortisol, Vitamin D.
Exercise program with resistance training is very effective in reversing frailty. Good nutrition can help with exercise, but the resistance, strength-training exercise is essential.
John Furber:: Many older people have arthritis. Are there special exercises? Do you know whether electrical stimulation can replace conventional exercise?
Rodríguez-Mañas: Many older people have osteoarthritis. At least 80% of older people can do resistance training, but only about 20% can do aerobic exercise. Resistance strength training show results in 6-10 weeks in elderly frail people. Many elderly frail have weakness in quadriceps, so they are unsteady on their feet.
Lester Packer: What form of strength training? Daily?
Rodríguez-Mañas: Twice/wk, 30-45 min. start with one repetition, but increase this as muscle strength increases. Bench press and leg press can be done by most elderly frail people. Also, it is important to work with balance, and if possible, also aerobic exercise.

(L21) **Reactive oxygen species production in skeletal muscle during contractile activity and aging**
Malcolm Jackson, *University of Liverpool, UK*

He collaborated with Arlan Richardson, Holly Van Remmen, Florian Muller, when they were at UTHSCSA.
SOD1-/- mice lose muscle mass with age. Their neuromuscular junctions are decreased in extent. However, selectively KO of SOD1 from muscle has very little effect.

They are investigating whether denervation of the NMJ on a few muscle fibers would affect nearby muscle fibers. The denervated fibers secrete H2O2, but so do the nearby fibers. Maybe there is redox crosstalk between the muscle fiber and the nerve. Maybe neurotropic factors.
Older mice overexpressing HSP10 maintain force and have larger muscle fibers.
How do ROS that are generated during contractions cause muscle growth?

26th June 2015
Session IV. Redox Biology in Vascular Disease

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

(L22) **Origin of mitochondrial ROS in ischemia reperfusion injury**

Michael Murphy, *MRC, Mitochondrial Biology Unit, Cambridge, UK*

Mitochondrial ROS have long been known to contribute to damage in conditions such as ischaemia-reperfusion (IR) injury in heart attack and stroke, but methods to stop this ROS production were limited. Over the past few years we have developed a mitochondria-targeted S-nitrosating agent, called MitoSNO, that we showed was effective in preventing ROS formation in IR injury with therapeutic implications
Standard heart attack treatment is currently to open up the blockage as quickly as possible. This results in reperfusion injury when oxygenated blood returns to the ischemic tissue. At that time, the ischemic mitochondria release a burst of free radicals, which damage the tissue. During ischemia, complex I goes through a deactive transition. During reperfusion, it turns on quickly, releasing a burst of free radicals.
They developed a mitochondria-targeted, S-nitrosating agent, called MitoSNO, which is effective in preventing ROS formation in I-R injury with therapeutic implications. MitoSNO is a new drug, which goes into mitochondria. (PNAS 2009) (Nat Med 2013) It locks the complex I OFF, for a while, so that it comes back on slowly.
Where are the electrons coming from to make the O2*- ? What metabolites build up during ischemia? Compare normoxic and ischaemic mouse tissues in heart, brain, liver, kidney. Succinate, xanthine, hypoxanthine build up in all 4 tissues. Actually 8 chemicals build up in heart. But succinate builds up the most. Succinate is rapidly oxidized during reperfusion.
Why does succinate build up during ischaemia? They used C13 tags to study this. The succinate is not from glucose or fats. Instead, electrons from NADH buildup pushes fumarate "backwards" to succinate in complex 2.
Why doesn't the succinate diffuse away or convert to something else? It would need GTP to drive it something else. It is at a low energy state.
The fumarate comes from 2 pathways: from aspartate or from malate.
Succinate is a key signature of ischaemia inside the cell. Trad used lactate, but that diffuses away out of the cell.
In the lab, it is known to feed cells with succinate if they want to generate O2*-. This forces the electrons backwards through complex I.
MitoB is a probe for H2O2, which turns it to mitoP inside the cell.
Malonate protects aconitase.
Therapeutic inhibition of succinate accumulation. Complex 2 inhibitor.
This succinate buildup is part of mitochondrial signalling under less dire conditions.

During reperfusion, succinate pushes to fumarate, forming O2*-.
[[[SEE PHOTOS OF HIS SLIDES on the OCC website.]]]
Henry Foreman: Also, dihydro-orotate builds up.
Murphy: Yes. Also phosphate builds up.
Lester Packer: Extensive succinylation and malonylation of proteins occur. Perhaps this helps regulate the damage?
Murphy: Succinylation comes from SuccinylCoA.
Q: Eventually, the complex 1 has to come back, becoming de-nitosylated slowly.
Murphy: Yes, the half life of S-nitrosylating is about 5 minutes, reactivation can be slower.

(L23) **Mechanisms of redox responses to endothelial shear stress: from peroxide to nitric oxide**
Santiago Lamas, *Centro de Biología Molecular Severo Ochoa,CSIC, Madrid, Spain*

Laminar shear stress (LSS) is a protective hemodynamic regulator of endothelial function. It limits the development of inflammatory diseases related to oxidative stress. Laminar shear stress triggers signalling inside the cell, which results in vasodilation. Several endothelial proteins have been proposed as potential shear stress mechano-sensors.
LSS triggering of p38 MAPK requires H2O2. Nox4 is a source of the H2O2.
LSS > H2O2 > p38 MAPK > eNOS4.
H2O2 is the signaling face of ROS.

(L24) **Vascular endothelial and smooth muscle cell redox signaling and function in health and disease**
Giovanni Mann, *British Heart Foundation Centre of Research Excellence, King's College London, UK.*
He did not show up. See abstract.

After 50 Years with GSH Peroxidases, or Distinct oxidized forms of GPxs revealed by DFT and MS/MS Analysis
Fulvio Ursini.

GPx1 universal antiox enzyme. Prevents cancer
GPx2 GI anti inflammatory, anti carcinogenic, anti apoptosis, but promotes cancer growth
GPx3 Extracellular
cGPx4
mGPx4
nGPx4 chromatin compaction
GPx5
GPx6
GPx7
GPx8
The GPxs are oxidized by peroxides. The enzymes are very fast and very stable. They need water or h2o2 to make the enzyme work. (Orian. FRBM 2015)
Upon oxidation, the mass of GPx1 and GPx4 shrinks by 2. The Selenocystein is close to the peptide backbone, so it attaches to a backbone N, releasing 2H.
Raphael: What are the relative roles of catalase vs GPx vs peroxiredoxins ?
Ursini: No definite answer. I like peroxiredoxins, too; I published a book about them.

Coffee break and poster session (III)

Oral communications III
Chairs: **Anne Negré Salvayre**, *University of Toulouse, France*
Teresa Mitjavila, *University of Barcelona, Spain*

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

Peroxisome proliferator activated receptor g co-activator 1a (PGC-1a) is a regulator of mitochondrial oxidative metabolism and reactive oxygen species (ROS) homeostasis that has been shown to play a role in angiogenesis.

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

The transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) is a master regulator of the cellular defenses against oxidative stress. In the presence of oxidizing agents or electrophiles, Nrf2 translocates to the nucleus, where it induces the transcription of genes involved in the defense against oxidative damage. 4-Hydroxy-2-nonenal (HNE) is a highly cytotoxic product of lipid peroxidation. Nevertheless, at low concentrations, it is able to activate protective pathways, including that of the transcription factor Nrf2. In addition, HNE activates uncoupling proteins (UCPs), mitochondrial inner membrane proteins that mediate uncoupling of oxidative phosphorylation and have been proposed to prevent excessive superoxide production and to protect against oxidative stress.

(O13) **Searching for biomarkers of Charcot-Marie-Tooth, a neuromuscular rare disease**
Seco-Cervera, Marta[1]; Ibáñez-Cabellos, Jose Santiago[2]; García-Giménez, Jose Luis[3]; Berenguer-Pascual, Ester[1]; Reyes-Palomares, Armando[4]; Rodríguez-López, Rocío[4]; Sánchez-Jiménez, Francisca[4]; Sevilla, Teresa[5]; Espinós, Carmen[6]; Palau, Francesc[7]; and Pallardó, Federico V.[1]. [1]*INCLIVA University of Valencia,;* [2]*SISTEMAS GENÓMICOS S.L.;* [3,6,7]*CIBERERUV;* [4]*University of Malaga, Spain;* [5]*La Fe hospital, Spain.*

Dr. Charcot, Dr. Marie, and Dr. Tooth first described this disease. CMT designates a heterogeneous group of disorders, which, despite some variability in their clinical features, share the same general phenotype, characterized by progressive loss of muscle tissue and touch sensation across various parts of the body. We observed significant differences in gelsolin levels between mild phenotype CMT patients and severe phenotype CMT patients. In addition, we found a correlation between the age of the patients and gelsolin protein levels.

(O14) **Oxidative stress and NOX4 up-regulation contribute to aortic wall injuries in Marfan syndrome aortic aneurysms**
Meirelles, Thayna[1]; Crosas-Molist, Eva [2]; Gorbenko del Blanco, Darya [1]; Hernández, Vanessa[1]; García-Calero, Carolina [3]; Condom, Enric[4]; Forteza, Alberto[5]; Rodríguez-Pascual, Fernando[6]; Brandes, Ralf[7]; Mas Stachurska, Aleksandra[8]; Sitges, Marta[8]; Sorolla , M. Alba[9]; Ros, Joaquim [9]; Laurindo, Francisco[10]; Fabregat, Isabel [2]; and Egea, Gustavo[1]. [1.4]*Universitat*

de Barcelona, Spain; [2]Institut d'Investigacions Biomèdiques de Bellvitge , Spain; [3]Hospital de Bellvitge-IDIBELL , Spain; [5]Hospital 12 de Octubre, Madrid , Spain; [6]Centro de Biologia Molecular Severo Ochoa, Madrid, Spain; [7]Goethe-University Frankfurt, Germany; [8]Hospital Clínic, IDIBAPS, Spain; [9]Universitat de Lleida, Spain; and [10] University of São Paulo, Brazil

Marfan syndrome (MFS) is characterized by ascending aortic aneurysms resulting from altered assembly of extracellular matrix microfibrils and chronic activation of TGF-ß signaling. TGF-ß is a potent regulator of reactive oxygen species (ROS) production and expression of some cardiovascular relevant NADPH oxidases, particularly the isoform 4 (NOX4).

(O15) **Nitrite/ascorbate redox interaction leads to nitric oxide production in the brain hippocampus, supporting neurovascular coupling. An in vivo study in real-time.**
Ferreira, Nuno R.; Lourenço, Cátia F.; Costa, Sérgio M.; Barbosa, Rui M.; and Laranjinha, João. *1Center for Neurosciences and Cell Biology, Faculty of Pharmacy University of Coimbra, Portugal*

The energetic demand of the brain requires a constant supply of oxygen (O_2) and glucose. To maximize energetic efficiency, metabolic requirements due to neuronal activation are satisfied through local and transient increase in cerebral blood flow (CBF), via the mechanism of neurovascular coupling (NVC). It has recently been shown that •NO produced by the neuronalsynthase (nNOS) mediates NVC in hippocampus under physiological conditions. However, when O_2 supply is diminished (e.g., stroke, ageing), this pathway for NVC is compromised.

Session V. Redox Biology in Neurodegenerative Diseases
Chairs: **Leonor Almeida**, *University of Coimbra, Portugal*
Giuseppe Valacchi, *University of Ferrara, Italy*

(L25) **Amyloid-ß disrupts calcium and redox homeostasis in brain endothelial cells**
Catarina Oliveira, *Center for Neuroscience and Cell Biology, University of Coimbra Portugal*

Abstract: Alzheimer's disease (AD) is a progressive and fatal brain disease, which, in sporadic form, is the most prevalent form of dementia in the elderly. Amyloid β-protein (Aβ) has been shown to accumulate in the brain of Alzheimer's patients, in senile plaques. But Aβ levels alone are not a reliable predictor of cognitive decline. Evidence exists showing that cerebrovascular function is altered in AD, often preceding the onset of cognitive impairment, contributing to neurodegeneration, and playing a major role in AD pathogenesis. Recent data in AD mice models demonstrated a direct correlation between microvascular impairment and Aβ accumulation and results obtained in human and animal cultured cells suggest that brain capillary endothelium dysfunction is due to the deleterious effect of Aβ peptide on endothelial cells. Taking into account our previous results, highlighting the involvement of Endoplasmic reticulum (ER) stress in neuronal dysfunction triggered by Aβ, we explored the hypothesis that endothelial cell damage occurring in AD is mediated through the induction of ER stress. In a rat brain endothelial cell, exposure to $A\beta_{1\text{-}40}$, which preferentially accumulates in brain vasculature, increased the levels of several markers of ER stress-induced unfolded protein response, activated mitochondria dependent and independent apoptotic cell death pathways and disrupted Ca_2 and redox cell homeostasis. The failure of ER stress- adaptive UPR led to a decrease in proteasome activity, promoted the accumulation of ubquitinated proteins and the impairment of the autophagic flux, culminating in endothelial cells apoptosis. Prolonged ER stress was shown to induce intracellular Amyloid Precursor Protein (APP) accumulation, which co-localizes with the ER chaperon GRP78, leading to β-secretase activation, increase in intracellular Aβ levels and apoptotic cell death. In conclusion, these data support that

endothelial cells dysfunction in AD arises from $A\beta_{1-40}$ induced ER stress, opening new strategies to prevent or delay the progression to AD.

Notes: amyloidβ > disrupts Ca++ homeostasis and UPR in brain endothelial cells > Alzheimer's Disease.
ER stress leads to intracellular retention of amyloidβ in brain endothelial cells (BECs).

(L26) **Possible role of oxysterols in the brain pathophysiology**
Giuseppe Poli, *University of Turin at S. Luigi Gonzaga Hospital, Italy*

Abstract: Oxysterols are a family of 27-carbon molecules originated from cholesterol oxidation by both enzymatic and non-enzymatic mechanisms, which, with respect to cholesterol, contain an additional hydroxy, epoxide, or ketone group in the sterol nucleus and/or a hydroxyl group in the side chain. This report focuses on 24-hydroxycholesterol (24OH) and 27-hydroxycholesterol (27OH), two oxysterols that are normally produced in human body, by enzyme-mediated oxidation of cholesterol, and are very good ligands of LXRα and LXRβ, two physiologically important nuclear receptors. 24OH is by far the main cholesterol metabolite in the brain and, unlike cholesterol, freely diffuses over the blood-brain barrier (BBB), by this way avoiding excess accumulation of brain cholesterol. 27OH is the most represented oxysterol in the blood stream and can pass the BBB into the brain. Both oxysterols are able to induce amyloid β_{1-42} synthesis, but 24OH acts against Tau phosphorylation while 27OH on the contrary stimulates Tau phosphorylation.
Hypercholesterolemia is a major risk factor for AD.
Enrique Cadenas: Is 27OHChl crossing the BBB?
Poli: Yes.
Enrique Cadenas: Is obesity a risk factor for Alzheimer's?
Poli: Yes, OBESITY is a PRIMARY risk factor for Alzheimer's.

(L27) **Alzheimer's Disease: from oxidative stress to apoE directed therapeutics**
José Viña, *University of Valencia, Spain*

Abstract: ApoE-mediated clearing of amyloid-β from brain is a major therapeutic target to treat Alzheimer's
disease. Brain ApoE is activated by the dimerization of the retinoid-X receptor (RXR) with peroxisome proliferator-activated receptor gamma (PPARgamma). Bexarotene binds to retinoid X receptor, lowers amyloid-β plaque area and rapidly reverses cognitive, social and olfactory deficits in Alzheimer's disease mouse models [4]. However, it has considerable undesirable side effects [5,6,7]. Genistein, which binds to the PPAR gamma moiety of the RXR/PPAR gamma dimer receptor [8,9], is extensively
used in clinical practice[10] and is devoid of significant side effects [11]. We found that treatment of an Alzheimer's mouse model with genistein results in a remarkable and rapid improvement in various parameters of cognition, such as hippocampal learning, recognition memory, implicit memory and odor discrimination. This is associated with a lowering of Aβ levels in brain, in the number and the area of amyloid plaques as well as in microglial reactivity.

Notes: His team is called "FRESHAGE". They have been working on aging for decades. Functional mitochondria are required for amyloidβ-mediated neurotoxicity (FASEB J 18Ap 2001) amyloidβ 1-42 causes ROS production in cells, which activates p38, which causes cell death. Estradiol or genistein rescues cells by deactivating p38. APO-E can clear amyloidβ from cells after it is activated by bexarotene, a drug , which binds to RXR/PPAR

gamma. Genestein also binds, with less toxicity and less cost than bexarotene. Genestein binds to ERbeta but not to ERalpha.
Cesar: Does Genistein cross the bbb?
Viña: Yes, we see Genestein in the brain. We use similar doses as prescribed to women to prevent hot flashes.
Enrique: Can you distinguish among Apo E2, 3, 4, so you don't make Alzheimer's worse.
Viña:: Yes, we don't want to activate the bad Apo E4.

Viña (in Hallway): It would be good to take Genestein as a preventative for Alzheimer's. Viña doesn't take Genestein himself yet, but maybe he will soon. Jarrow Rogovin thinks that he probably gets sufficient Genestein from the natto that he eats almost every morning for breakfast. It probably would not worsen a person with ApoE4, but not make them better. So really, it is not so essential to test for ApoE4 before deciding to take Genestein. Anthony Thomas says that Genestein can also enhance the activity of Vitamin D through its 24-hydroxylase antagonism. Vitamin D is deactivated when it is 24-hydroxylated.

(L28) **Neurotransmission-dependent metabolic and redox coupling between neurons and astrocytes**
Juan Bolaños, *Institute of Functional Biology and Genomics, University of Salamanca-CSIC, Spain*

Energy and redox conservation in the brain requires metabolic cooperation between distinct cell types. We have identified mechanisms and factors that maintain cell-specific programs to allow this metabolic-redox collaboration. Neurons show a high dependence on mitochondrial oxidative metabolism for survival, whereas astrocytes resist to almost complete inhibition of mitochondrial respiration.

5-8 Sep 2016 will be the 39th Congress of the Spanish Biochemistry and Molecular Biology Society in Salamanca.

(L29) **The role and mechanisms of mitochondrial cholesterol in neurodegeneration**
José Carlos Fernández-Checa, I*nstituto Investigaciones Biomedicas de Barcelona (IIBB)-CSIC, Barcelona, Spain; and University of Southern California, Los Angeles, USA*

Symptoms of Alzheimer's can be caused in mice be giving them upregulated mitochondrial cholesterol transporters, so that cholesterol levels increase in mitochondria. This lowers GSH in mitochondria. Symptoms include amyloidβ accumulation, tau phosphorylation, and cognitive decline.
The Niemann-Pick disease type C (NPC) is a lysosomal storage disorder that affects the cerebellum and peripheral organs that results in neurodegeneration and premature death. NPC disease is caused by loss-of-function of the endolysosomal proteins NPC1/2 involved in cholesterol homeostasis and trafficking that leads to lysosomal cholesterol accumulation. The can cause fatal fatty liver disease. (Mari. Cell Metabolism 2006). Also problems in brain (Yu. JBC 2005). They did additional experiments with mice NPC1-KO. GSH ethyl ester partly restores motor capacity and life span in NPC closer to levels of normal controls. But it doesn't work as well as **cyclodextrine**, which is believed to remove excess cholesterol from the cells. Cyclodextrine is the standard treatment for NPC disease in the US.

17:00 – 18:00 h. Closing Keynote Lecture

Introduction by the Chair: **Josiane Cillard**, *University of Rennes 1, France*
Prof Davis got his PhD from UC Berkeley 1981, under Lester Packer and Bruce Ames.
He has pioneered the concept of impaired "adaptive Homeostasis" as a major factor in aging. The decline in Lon protease and proteasome expression contributes to senescence.
He was the founding Editor-in-Chief of FRBM. He has a nice sailboat.

(L30) **Upregulation of mitochondrial Lon protease allows adaptation to acute oxidative stress but dysregulation is associated with aging and chronic stress**
Kelvin Davies, *Leonard Davis School of Gerontology of the Ethel Percy Andrus Gerontology Center; and Division of Molecular and Computational Biology of the Dornsife College of Letters, Arts, and Sciences, University of Southern California, Los Angeles, USA*

Mitochondria are major sites of free radical generation and mitochondrial proteins undergo significant oxidation. To minimize the accumulation of oxidative damage, mitochondria utilize reducing enzymes, repair systems, and targeted proteolysis of damaged proteins to maintain function. A key mitochondrial-matrix proteolytic enzyme is the nuclear-encoded Lon protease: an ATP-stimulated protease that preferentially degrades oxidized proteins. We have shown that Lon is a stress response protein whose increased levels and activity during stress provides significant protection for cultured mammalian cell lines, primary cells, worms (*C. elegans*), flies (*D. melanogaster*), and rodents.

Adaptive response: Yeast cells can be mostly killed by a high dose of H2O2. But if they are first pre-treated with a low dose, then they survive later high dose.
This is **not hormesis**, in which sublethal dose causes damage, which is repaired stronger.
This is much lower levels than hormesis. This involves **signaling**, not damage repair.
Homeostasis keeps parameters within a range: Adaptive response is slightly beyond the homeostasis range.
During adaptation for oxidative stress, we see induction of several classes of genes, including proteases and signal transduction.
Mitochondria have no proteasome, but they have instead Lon protease. With Tilman Grune, they found proteasome collection in the cell is highly adaptive to oxidative stress. (H2O2)
Lon protease selectively degrades oxidized proteins in the mitochondria.
In non-stress, Lon is located in the mitochondrial D loop, where it is essential for mitochondrial proliferation. (G. Attardi)

Preconditioning: After Lon is induced, the cells are better protected from future oxidative challenges.

Adaptation in C. elegans and Drosophila: Fly ortholog of mammalian Nrf2.
In Drosophila, females adapt, but males don't adapt.
Perhaps mitochondrial genes are better suited for females, and perhaps nuclear genes are better suited for males, because mitochondria are maternally inherited.

Aging: We see the damage accumulate in the last 1/3 of life. Why don't damage repair systems work then as well as they do in the first 2/3? Is it a cause or effect of aging? Lon levels decreases in mouse muscle in old age. In cell culture, Lon levels decline with senescence. The inducibility and adaptability of the Lon protease declines with age. This decline is linked with decreased robustness, healthspan, and lifespan. Thus, Lon appears to be very much like the Proteasome in its age-dependent loss of activity and loss of inducibility. If you turn Lon expression down by 70%, you see significantly reduced mouse lifespan. However, overexpression of Lon does not appear to extend lifespan. What are the effects of age

dependent loss of Lon? Can we model this? All 4 ETC complexes get decreased in activity.
The inducibility of Lon and proteasome decreases with old age.
What causes loss of Nrf2 responsiveness in old age? (with Henry Foreman)
We might want to reconsider the Free Rad Thy of Aging, in which ox damage increases exponentially over lifespan, causing accumulation in the last third of life.
Collaborates with: Tilman Grune, Henry Foreman, John Tower, Derek Sieburth, Trisha Staab, Hongqiao Zhang.
Dean Jones: How do proteases turn each other over?
Davies: Proteasomes can degrade each other.
Josiane Cillard: Did you look for Lon non-aging cancer cells?

PRIZES AND AWARDS

Several prizes and awards were presented at the end of the Valencia meeting.
All of the awards and awardees, along with photographs, are displayed in a PDF, available on the OCC website.

http://oxyclubcalifornia.org/OCC/past_OCC_meetings.php

The 2015 Health Sciences Prize was presented by John MaGuire, **Oxygen Club of California,** and Jarrow Rogovin, **Jarrow Formulas.** The winner of the **Health Sciences** award of $12,500 is Professor **Giuseppe Poli.**
Lifetime memberships in the OCC were presented to **Juan Sastre** and **João Laranjinha.**
Please see the PDF for details and photograph of these and other awards:

- **Closing Remarks**

Juan Sastre, Josiane Cillard, João Laranjinha

Abbreviations:

aa = amino acids; ab = antibodies.
AD = Alzheimer's Disease; Alz = Alzheimer's.
Abeta = Aβ = amyloid beta.
apop = apoptosis.
Apgy = autophagy
BBB = blood-brain barrier
bp = base-pairs of DNA
C.e. = C.elegans = nematode = worm.
CR = calorie restriction = DR = dietary restriction
cv = cardiovascular; cvd = cardiovascular disease
dsDNA=double-stranded DNA; ssDNA = single-stranded DNA; nDNA=nuclear DNA; mtDNA=mitochondrial DNA
Dros = Drosophila melanogaster = fly.
EtBr = Ethidium Bromide
ESC = embryonic stem cell
exp = gene expression.
fn = function
GSH = Glutathione (reduced); GSSG = Glutathione (oxidized)
GPCR = G-protein coupled receptor
GWAS = Genome-wide association study
Hb = hemoglobin, the oxygen-carrying protein in RBCs.
HBO = Hyperbaric oxygen (greater than atmospheric pressure)
HSC = hematopoietic (blood-forming) stem cell

IGF = insulin-like growth factor
IP = intellectual property
iPSC = iPS cells = iPC = induced pluripotent stem cells
KD = gene knock down; KO = gene knockout.
life ext= lifespan extension.
LF = lipofuscin, a heterogeneous polymer that accumulates in aging lysosomes.
miR = miRNA = microRNA
mito = mitochondrion; mt = mitochondrial; mtDNA = mitochondrial DNA.
MSC = mesenchymal stem cells
mTOR = mammalian Target Of Rapamycin
NAC = N-acetyl-L-cysteine
NIA = National Institute on Aging of the U.S. National Institutes of Health
phosylate = phosphorylate = covalently bind a phosphate group to a molecule
RBC = red blood cell = erythrocyte
NAC = N-acetyl-cysteine
NO* = *NO = nitric oxide radical; ONOO = peroxynitrite
NO_2^- = nitrite ion; NO_3^- = nitrate ion
RNS = reactive nitrogen species
ROS = reactive oxygen species or free radicals
SASP = senescence associated secretory phenotype
Tase = telomerase; Tmere = telomere
TLN = translation of RNA to protein; TXN= transcription of DNA to RNA.
vs = versus, compared with
w = with ; wo = without.
wt = wild-type gene. +/+ = homozygous normal gene. KO = gene knockout.
KD = gene knock down; -/- = homozygous KO; +/- = heterozygous gene.
8-oxo-dG = 8OHdG = marker of oxidized DNA.
In biochemical or genetic pathways:
blockage or inhibition -| Activation or causation =>
Q: or Name: question or comment from the audience.
A: answer from the speaker
[JF: Editorial comments by John Furber.]

