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# SFRR-Europe 2013 Poster Presentations

PP01

## 1,3-Disubstituted-5-nitroindazole derivatives are inhibitors and subversive substrates of trypanothione reductase from *Trypanosoma cruzi*

B. Aguilera-Venegas [a,b], C. Olea-Azar [b], H. Speisky [a], V.J. Arán [a], R.L. Krauth-Siegel [d]

[a] *Laboratory of Antioxidants, Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile*
[b] *Laboratory of Free Radicals, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile*
[c] *Medicinal Chemistry Institute (IQM), Superior Council of Scientific Research (CSIC), Madrid, Spain*
[d] *Biochemie-Zentrum der Universität Heidelberg (BZH), Heidelberg, Germany*
*E-mail address:* benya@ciq.uchile.cl (B. Aguilera-Venegas)

American trypanosomiasis, commonly known as Chagas disease (CD) is considered one of the most prevalent parasitic neglected tropical diseases (NTD). Although its greatest impact has been focused on Latin America, the spread of this disease to the old continent and first-world countries has become CD into a global problem. Moreover, the lack of public policies and the disinterest from the pharmaceutical industry has triggered a crisis for lack of drugs, which is reflected in the absence of effective treatments. The protozoan parasite *Trypanosoma cruzi* (*T.cruzi*) is the etiological agent of CD and possess a complex antioxidant machinery based on a low molecular weight thiol, trypanothione (T[SH]). T[SH] levels are maintaining either via *de novo* synthesis or by the 2-electron reduction of oxidized trypanothione (T[S]$_2$) in a reaction catalyzed by the enzyme trypanothione reductase (TR). Herein, 1,3-disubstituted-5-nitroindazole were assessed as inhibitors and subversive substrates of TR from *T.cruzi*. Kinetic studies on the recombinant enzyme reveal that indazole ring binds to TR and inhibits it by competitive or mixed-type mechanisms. The specific type of inhibition mechanism was shown to be dependent on the 3-substituent, whereas the 1-substituent modulates the affinity of the inhibitor toward the ES complex (for mixed-type inhibitors). Furthermore, regardless of the substituents, 5-nitroindazoles were reduced by TR, which led to an increment in oxidase activity and the release of reactive oxygen species. Additionally, the extent of the variation was strongly dependent on the 3-substituent but slightly on the 1-substituent due to chemical-coupled reactions. Thus, these results disclose indazole structure as a promising scaffold for further chemical modifications in the search of novel TR inhibitors and potential anti-*T. cruzi* drugs.

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PP02

## Effects of hypotaurine on carbonate radical anion and nitrogen dioxide radical generated by peroxidase activity of Cu,Zn-superoxide dismutase

Alessia Baseggio Conrado, Laura Pecci, Mario Fontana

*Dipartimento di Scienze Biochimiche, Sapienza Università di Roma, Rome, Italy*
*E-mail address:* alessia.baseggioconrado@uniroma1.it (A.B. Conrado)

Cysteine sulfinate (CSA) and hypotaurine are recognized as key intermediates in the metabolic pathway leading from cysteine to taurine. The oxidation of sulfinic group to the respective sulfonate is a crucial point for generation of taurine in mammalian tissues. The mechanism of sulfinic group oxidation could not be related to specific enzymatic activities. However, oxidizing agents, such as hydroxyl radical, photochemically generated singlet oxygen and peroxynitrite have been reported to accomplish such oxidation in good yield.

Carbonate radical anion ($CO_3^{\bullet-}$) is receiving increasing attention as important mediator of biological processes and is a potent one-electron oxidant that is able to oxidize a variety of biotargets. Nitrogen dioxide radical ($^{\bullet}NO_2$) is well known as a reactive species capable to initiate both oxidation and nitration reactions. The pathogenic role of $^{\bullet}NO_2$ has been related mostly to the increased level of nitrated proteins detected under many disease conditions.

The interaction of sulfinates with $CO_3^{\bullet-}$ or with $^{\bullet}NO_2$, generated separately by the peroxidase activity of Cu,Zn-Superoxide Dismutase, has been studied. It is observed that $CO_3^{\bullet-}$ and $^{\bullet}NO_2$ mediates the oxidation of hypotaurine and CSA, producing the respective sulfonates, taurine and cysteic acid. Both radicals react by one-electron mechanism with sulfinates to form sulfonyl radicals as transient intermediates. These results indicate that hypotaurine and CSA, already known as protective agents against reactive oxygen and nitrogen species, can also act as $CO_3^{\bullet-}$ scavengers. In order to investigate the sulfinate ability to prevent $CO_3^{\bullet-}$-mediated oxidation, their effect on tyrosine dimerization and ABTS oxidation has been studied.

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PP03

## Oxidative stress cause liver damage in adult β-thalassemia major at Dubai thalassemia center

A.L. Altaie [a], F.A.A Lkhaja [b]

[a] *Zayed University, Abu Dhabi, UAE*
[b] *Dubai Thalassemia Center, Dubai, UAE*
*E-mail address:* Ala.altaie@zu.ac.ae (A.L. Altaie)

β thalassemia is a genetic disorder that affects the synthesis of normal hemoglobin (Hb) rendering the patient blood transfusion dependent for lifelong. This Regular blood transfusion generates Reactive oxygen species (ROS) and consequently tissue damage. We studied the oxidative status, antioxidant and serum hemoxygenase-1 (HO-1) an antioxidant protein in 17 β thalassemia patients with liver damage following in Dubai Thalassemia Center and 18 normal controls. Our results showed significant increase in superoxide dismutase (SOD) activity and decrease in the catalase (CAT) activity in patients compared to controls. Elevated serum ferritin showed positive correlation with SOD activity. Similarly serum glutamic pyruvic transaminase (sGPT) showed positive correlation with serum ferritin and SOD activity and negative correlation with CAT. The serum HO-1 showed no significant difference between the two groups. In conclusion we clearly demonstrated that iron overload due to regular blood transfusions, leads to high levels of oxidative stress and decrease the antioxidant activity in β thalassemia patients. This generated oxidative stress is the cause of liver damage in those chronically transfused patients.

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PP04

## Characterization of new alkanal-phosphatidylethanolamine adducts

Andrea Annibal [a,b,c,d], Ralf Hoffmann [a,b,d], Jürgen Schiller [b,c,d], Maria Fedorova [a,b]

[a] *Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig,Leipzig, Germany*
[b] *Center for Biotechnology and Biomedicine, Universität Leipzig, Leipzig, Germany*
[c] *Institute for Medical Physics and Biophysics, Faculty of Medicine, Universität Leipzig, Germany*
[d] *LIFE-Leipzig Research Center for Civilization Diseases, Universität Leipzig, Germany*

Aldehydes generated as a result of lipid peroxidation represent promising biomarkers of oxidative stress that plays a major role in many human diseases and inflammation. Reactive oxygen species (ROS) can oxidize fatty acyl residues of phospholipids yielding a heterogeneous class of lipids peroxidation products (LPPs) including the cleavage of free unsaturated and saturated aldehydes. Carbonyl groups present in LPPs are highly reactive to nucleophilic groups in other biomolecules, such as the side chains of lysine, cysteine and histidine residues in proteins or amino groups in phosphatidylethanolamines (PE). Whereas the reactivity of unsaturated aldehydes has been well investigated, only few data are available for saturated aldehydes. Here we report new alkanal-PE adducts identified by mass spectrometry (MS) using consecutive fragmentations ($MS^n$) as well as their formation quantified by multiple reaction monitoring (MRM) obtained by incubating dipalmitoylphosphatidylethanolamine (DPPE, 0.1 mmol/L) with hexanal (0.4 mol/L) in aqueous solutions (1 h, 37°C). Lipids were extracted and analyzed by ESI-LTQ-Orbitrap-MS (shotgun lipidomics). Surprisingly, eight different products were identified including two previously reported (Schiff-base and amide) and six new compounds. The new PE-hexanal adducts contained dimeric and trimeric hexanal conjugates formed by consecutive β-aldol condensation.

In order to study the biological relevance of hexanal-adducts, trimeric hexanal-PE was purified and added to multilamellar vesicles of dipalmitoleoylphosphatidylethanolamine (DiPoPE, 5.5 g/L). Differential scanning calorimetry (DSC) enabled to monitor the membrane curvature change induced by the incorporation of trimeric hexanal-PE into lipid vesicles. It was shown that trimeric hexanal modification appears to be sufficient to increase the negative curvature of multilamellar vesicles *in vitro*. This indicates that formation of the hexanal-PE adduct could influence the structure and the status of biological membranes leading to a change in macroscopic structure, stability and function.

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PP05

## Specific kidney cortico-medullary distribution of NADPH oxidase-gp$^{91phox}$ contributes to age-related hypertension and salt-sensitivity in Fischer Brown Norway (FBN) rats

M. Asghar, I. Pokkunuri, G. Chugh, M. Lokhandwala

*Department of Pharmacological and Pharmaceutical Sciences Heart and Kidney Institute, University of Houston, Houston, TX, USA*
*E-mail address:* masghar@uh.edu (M. Asghar)

We recently reported that oxidative stress is causal to hypertension and impaired kidney angiotensin AT1 receptor (AT1R) and dopamine D1 receptor (D1R) functions in aging FBNs. These renal receptors by counter-regulating each other's functions maintain sodium homeostasis and normal blood pressure (BP). Here, we tested whether or not these rats develop salt-sensitivity and examined cortico-medullary distribution of these receptors and oxidant producing enzyme NADPH oxidase-gp$^{91phox}$ in response to 4-week normal-salt (NS, 0.4% NaCl) and high-salt (HS, 8% NaCl) feeding in adult (3-month) and old (21-month) FBNs. BP in conscious animals was measured by radiotelemetry. Distribution of D1R, AT1R and gp$^{91phox}$ were determined by RT-PCR and western blotting. We found that NS-fed old rats had higher BP than NS-fed adult rats, which further increased with HS feeding in old rats. BP did not change with HS feeding in adult rats. The levels of D1R mRNA in cortex or in medulla were not different between NS-fed adult and old rats, but decreased in both cortex and medulla only in HS-fed old rats. Contrary to this, AT1R mRNA levels were higher only in cortex of old rats fed either NS or HS. Moreover, despite similar gp$^{91phox}$ mRNA levels in cortex and medulla between adult and old rats fed either NS or HS, gp$^{91phox}$ protein levels were higher in the cortex of old rats irrespective of salt treatment and increased only in the medulla of old rats with HS in these rats. Our results suggest that age-related hypertension and salt-sensitivity is associated with specific cortico-medullary distribution of gp$^{91phox}$, which, by a redox mechanism, may cause transcriptional and functional dysregulation of AT1R and D1R contributing to hypertension in the old animals.

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PP06

## The role of α-tocopherol in mediating anti-proliferative effects through mitochondrial targeting in HepG2 cells

Ruth Banks [a], Marc Birringer [c], Regina Brigelius-Flohè [d], Damon Lowes [b], John R. Speakman [a], Colin Selman [e]

[a] *Integrative Environmental Physiology, Institute of Biological and Environmental Sciences, University of Aberdeen, UK*
[b] *Medicine and Therapeutics, Institute of Medical Sciences, University of Aberdeen, UK*
[c] *Department of Nutritional, Food and Consumer Studies, University of Applied Sciences, Fulda, Germany*
[d] *Biochemistry of Micronutrients Department, German Institute of Human Nutrition, Potsdam- Rehbruecke, Germany*
[e] *College of Medical, Veterinary and Life Sciences, University of Glasgow, UK*
*E-mail address:* r.banks@abdn.ac.uk (R. Banks)

Vitamin E comprises a family of eight steroisomeric compounds, including the tocopherols and tocotrienols (α-, β-, δ-, γ-). α-tocopherol has gained considerable interest extending beyond its antioxidant properties; previously we showed that long-term α-tocopherol supplementation in C57BL/6 mice induced a transient increase in xenobiotic metabolism, which was associated with a 15% increase in median lifespan. Furthermore, α-tocopherol metabolites, in particular the long-chain α-tocopheryl acid (α-13'-COOH), were recently shown to possess anti-cancer activity *in vitro*, highlighting the potential association of vitamin E metabolism with longevity in mammals. In the present study, we have used the HepG2 cell line as an *in vitro* model to further understand this growth-preventative response and to elucidate the mechanism through which α-tocopherol may act to promote lifespan extension. Key mitochondrial signaling pathways governing energy metabolism and biogenesis were examined in response to 'physiological' doses (2 μM) and 'pharmacological' doses (20 μM) of α-13'-COOH. Exposure to physiological levels of α-13'-COOH increased oxygen consumption, proton leak and caused subsequent depletion of the cellular reserve capacity in HepG2 cells. Elevated SIRT-1 and PGC-1α levels implied adaptive energy metabolism and increased UCP-3 and ANT protein levels suggested cellular adaptation to increased substrate oxidation and/or sensitization to apoptosis. Pharmacological doses of α-13'-COOH significantly increased the rate of ROS production and caused complete loss of cellular mitochondrial reserve capacity. Furthermore, the presence of high levels of tyrosine residue nitration and considerably reduced cell viability could indicate damage to bioenergetic components. Cell cycle arrest was indicated by increased levels of p-P53[46] and P21 levels in association with reduced cyclin D1 levels. These results further clarify the anti-proliferative role of α-tocopherol and highlight the significance of its metabolism as a potential mechanism of longevity assurance in mammals.

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PP07

## Characterization and Kinetic Studies of New Heteroaryl Nitrones as Spin Traps

G.Barriga González [a], E. Chamorro [a], C. Olea-Azar [b], E. Norambuena [c], H. Cerecetto [d], M. González [d], W. Porcal [d]

[a] *Departamento de Ciencias Químicas, Facultad de Ciencias Exactas, Universidad Andres Bello, Santiago, Chile*
[b] *Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile*
[c] *Departamento de Química, Facultad de Ciencias Básicas, Universidad Metropolitana de Ciencias de la Educación, Santiago, Chile*
[d] *Departamento de Química Orgánica, Facultad de Química - Facultad de Ciencias, Universidad de la Republica, Montevideo, Uruguay*
*E-mail address:* gbarriga@ciq.uchile.cl (G.Barrig. González)

Although nitrones constitute a structural group highly used in trapping of short-life-free radicals some of the currently available nitrones, used as "Spin Traps" (ST), display problems of solubility in aqueous media, selectivity and bioavailability. There is an extended variety of STs designed in the last 15-20 years as DMPO's or PBN's modifications, for instance EMPO, DEPMPO, POBN, SPBN. In spite of these modifications, STs have some limitations mainly related to the short half-life of their spin adducts which does not allow a better study of free radicals with short lives and specific phenomena. In this work we present three novel nitrones belonging to two structurally different heterocyclic families, these new nitrones have longer spin adducts half-lives than the currently used ST. The first studied nitrone, NT1, presents a spin adduct with a half-life for the $^{\bullet}$OH radical near to 2-hours and 3.4-times better trapping ability than DMPO according to the competition studies. The second nitrone, NT2, is 4.7 times better than DMPO in the competition studies, and finally the third nitrone, NT3, is 5.0 times better than DMPO in the competition studies. These new heteroaryl nitrones are able for trapping free radicals centered on O, N, C and S atoms and are soluble in aqueous media/acetonitrile mixture. These new nitrones have shown a good capacity for trapping free radicals in different biological media.

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PP08

## Plasma Malondialdehyde and Erythrocyte Superoxide Dismutase Levels in Obesity

Eda Becer, Güldal Mehmetçik

*Near East University, Faculty of Pharmacy, Department of Biochemistry, Nicosia, Cyprus*

Obesity may be a state of chronic oxidative stress. Hyperglycemia, elevated plasma lipid levels and inadequate antioxidant defenses promotes oxidative stress in obesity. The aim of this study was to determine the relationship between anthropometric parameters, plasma lipid levels and markers of oxidant/antioxidant status in obese subjects compared with non-obese subjects and its relationship with obesity-linked insulin resistance. The study included 110 obese and 90 non-obese subjects. Oxidative stress were assessed by measuring the concentration of plasma malondialdehyde (MDA). The cytoprotectic enzyme, erythrocyte superoxide dismutase (SOD) activities were measured as biological markers of antioxidant status. We also evaluated antropometric parameters and plasma lipid levels. The obese subjects had significantly higher plasma MDA levels than non-obese subjects. Erythrocyte SOD activities significantly lower in obese group compared ton non-obese group. Non-obese subjects had significantly lower HOMA-IR compared to obese subjects. Plasma MDA levels were

significantly positively correlated with body mass index (BMI) and hip circumference in obese group. Erythrocyte SOD showed significant negative correlation with BMI in obese group. Furthermore, erythrocyte SOD were significantly negative correlated HDL-cholesterol in non-obese group. The increase in adipose tissue as a result of obesity causes a chronic inflammation which in turn may cause increases in lipid peroxidation and decreases in erythrocyte cytoprotectic. So our result suggest that obesity even in the absence of other confounding factors such as diabetes and hypertension, is an independent risk factor for lipid peroxidation and increase in oxidative stress.

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PP09

## Serum VEGF, CD40L and NO levels in Peripheral Arterial Disease

Eda Becer [a], Elif Özkök [b], Fatih Yanar [c], Yılmaz Başar [c], Nihal Salmayenli [d], Güldal Mehmetçik [a]

[a] *Department of Biochemistry, Faculty of Pharmacy, Near East University, Nicosia, Cyprus*
[b] *Department of Neuroscience, Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey*
[c] *Department of General Surgery, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey*
[d] *Central Laboratory of Clinical Biochemistry, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey*

Peripheral arterial disease (PAD) is closely associated with systemic atherosclerosis and thrombosis. It has been shown that serum CD40 ligand, a transmembrane protein expressed on endothelial cells, is related to atherosclerosis from early stages to late severe thrombosis. Atherogenesis begins with endothelial dysfunction. The vascular endothelium plays a critical role in the atherothrombogenesis. The production of endothelial mediators are affected by injury of endothelium. Vascular endothelial growth factor (VEGF) is a potent endothelial cell-specific mitogen. It has been shown that VEGF plasma concentrations increased in patients with PAD. In the light of these findings, we aimed to research the levels of serum CD40L, VEGF and nitrite/nitrate levels in patients with PAD. We enrolled 106 patients with PAD and 65 age-matched healthy controls into study. The concentrations of VEGF, sCD40 ligand were determined using with ELISA method in serum samples. The final products of NO in vivo are nitrite ($NO_2$) and nitrate ($NO_3$). We measured serum nitrite ($NO_2$) and nitrate ($NO_3$) levels of the final product of NO by colorimetric method. Statistical analyses were performed using the SPSS software package, revision 15.0. p value $< 0.05$ was considered significant. There was no difference in age between controls and patients. The mean plasma concentrations of VEGF ($p < 0.001$), sCD40L ($p < 0.001$) were higher in PAD patients than those of the controls. In contrast, the concentrations of total nitrate and nitrite were lower in patients with PAD than those of the controls ($p < 0.05$). Increased CD40L that could play an important role in human atherosclerosis may be an indicator for PAD. Also, increased VEGF levels were compatible with the results of the other studies in PAD. However, decreased total nitrate/nitrite levels may be due to reduced synthesis and inactivation of NO in patients with PAD.

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PP10

## Carbonylated HeLa cell proteome

Ravi Chand Bollineni [a,b], Ralf Hoffmann [a,b], Maria Fedorova [a,b]

[a] *Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Center for Biotechnology and Biomedicine, Universität Leipzig, Leipzig, Germany*
[b] *Center for Biotechnology and Biomedicine, Universität Leipzig, Leipzig, Germany*
*E-mail address:* ravi.bollineni@bbz.uni-leipzig.de (R.C. Bollineni)

Protein oxidation is considered as a major hallmark of aging and age-related disorders. Carbonylation, a major class of oxidative protein modifications, is widely accepted as biomarker of oxidative stress, aging and age-related disorders. However, studies of cellular/protein distribution and biological role of protein carbonylation are limited. Here we present a proteome-wide profiling of carbonylated proteins from HeLa cells treated with hydrogen peroxide, using our previously published LCxLC-(LDI-TOF)-ESI-Orbitrap-MS/MS approach [1]. This high throughput proteomic approach identified a total of 210 carbonylated proteins. Surprisingly, when compared with entries of cell death database (2480 entries), one third of carbonylated HeLa proteins were shown to be regulated during apoptosis, whereas 41 were involved in autophagy (19%) and nine in mitotic catastrophe (4%). STRING functional interaction analysis of the first set of carbonylated proteins regulated during apoptosis revealed several nodes with proteins involved in nuclear-cytoskeleton anchoring, protein synthesis, vesicle transport and cell proliferation/growth, moreover proteins involved in autophagy were mostly ribonuclear and cytoskeletal proteins. Main functional interaction between carbonylated proteins not present in cell death database (119 proteins) were connected with centrosome/spindle organization (chromosome segregation during cell division). Additionally, high throughput MS based approach allowed us to identify 643 carbonylation sites within 210 proteins, out of which 284 modification sites were found on lysine (44%), 121 on arginine and threonine (19% each), 117 on proline (18%). This high number of carbonylated sequences allowed us to perform a statistically significant analysis of sequence motifs associated with carbonylation sites. Motif analysis revealed significant enrichment of basic amino acids (lysine and arginine) around $\pm$ 10 positions from the modification sites (especially at +7, +8, -5, and -7). In conclusion, these results provide interesting insights into the specificity of cellular protein carbonylation associated with cell death and the effect of surrounding amino acids on modification frequencies.

### References

[1] Bollineni, R.; Hoffmann, R.; Fedorova, M.; Identification of protein carbonylation sites by two-dimensional liquid chromatography in combination with MALDI- and ESI-MS. J Proteomics 74 (2011) 2338-2350; 2011.

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PP11

## Roles for hypoxia inducible factor 1 and glucose metabolism in tumor cell survival following photodynamic therapy

Mans Broekgaarden [a], Milan Kos [a], Ruud Weijer [a], Massis Krekorian [a,b], Thomas M. van Gulik [a], Michal Heger [a]

[a] *Department of Experimental Surgery, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands*

[b] *Institute for Life Sciences and Chemistry, Utrecht University College, Utrecht, The Netherlands*

Photodynamic therapy (PDT) is a tumor treatment modality based on the photochemical production of reactive oxygen species at the tumor site. By irradiating tumor-replete photosensitizers, the tumor tissue becomes exposed to severe oxidative stress, vascular shutdown and corollary hypoxia, and a prolonged anti-tumor immune response. Independently of PDT, these types of stress induce survival responses that are partly mediated by the transcription factors hypoxia inducible factor 1 (HIF-1) and nuclear factor κB (NFκB), which regulate anaerobic glucose metabolism, angiogenesis, and inflammation to ensure tumor cell survival. The goal of this study was to investigate whether HIF-1 and NFκB were activated by PDT and whether increased glucose metabolism is involved in tumor cell survival.

Human epidermoid carcinoma cells (A431) and human extrahepatic cholangiocarcinoma cells (Sk-Cha1) were incubated with zinc phthalocyanine (ZnPC)-encapsulating cationic liposomes and subjected to PDT (671nm, 30J/cm$^2$). Immunoblotting and the use of an oxygen tension-dependent degradation domain-containing reporter construct, which produces a green fluorescent protein during hypoxia, revealed activation of HIF-1α as a result of PDT + hypoxia, but not PDT itself at normoxia. Moreover, glucose uptake was enhanced following PDT in Sk-Cha1 cells but not in A431 cells. Glucose depletion following PDT was able to exacerbate the extent of cell death in A431 cells.

This study confirmed that HIF-1 is activated in cancer cells following PDT with ZnPC-liposomes. Furthermore, this study is the first to demonstrate a role for anaerobic glucose metabolism in cancer cell survival post-PDT. Experiments on the activation of NFκB are currently performed. Overall, these findings encourage follow-up studies on the inhibition of these survival pathways following PDT as a means to enhance therapeutic outcomes of PDT.

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PP12

## The apoptotic effects of resveratrol in colon cancer cell line

C. Corek [a,b], E.M. Altundag [a,b], B. Catalgol [a,b], S. Kocturk [a,c], N.K. Ozer [a,b], Y. Taga [a,b]

[a] *Genetic and Metabolic Diseases Research and Investigation Center (GEMHAM), Marmara University, Istanbul, Turkey*
[b] *Dept. of Biochemistry, Faculty of Medicine, Marmara University, Istanbul, Turkey*
[c] *Dept. of Biochemistry, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey*
*E-mail address:* ceydacorek@gmail.com (C. Corek)

Colon cancer has the third highest incidence among cancers and also is the third leading cause of mortality from cancer deads worldwide. Cancer chemoprevention has been considered as a promising strategy to controlling cancer death. Epidemiological studies have linked resveratrol intake with reduced risk of colon cancer by the inhibition of DNA synthesis and decreasing the expression levels of inflamatory genes. In this study, the effect of resveratrol on apoptosis has been tested in a colon cancer cell line, HCT-116. First, WST-1 as viability analysis and flow cytometric count of Annexin V / Propidium iodide (PI) as apoptosis and cell cyle analysis has been performed. The production of reactive oxygen species was monitored with the fluorescent probe DCFH-DA. The protein levels of PARP has been determined in the resveratrol treated cells. We observed that resveratrol inhibits the growth of HCT-116 cells. Data show that treatment of HCT-116 cells with resveratrol resulted in a dose dependent increase in cell death, and the IC50 value was estimated to be 50 μM. Resveratrol significantly induced apoptosis and inbited cell cycle progression(G0/G1) and caused accumulation of cells in S phase. ATPlite assay showed decrease in the level of ATP amounts in resveratrol treated HCT-116 cells. Western analyses demonstrated an increase in PARP cleavage. In this direction, resveratrol induced apoptosis in HCT-116 cells were shown to be reactive oxygen species dependent.

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PP13

## Polyacrylic acid coated and naked iron oxide nanoparticles: Their effects on neutrophils' oxidative burst and inflammatory process

Diana Couto [a], Irene Dias [a], Marisa Freitas [a], Graça Porto [b], M.Arturo Lopez-Quintela [c], José Rivas [d], Paulo Freitas [d], Félix Carvalho [e], Eduarda Fernandes [e]

[a] *REQUIMTE, Deparment of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal*
[b] *Service of Clinical Hematology, Santo António Hospital, Porto, Portugal*
[c] *Department of Physical Chemistry, University of Santiago de Compostela, Santiago de Compostela, Spain*
[d] *International Iberian Nanotechnology Laboratory, Braga, Portugal*
[e] *REQUIMTE, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal*

The potential use of iron oxide nanoparticles is becoming increasingly attractive in several fields of biomedicine, namely in cancer therapy, drug delivery and as contrast agents in imagiologic techniques, such as magnetic resonance imaging. Currently, there are several types of iron oxide nanoparticles available in the market, with different sizes and coatings. These different structural and size features may be determinant for their biomedical efficacy and safety. However, human safety concerns involving the use of these nanoparticles is an unsolved issue, as the studies evaluating the toxicity of these nanoparticles are still scarce, particularly concerning their putative pro-inflammatory properties [1]. The production of cytokines as well as chemotaxis and induction of oxidative burst in neutrophils, are known to play an important role in inflammatory processes [2]. For this reason, the aim of this study was to evaluate the effect of two different types of superparamagnetic iron oxide nanoparticles (polyacrylic acid coated and naked) on human neutrophils' chemotaxis and oxidative burst, and on the induction of cytokine production, through the determination of IFN-γ, TNF-α, IL-6, IL-10, IL-1β and IL-8 production in human blood. For that purpose, the modulation of the neutrophils' oxidative burst was studied using the probe dihydrorhodamine 123, neutrophils' chemotaxis was evaluated using a fluorometric kit (CytoSelect™ 96-well cell migration assay) and the induction of cytokine production in whole human blood was evaluated using a Multi-Analyte ELISArray kit. The obtained results demonstrated that, while chemotaxis was not observed, the studied iron oxide nanoparticles have the ability to activate neutrophils' oxidative burst and to activate all the cytokines tested. The obtained results suggest that the studied iron

oxide nanoparticles may have considerable implications in the inflammatory process during the above mentioned applications of iron oxide nanoparticles for medical purposes.

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**References**

[1] Shubayev, V.I.; Pisanic, T.R.; Jin, S.; Adv. Drug. Deliver. Rev 61 (6) (2010) 467-477.
[2] Escobar, J.; Pereda, J.; Arduini, A.; Sandoval, J.; Sabater, L.; Aparisi, L.; López-Rodas, G.; Sastre, J. Curr. Pharm. Des 15 (26) (2100) 3027-3042.

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PP14

## Cellular stress responses for monitoring and modulating ageing

Dino Demirovic [a], Sylvianne Schnebert [b], Carine Nizard [b], Suresh I.S. Rattan [a]

[a] *Laboratory of Cellular Ageing, Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark*
[b] *LVMH Recherche, Saint Jean de Braye, France*
*E-mail address:* did@mb.au.dk (D. Demirovic)

Cellular stress response is a crucial factor in maintaining efficient homeodynamics for survival, health and longevity. Both the immediate and delayed responses to external and internal stressors effectively determine the molecular biochemical and physiological stability in a dynamic and interactive manner.

There are three main aspects of stress responses: (i) immediate stress response involving extra- and intra-cellular signaling during the period of disturbance and exposure to the stressors; (ii) delayed stress response involving sensors and modulators in the presence of stressors or after the removal of the stressors; and (iii) down-stream effectors for counteracting the effects of disturbance and for re-establishing homeodynamics. At the present it is not known how these three steps are maintained interactively in terms of kinetics and intensity, and how these may alter during growth, development and ageing.

Our aim is to define and establish the immediate and delayed stress profiles of normal human skin fibroblasts undergoing ageing *in vitro*. This is done efficiently by using various cellular, molecular and antibody-based detection methods, combined with functional assays, such as wound healing *in vitro* by fibroblasts, and induction of differentiation of telomerase-immortalised stem cells. Furthermore, immediate and delayed stress profiles need to be established at several age points during the replicative senescence of cells in culture, which can then be the basis for testing potential protectors and stimulators of homeodynamics, and create a kind of "gold-standard" for monitoring the efficacy of other potential anti-ageing and pro-survival natural and synthetic compounds.

We have so far standardised an effective method for detecting all seven stress response pathways, by several biochemical methods, detecting one or more proteins exclusively involved in the specific stress response pathways. The results indicate that the ageing phenotype is a result of an ineffective probability for cells to respond to stress.

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PP15

## Implication of the circadian system in the modulation of the intracellular load of oxidized protein and its removal by the proteasome

A. Desvergne, N. Ugarte, I. Petropoulos, B. Friguet

*UR4-UPMC Vieillissement, stress et inflammation, Paris, France*
*E-mail address:* audrey.desvergne@snv.jussieu.fr (A. Desvergne)

The circadian clock generates rhythms with a periodicity of 24 hours of various biochemical and physiological processes. Recent data suggest a mutual influence between the circadian clock and the cell cycle, and provides a functional link between the circadian clock, cancer and ageing [1]. Circadian rhythmicity of antioxidant mechanisms has also long been reported [2]. The established link between the circadian clock and anti-oxidative defence suggests that elements of the redox homeostasis, including oxidized protein repair and degradation pathways such as the proteasome, could be modulated by the circadian clock. Using HEK cells synchronized by a serum shock as an initial cellular model for studying the circadian influence on protein maintenance, we have shown that the level of carbonylated protein varies rhythmically following a 24 hours period and the proteasome exhibits circadian rhythmicity in its peptidase activities. Interestingly, the rhythms match the circadian oscillations observed for protein oxidative damage. Moreover, it has been shown recently that adaptation to a Nrf2-dependent oxidative stress cause an increase in the cellular capacity to degrade oxidized proteins that are attributable to an increased expression of the 20S proteasome and its activator Pa28αβ (Pickering et al., 2011). So, using synchronized cellular models to define more precisely the modulation of proteasome function mediated by the circadian clock, we have shown that both Nrf2 and Pa28αβ exhibit a circadian expression. If as we envisage, circadian rhythmicity is involved in protein maintenance, the age-associated alteration of the circadian system may therefore contribute to the accumulation of oxidized proteins and the decline of intracellular protein maintenance. Hence, strategies that could restore this vital function may be effective in slowing ageing and the onset of diseases for which a defect in the protein homeostasis has been proposed to play a key role.

**References**

[1] Sahar, S.; Sassone-Corsi, P.; Nat Rev Cancer. 9 (2099) 886-96; 2009.
[2] Hardeland, et al., Chronobiol Int. 2 (2003) 921-62; 2003.

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PP16

## Spectroscopic study of the structural changes induced by free radical stress on oligopeptides for bone regeneration

M. Di Foggia [a,b,c,d], A. Torreggiani [a,b,c,d], P. Taddei [a,b,c,d], M. Dettin [a,b,c,d], S. Sanchez-Cortes [a,b,c,d], A. Tinti [a,b,c,d]

[a] *Dipartimento di Biochimica, Università di Bologna, Bologna, Italy*
[b] *ISOF-CNR, Bologna, Italy*

[c] *Dipartimento di Processi Chimici dell'Ingegneria, Università di Padova, Padova, Italy*
[d] *Departamento de Espectroscopia Vibracional y Procesos Multifotonicos-CSIC, Madrid, Spain*
*E-mail address:* micheled@chem.unibo.it (M. Di Foggia)

Regular oligopeptides with polar and nonpolar residues alternating as the EAK16 $(AEAEAKAK)_2$ are interesting biomimetic materials for bone implants, since they are able to self-assemble, forming a macroscopic insoluble and highly biocompatibile membrane. Their ability to create stable structures derives from both hydrophobic interactions between the aliphatic groups of non-ionic residues and ionic interactions between charged amino acids side-chains. This biomimetic membrane (mainly organized into beta-sheet secondary structure), may be subjected to oxidative radical stress during the phases of colonization and growth of bone cells (osteoblasts) as result of inflammatory phenomena. This stress could lead to alterations in the membrane structure with a consequent decrease of its biomimetic properties. In the physiological environment, the hydroxyl radical ($^{\bullet}OH$) is considered one of the most reactive oxidative species and therefore one of the most harmfull. In this context, we have studied the structure alterations suffered by 7 oligopeptides derived by EAK16 (through selective substitution of amino acids) under conditions of high oxidative radical stress. The radical species have been produced by γ-radiolysis of aqueous solutions. Structural damages, especially in terms of variation of the secondary structure and interactions between side chains, caused by the attack of $^{\bullet}OH$ radicals generated at different concentrations, were evaluated by means of IR, traditional Raman and Surface-Enhanced Raman (SERS) spectroscopies. The results have showed a different response of the oligopeptides to oxidative stress. In particular, the sequences in which aspartic acid replaces glutamic acid residue (i.e. with a shorter lateral chain) are very sensitive to radical stress exposure. In contrast, other oligopeptides showed no significant changes, thus revealing a higher resistance to degradation due to radical stress.

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PP17

## Modified lipids from LDL in the blood during mid-life increase blood brain barrier permeability

H.K.I. Dias [a], M.C. Polidori [b], G.Y.H. Lip [c], H.R. Griffiths [a]

[a] *Life and Health Sciences, Aston University, Birmingham, UK*
[b] *Institute of Biochemistry and Molecular Biology I, Heinrich-Heine University, Düsseldorf, Germany*
[c] *University of Birmingham Centre for Cardiovascular Sciences, City Hospital, Birmingham, UK*
*E-mail address:* h.k.i.dias1@aston.ac.uk (H.K.I. Dias)

Low density lipoprotein levels (LDL) are consistently elevated in cardiovascular disease. It has been suggested that those with high circulating LDL levels in mid-life may be susceptible to develop neurodegenerative diseases in later life. In the circulation, high levels of LDL are associated with increased oxidative modification (oxLDL) and nitration. We have investigated the hypothesis that disruption of blood brain barrier function by oxLDL and their lipids may increase risk of neurodegeneration in later life and that statin intervention in mid-life can mitigate the neurodegenerative effects of hyperlipidaemia. Blood from statin-naïve, normo- and hyperlipidaemic subjects (n = 10/group) was collected at baseline. Hyperlipidaemic subjects received statin-intervention whereas normolipidaemic subjects did not prior to a second blood sampling, taken after 3 months. The intervention will be completed in June 2013. Plasma was separated by centrifugation (200g, 30min) and LDL was isolated by potassium bromide density gradient ultracentrifugation. Total homocysteine, LDL cholesterol, 8-isoprostane F2α levels were measured in plasma using commercial kits. LDL were analysed by agarose gel electrophoresis. LDL-lipids were extracted by partitioning in 1:1 chloroform:methanol (v/v) and conjugated to fatty acid free-BSA in serum-free EGM-2 medium (4hrs, $37^0$C) for co-culture with human microvascular endothelial cells (HMVEC). HMVEC were maintained on polycarbonate inserts for two weeks to create a microvascular barrier. Change in barrier permeability was measured by trans-endothelial electrical resistance (TER), FITC-dextran permeability and immunohistochemistry. HMVEC glutathione (GSH) levels were measured after 2 hours by GSH-glo assay. LDL isolated from statin-naïve hyperlipidaemic subjects had higher mobility by agarose gel electrophoresis (Rf;$0.53 \pm 0.06$) and plasma 8-isoprostane F2α ($43.5 \pm 8.42$ pg/ml) compared to control subjects ($0.46 \pm 0.05$ and $24.2 \pm 5.37$ pg/ml; $p < 0.05$). Compared to HMVEC treatment with the LDL-lipids (5μM) from normolipidaemic subjects, LDL-lipids from hyperlipidaemic subjects increased barrier permeability ($103.4 \pm 12.5$ $\Omega cm^2$ v $66.7 \pm 7.3$ $\Omega cm^2$,$P < 0.01$) and decreased GSH (18.5 nmol/mg v 12.3 nmol/mg; untreated cells $26.2 \pm 3.6$ nmol/mg).

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PP18

## Effects of renal function and antioxidant status on activation of nuclear factor κB in peripheral blood mononuclear cells

G. Faustmann [a], H. Hafner-Giessauf [b], H.J. Gruber [c], J. Grabher [b], A.R. Rosenkranz [b], B. Tiran [c], J.M. Roob [b], B.M. Winklhofer-Roob [a]

[a] *Human Nutrition & Metabolism Research and Training Center, Institute of Molecular Biosciences, Karl-Franzens University of Graz, Austria*
[b] *Clinical Division of Nephrology, Department of Internal Medicine, Medical University of Graz, Austria*
[c] *Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Austria*

Background: The nuclear factor κB (NF-κB) signaling pathway plays a central role in chronic inflammation and is an important interface between inflammation and oxidative stress. The heterodimer p65-p50 is the most abundant transcription factor of the NF-κB family. Purpose: The purpose of this cross-sectional study of the FP7 EU project BIOCLAIMS was to investigate the activation of both the p65 and p50 dimers in individuals with normal and impaired renal function and possible associations with antioxidant status. Methods: Peripheral blood mononuclear cells were collected from 229 (104 M, 125 F) study subjects, aged $52.1 \pm 15.1$ years, not taking statins or angiotensin-converting enzyme inhibitors (which have been shown to have an effect on NF-κB activation), and with estimated glomerular filtration rate (eGFR, MDRD) of $72 \pm 22.9$ (19.3-123.4) mL/min/1.73 $m^2$. Activation of NF-κB dimers containing p65 and p50 subunits was determined in whole cell extracts using an ELISA-based assay (TransAM Active Motif), in which activated dimers bind in a specific manner to NF-κB consensus binding sites on immobilized nucleotides and

can be detected by antibodies directed against either p50 or p65. Plasma antioxidants, including ascorbate, α- and γ-tocopherol, α- and β-carotene, lycopene, β-cryptoxanthin, and lutein/zeaxanthin, were determined by HPLC. Results: Activation of p50-containing NF-κB dimers increased significantly with declining eGFR ($r$=-0.215, $P<0.001$), while activation of p65-containing NF-κB dimers did not change. Activation of p50-containing dimers decreased with increasing plasma ascorbate ($r$=-0.167, $P$=0.012) and lycopene concentrations ($r$=-0.207, $P$=0.002). Both ascorbate ($r$=0.345, $P<0.001$) and lycopene concentrations ($r$=0.193, $P$=0.003) decreased with declining eGFR. Plasma ascorbate and lycopene concentrations were 68 ± 18.7 (9.76-121) μmol/L and 0.60 ± 0.30 (0.06-1.68) μmol/L, respectively. Conclusions: These data indicate that impaired renal function has an impact on the activation of p50-containing NF-κB dimers, which could be explained by impaired vitamin C and lycopene status.

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PP19

## Carotenoids inhibit lipid peroxidation induced by *tert*-butyl hydroperoxide, but not the depletion of glutathione induced by peroxyl radicals in human erythrocytes

R.C. Chisté [a,b], M. Freitas [a], A.Z. Mercadante [b], E. Fernandes [a]

[a] *REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto (UP), Porto, Portugal*
[b] *Department of Food Science, Faculty of Food Engineering, University of Campinas (UNICAMP), Campinas-SP, Brazil*
*E-mail address:* renanchiste@gmail.com (E. Fernandes)

Despite the presence of endogenous antioxidants in erythrocytes, such as glutathione, these cells are highly susceptible to oxidative damage. Here, we evaluated the potential of some natural antioxidants: β-carotene, zeaxanthin, lutein, β-cryptoxanthin and lycopene, which are carotenoids usually detected in human blood plasma, to prevent both lipid peroxidation and depletion of glutathione in erythrocytes caused by *tert*-butyl hydroperoxide (tBHP) and peroxyl radicals ($ROO^{\bullet}$)-induced oxidative damage, respectively. Human erythrocytes were isolated from fresh blood from healthy volunteers (n=4) and diluted to a known density [$500x10^6$ cells/mL - lipid peroxidation assay - and $30x10^6$ cells/mL - reduced glutathione (GSH) and oxidized glutathione (GSSG) assays]. Erythrocytes were exposed to carotenoids (0.09-3 μM), for 30 min, at 37°C, in a water bath and then to 0.2 mM tBHP/30 min (lipid peroxidation) or 17 mM AAPH (α,α'-azodiisobutyramidine dihydrochloride)/15 min (glutathione depletion) as $ROO^{\bullet}$ generator. The lipid peroxidation was measured by the thiobarbituric acid-reactive substance (TBARS) assay and the levels of GSH and GSSG in erythrocytes suspension by a spectrophotometric recycling enzymatic assay. Regarding inhibition of TBARS formation, lycopene was the most efficient carotenoid ($IC_{50}$ = 2.20 ± 0.4 μM) and presented higher antioxidant capacity than ascorbic acid (positive control, $IC_{50}$ = 10 ± 2.9 μM). The other carotenoids were less efficient and at 3 μM β-cryptoxanthin presented 37 ± 2.0% of inhibition capacity, followed by lutein (27 ± 2.8%), zeaxanthin (19 ± 3.2%) and β-carotene (5 ± 2.4%). On the other hand, the tested carotenoids did not prevented the $ROO^{\bullet}$ mediated GSH depletion and GSSG formation. This lack of interaction may be explained due to the difference of polarity between the carotenoids (apolar) and glutathione (polar). This hypothesis was confirmed since trolox (polar synthetic antioxidant) prevented GSH depletion ( ≈ 30%) and GSSG formation ( ≈ 84%) at the highest tested concentration (16 μM). These results suggest that carotenoids have important antioxidant properties to prevent oxidative damages in erythrocytes through interaction with cellular lipophilic compartments.

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PP20

## Vitamin E metabolism is the major determinant of γ-tocopherol concentrations in mammals but appears to interact with α-tocopherol transfer protein

Nadine Grebenstein [a,b], Monika Schumacher [a], Lutz Graeve [a], Jan Frank [a,b]

[a] *University of Hohenheim, Institute of Biological Chemistry and Nutrition, Stuttgart, Germany*
[b] *University of Bonn, Department of Nutrition and Food Science, Bonn, Germany*
*E-mail address:* jan.frank@nutrition-research.de (J. Frank)

The mechanisms responsible for the preferential retention of a single (α-tocopherol, αT) of the eight vitamin E compounds in the body are poorly understood. Observations from our group and others suggest that vitamin E-metabolism rather than the hepatic α-tocopherol transfer protein (TTP), as formerly suggested, drives the discrimination against non-αT congeners in mammals. Studying homozygous and heterozygous TTP-knockout mice vs. wildtype mice, we observed that vitamin E-metabolism is indeed the main force behind the selective retention of αT in mammals and noticed an interaction of TTP with vitamin E-metabolism. We then used TTP-transfected and non-TTP-expressing liver cells in combination with the vitamin E metabolism-inhibitor sesamin and confirmed the dominant role of metabolism, but unexpectedly observed that vitamin E-degradation decreases with increasing TTP expression. Competitive displacement of γ-tocopherol (γT) from TTP with increasing concentrations of αT in TTP-expressing HepG2 cells enhances the secretion of the short-chain metabolite γ-carboxyethyl hydroxychromanol. In conclusion, the data from our in vivo and in vitro experiments supports that vitamin E-metabolism is the major determinant of γT concentrations in mammals and that hepatic vitamin E metabolism interacts with the TTP. Based on our observations, we propose that TTP may bind and partly protect γT from the degrading enzymes during intracellular trafficking.

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PP21

## Excitotoxicity in AD is partially caused by the inactivation of APC/C-Cdh1 E3 Ubiquitin Ligase

T. Fuchsberger, E. Giraldo, A. Lloret, J. Viña

*University of Valencia, Faculty of Medicine, Department of Physiology, Valencia, Spain*
*E-mail address:* f.tanja@gmx.at (T. Fuchsberger)

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.

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PP22

## Lipophilic caffeic acid derivatives protect cells against $H_2O_2$-induced DNA damage by chelating intracellular "labile iron"

N. Kitsati [a], A. Barbouti [b], D. Galaris [a]

[a] *Laboratory of Biological Chemistry, University of Ioannina Medical School, Ioannina, Greece*
[b] *Laboratory of Anatomy-Histology-Embryology, University of Ioannina Medical School, Ioannina, Greece*
*E-mail address:* dgalaris@uoi.gr (D. Galaris)

Humans, take up daily huge amounts of hydroxyl-cinnamate derivatives, mainly in the form of caffeic acid esters. Naturally occurring cinnamic acid derivatives are ubiquitously distributed in the plant kingdom and it has been proposed that their consumption contributes to the maintenance of human health, through modulation of a variety of molecular mechanisms. However, the exact mode of biochemical action for each of the numerous compounds contained in food remains obscure. In this investigation, we evaluated the ability of several cinnamic acid derivatives (trans-cinnamic, p-coumaric, caffeic and ferulic acids), as well as synthetic caffeic acid -methyl, -propyl and -phenethyl esters to protect nuclear DNA in cells exposed to $H_2O_2$. It was observed that effective protection was dependent on the ability of each compound: (i) to penetrate cell membrane and (ii) to chelate intracellular "labile" iron. These results support the notion that numerous lipophilic iron chelating compounds, present abundantly in Mediterranean diet plant-derived components, may protect cells in conditions of oxidative stress by inhibiting the formation of extremely reactive free radicals from less reactive intermediates of oxygen reduction. Whether this pathway may be important contributor toward maintenance of human health is not clear at present and need further investigation.

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PP23

## Implication of "labile iron" in $H_2O_2$-induced cell signaling

M.D. Mantzaris [a], I. Verginadis [a], V. Skiada [a], S. Bellou [b], S. Kitsati [a], D. Galaris [a]

[a] *Laboratory of Biological Chemistry, University of Ioannina Medical School, Ioannina, Greece*
[b] *Foundation for Research & Technology-Hellas, Institute of Molecular Biology & Biotechnology, Department of Biomedical Research, Ioannina, Greece*
*E-mail address:* dgalaris@uoi.gr (D. Galaris)

The role of hydrogen peroxide ($H_2O_2$) as second messenger in cell signaling has been studied extensively the last years and it has been established to participate in main redox signaling pathways, including cytokine, growth factor and transcriptional factor associated signals. However, the exact molecular mechanisms underlying these processes remain poorly understood and need further investigation. Our results indicate that intracellular labile iron plays critical role in pathways associated with $H_2O_2$-induced cell signaling. Modulation of the intracellular level of "labile iron" either by iron chelator treatment or by genetic manipulation of proteins associated with iron homeostasis induced profound effects on cell signaling. In order to identify the points of action and to elucidate the exact molecular mechanisms of these iron effects, we investigated several steps of $H_2O_2$-induced apoptosis (chromatin condensation and fragmentation, activation of the caspase cascade, release of cytochrome c from mitochondria, translocation of BAX from cytosol to mitochondria and phosphorylation of mitogen- activated protein kinases - MAPKs). In addition, since the activation of MAPKs is regulated by the activity of upstream kinases and their relevant phosphatases, we examined the effect of iron deprivation on selected members of these proteins. It was observed that labile iron is implicated both in the activation of "apoptosis signaling regulating kinase-1" (ASK-1) and the inactivation of "dual specificity protein phosphatase-1" (DUSP-1). It is concluded, that the level of intracellular "labile iron" represents a key player in redox signaling, able to determine the final effect of certain signals. However, the underlying molecular mechanisms remain unclear and need further investigation.

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PP24

## Enhanced proteasome degradation extends *Caenorhabditis elegans* lifespan and ameliorates neurodegeneration

K. Georgila [a1], N. Chondrogianni [a1], N. Kourtis [b], N. Tavernarakis [b], E.S. Gonos [a]

[a] *Institute of Biology, Medicinal Chemistry & Biotechnology, National Hellenic Research Foundation, Athens Greece*
[b] *Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece*
*E-mail address:* kgeorgila@gmail.com (K. Georgila)

Aging is associated with decline of proteostasis and accumulation of damaged macromolecules. The proteasome is the major cellular protease implicated in the removal of both normal and damaged proteins, having an impaired function during aging. In previous reports using human cells, we demonstrated that proteasome activation through overexpression of proteasome subunits confers lifespan extension and resistance to oxidative stress. In this study, we sought to investigate the impact of enhanced proteasome degradation on a multicellular organism and employed *Caenorhabditis elegans* as an established model of organismal aging. We found enhanced proteasome activity upon overexpression of a single core proteasome subunit in wild type worms. We explored the effects of proteasome activation on lifespan and on animal survival under proteotoxic conditions. Finally, we examined the impact of enhanced proteasome activity on age-associated pathologies by exploiting established models of neurodegeneration. Understanding the mechanism by which restoration of proteostasis via increased proteasome function, decelerates the aging process may lead to new therapeutic and anti-aging interventions.

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[1] Equal contribution.

PP25

## Mitochondria-related effects and epigenetic changes induced by incretins and humanin in pancreatic mouse beta cells

J. Góralska, A. Śliwa, A. Gruca, B. Zapała, T. Staszel, U. Raźny, A. Zdzienicka, B. Kieć-Wilk, A. Dembińska-Kieć

*Department of Clinical Biochemistry, Jagiellonian University Medical College, Krakow, Poland*
*E-mail address:* jgoralska@cm-uj.krakow.pl (J. Góralska)

Background: Glucose-dependent insulinotropic peptide (GIP) and Glukagon-like peptide-1 (GLP-1) are incretin hormones targeting pancreatic β-cells, where they enhance insulin secretion in a glucose depended manner. Recent studies reported that excess of GLP-1 exerted other beneficial effects, including antiapoptotic and proliferative effects on β-cells. Humanin, the mitochondrial DNA derived 24-amino acid peptide is reported to act by suppressing apoptosis as well as exerting anti-inflammatory properties, in which some mechanism improving mitochondrial bioactivity could be involved. We investigated the effects of incretins and humanin on β-cells function and survival under cytokine-induced stress. Methods: The mouse line of pancreatic beta-cells BTC6 were preincubated with humanin HNG or GIP/GLP-before the challenge with TNF-α for the following 24h. Apoptosis was measured using Annexin V /propidinum iodine staining by flow cytometry, as well as caspases activity. The mitochondrial membrane potential was measured by JC-1 fluorescence by flow-cytometry. Oxygen consumption in intact cells was measured using high resolution respirometry and ATP-generation was monitored. Glucose-stimulated insulin secretion, central to normal control of metabolic fuel homeostasis, was also studied. Epigenetic changes were studied measuring global DNA methylation using MethylFlash Methylated DNA Quantification Kit. Results: In the TNF-α induced model of apoptosis, prevention of especially late apoptosis of BTC6 cells by GIP as well as by humanin, was observed. Caspase-9 and caspase-8 activity together with mitochondrial membrane potential changes revealed the pathway of apoptosis. Humanin was also found to enhance DNA methylation. GLP-1 potentiated GSIS, also in presence of TNF-α. Conclusions: These results suggest that humanin exert some beneficial effects promoting β-cells survival under cytokine-induced stress. Incretins (GIP and GLP-1) prevent β-cells dysfunction and consistently insulin secretion, partially through the mitochondria-related pathways. Different mechanism may be involved in these protective effects, including epigenetic modification –methylation of DNA.

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PP26

## Stimulation of insulin-like action in 3T3-L1 cells exposed to mild dose of lipopolysaccharide

Gowri Kanagasabapathy [a,b], Chua Kek Heng [a,b], Umah Rani Kuppusamy [a,b]

[a] *Department of Biomedical Science, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia*
[b] *Mushroom Research Centre, University of Malaya, Kuala Lumpur, Malaysia*
*E-mail address:* umah@um.edu.my (G. Kanagasabapathy)

Excessive differentiation of adipocytes lead to obesity and this phenomenon has been linked to oxidative stress and chronic low grade inflammation. Studies using murine preadipocytes (3T3-L1 cell line) have demonstrated that lipopolysaccharide (LPS) stimulate adipose tissue-derived cytokines involved in differentiation and inflammation. In this study, the effects of LPS (Escherichia coli [055:B5]) on 3T3-L1 preadipocyte differentiation and the expression of glutathione peroxidise (GPx3), lipogenesis markers and inflammatory cytokines were investigated. Fully differentiated adipocytes were treated with various concentrations of LPS for 3 days and the lipid accumulation was measured using Oil Red O staining. The RNA was extracted and the expression of markers was quantified using Real-Time Taqman-PCR. Insulin was used as a positive control. LPS treated adipocytes stimulated lipogenesis at lower concentrations (0.001- 0.01μg/ml) but inhibited lipogenesis at higher concentrations (0.1- 10μg/ml). Peroxisome proliferator activated-receptor-gamma, sterol regulatory binding protein-1c, lipoprotein lipase, adiponectin, glucose transporter-1 and glucose transporter-4 were up-regulated in 0.001 μg/ml and 0.01 μg/ml LPS treated adipocytes (similar to insulin effect) however, the effect was more enhanced at a lower dose (0.001μg/ml). The expression of anti-inflammatory cytokine, interleukin 10 (IL-10)

was increased in 0.001μg/ml LPS treated adipocytes and the pro-inflammatory cytokine, interleukin 6 (IL-6) was increased in 0.01 μg/ml LPS treated adipocytes. The expression of both IL-6 and IL-10 cytokines were not evident in insulin treated adipocytes. The expression of GPx3 was increased in low dose LPS and insulin treated adipocytes possibly as a protective mechanism to counteract inflammatory signals and/or free radicals. Suppression or absence of GPx3 expression upon exposure to inflammatory stimuli might increase local reactive oxygen species accumulation in adipocytes. This in turn may lead to oxidative stress, metabolic dysregulation and ultimately obesity. In conclusion, lower concentration of LPS may have insulin-like properties. However at higher concentrations, LPS may cause inflammation in adipocytes which may potentially lead to obesity.

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PP27

## A novel recombinant form of the human manganese superoxide dismutase protects liver grafts procured for transplantation

D. Hide [a], S. Guixé-Muntet [a], C. Fondevila [b], A. Mancini [c], J. Bosch [a], J. Gracia-Sancho [a]

[a] *Barcelona Hepatic Hemodynamic Lab, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) - CIBEREHD - University of Barcelona, Barcelona, Spain*
[b] *Hepatobiliary and Liver Transplant Surgery, Hospital Clinic de Barcelona, Barcelona, Spain*
[c] *Department of Molecular Biology and Biotherapy, National Cancer Institute "G. Pascale", Naples, Italy*
*E-mail address:* jgracia@clinic.cat (J. Gracia-Sancho)

Introduction Ischemia-reperfusion during liver transplantation causes hepatic injury and early graft dysfunction. The mechanisms involved include vascular dysfunction and oxidative stress. A new recombinant human manganese superoxide dismutase (rMnSOD) has been generated. This protein form freely enters the cells and is constitutively active. The present study aimed at evaluating the protective effects of rMnSOD on the hepatic and endothelial function and viability of liver grafts undergoing cold storage and warm reperfusion injuries. Methods 1- Effects of rMnSOD on oxidative stress levels and nitric oxide bioavailability were tested in freshly isolated rat liver sinusoidal endothelial cells (SEC) preserved in cold storage conditions. 2- Rats were i.v. treated with rMnSOD, or its vehicle, 30 min before liver graft procurement and cold preservation for 16h. Afterwards, grafts were warm reperfused for 1h and hepatic injury, endothelial function, antioxidant capacity, oxidative stress, inflammation, and nitric oxide bioavailability were evaluated. 3- Antioxidant capability of rMnSOD as supplement of a preservation solution was evaluated in rat and human hepatic biopsies cold stored for transplantation. Results 1- Cold storage induced a marked increase in $O_2^-$ levels and a decrease in nitric oxide bioavailability in SEC, those detrimental effects were abolished in cells preserved with rMnSOD. 2- In rats, administration of rMnSOD ameliorated hepatic injury and endothelial dysfunction derived from cold storage and warm reperfusion injuries. The beneficial effects of rMnSOD were associated with a reduction in hepatic oxidative stress and inflammation together with an improved antioxidant activity and nitric oxide bioavailability. 3- rMnSOD added to a conventional preservation solution maintains its marked antioxidant activity avoiding oxidative stress formation in rat and human hepatic tissue preserved for transplantation. Conclusion: rMnSOD represents a new therapeutic strategy to protect liver grafts undergoing transplantation.

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PP28

## Altering redox homeostasis in humans: the repeated eccentric exercise model

Gerasimos V. Grivas [a], Antonios Kyparos [a], Vassilis Paschalis [b,c], Anastasios A. Theodorou [c], George Panayiotou [c], Nikos V. Margaritelis [a], Andreas Zafeiridis [a], Konstantina Dipla [a], Michalis G. Nikolaidis [a], Ioannis S. Vrabas [a]

[a] *Exercise Physiology and Biochemistry Laboratory, Department of Physical Education and Sports Science at Serres, Aristotle University of Thessaloniki, Serres, Greece*
[b] *Department of Physical Education and Sports Science, University of Thessaly, Karies, Trikala, Greece*
[c] *Laboratory of Exercise, Health and Human Performance, Research Center, European University of Cyprus, Nicosia, Cyprus*
*E-mail address:* grivasger@hotmail.com (G.V. Grivas)

Several oxidant stressors have been repeatedly employed in the field of free radical biology to perturb redox homeostasis. Most of these oxidant stressors are of pharmacological nature and produce toxic effects, limiting their use to cells and animals. Hence, the development of a physiological oxidant stimulus applicable to humans would be useful in redox biology. Exercise is probably the most frequently used experimental model to induce oxidative stress in a physiological manner. Nevertheless, whether the exercise model serves this purpose has been rarely tested. In fact, several exercise studies have failed to induce oxidative stress. To our opinion, the most important reason for this failure is the use of "aerobic" exercise (accompanied by limited muscle damage) producing short-lived effects (lasting less than 2 h after exercise) on redox homeostasis. Taking into account a series of studies conducted in our laboratory, we suggest that eccentric exercise that induces severe muscle damage may be a more appropriate model to study the dynamics of redox homeostasis than other forms of exercise that induce limited muscle damage. The reason is that eccentric exercise induces alterations in redox homeostasis that are characterized by long-lasting (up to four days after exercise) and large (even up to 40% compared to rest) increases in oxidant biomarkers. Equally important, eccentric exercise induces rapid adaptations in redox homeostasis even after a single exercise bout, permitting the investigators not only to monitor the responses but also the adaptations to an oxidant stimulus using merely two bouts of eccentric exercise. It is noteworthy that other forms of exercise require more than a month of training to induce adaptations of similar magnitude. In conclusion, the repeated eccentric exercise model may be a useful and practical physiological tool to study redox biology in humans.

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PP29

## GSH is essential for preserving nuclear functions and thus cell survival under oxidative stress

E. Hatem [a], V. Berthonnaud [a], M. Dardalhon [b], G. Lagniel [a], P. Baudouin-Cornu [a], M.E. Huang [b], J. Labarre [a], S. Chédin [a]

[a] *CEA Saclay - DSV / iBiTec-S / SBIGeM / LSOC/PEPS, Gif-sur-Yvette, France*
[b] *Centre National de la Recherche Scientifique, UMR3348 "Genotoxic Stress and Cancer", Orsay, France*

The role of Glutathione (GSH), the thiol-redox guardian of the cell, has been recently revaluated and found to be essential for iron metabolism and maintenance of mitochondrial DNA under non-stress conditions. To determine the essential function of GSH under oxidative stress conditions, we used two different strategies that aimed to deplete yeast cells of GSH and decrypted their physiological response to $H_2O_2$ treatment. We observed that, under GSH depleted conditions, oxidative stress highly impacted cells leading to accumulation of carbonylated proteins and increase in cell mortality. Surprisingly, scarce amount of intracellular GSH is sufficient to rescue yeast cells viability but do not have any effect on protein oxidation. By following the translational and transcriptional responses to $H_2O_2$ under GSH depleted conditions, we show that cell survival tightly correlates with the cell ability to induce a transcriptional response to oxidative stress. Additional experiments led us to propose that the essential function of GSH during oxidative stress conditions reside in its ability to shield nuclear functions and DNA from oxidation. Only trace amounts are sufficient to assure this vital function suggesting that high levels of GSH are mainly required to preserve cytosolic redox equilibrium and thus allowing the synthesis of the ROS detoxification machinery. The impact of GSH variations on compartmental redox homeostasis and subsequently on $H_2O_2$ resistance will be discussed.

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PP30

## Novel quercetin derivatives in treatment of peroxynitrite-oxidized calcium pump

Lubica Horakova [a], Petronela Zizkova [a], Magdalena Majekova [a], Lucia Rackova [a], Miroslav Veverka [b], Lubomir Svorc [c], Dusan Blaskovic [a]

[a] *Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia*
[b] *EUROFINS Bel/Novamann International, s.r.o.*
[c] *Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Bratislava, Slovakia*
*E-mail address:* lubica.horakova@savba.sk (L. Horakova)

Sarco/endoplasmic reticulum calcium ATP-ase (SERCA) is regulated by low concentrations of peroxynitrite and inhibited by high levels, as indicated in human diseases. We studied quercetin (Q) and its novel derivatives monochloropivaloylquercetin (MPQ) and chloronaphthoquinonequercetin (CHQ) as agents with expected preventive properties against peroxynitrite induced SERCA impairment.

Q and MPQ protected the SERCA1 against peroxynitrite induced activity decrease, while CHQ potentiated the inhibitory effect of peroxynitrite. Quercetin derivatives were found to be weaker antioxidants compared with Q, as indicated by their ability to scavenge peroxynitrite and prevent of SERCA1 carbonylation, both decreasing in the order (Q > MPQ > CHQ). Quantum chemical values of theoretical parameter $E_{HOMO}$ also indicated lower antioxidant capacities for MPQ and CHQ. Prooxidant properties estimated by calculations of frontier molecular orbitals ($E_{LUMO}$) correlated with experimentally determined SH-group decrease induced the by compounds studied. Both methods showed a decrease of prooxidant properties as follows: CHQ > MPQ > Q. In addition, experimentally measured half-wave potentials indicated stronger prooxidant properties of quercetin derivatives compared to Q.

More expressive alterations of conformation in the transmembrane region of SERCA1 induced by quercetin derivatives, as compared with Q, may at least partially correlate with their higher lipophilicities. The protective effects of Q and MPQ on SERCA1 activity may be useful in prevention and treatment of inflammation or muscle diseases. The inhibitory effect of CHQ on SERCA1 may be beneficial in therapeutic approaches aimed at anti-tumor treatment.

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PP31

## The membrane composition affects the NADPH oxidase functionning

R. Masoud, T. Bizouarn, L. Baciou, C. Houée-Levin

*Laboratoire de chimie physique, Université Paris Sud, Orsay, France*

The NADPH oxidase is the main source of non-mitochondrial superoxide ions and thus oxygen free radicals. It is at the origin of the non-specific immune defence in neutrophils and of the intercellular communications. It is constituted of two membrane proteins (GP91 and p22phox, also called Nox2, the redox effector) and several cytosolic ones that assemble upon activation. We have constructed a cell-free system, which allows us to study in detail the functioning and the activation process of the system. We show that the lipid composition of the membrane modulates the superoxide production. For instance it is known that cis arachidonic acid activates it and in cell free systems, it can replace the phosphorylations.

These fatty acids act in particular by modifying the cytosolic protein structure. Interestingly, the trans isomer inactivates it, which raises the problem of the consequences of ingestion of "junk food". Addition of Cholesterol leads to a weakening of the activity, however NADPH oxidase still produces superoxide anions. This effect can be related to the stiffness of the membrane in which the redox effector GP91 is embedded, because cholesterol does not change much the structure of the cytosolic proteins. These data show that the composition of the membrane has an influence on the defence against pathogens and on the cellular communications.

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PP32

## Effects of Advanced Glycation Endproducts on the proliferation and ageing of human vascular smooth muscle cells

Kathleen Jacobs, Britt Hofmann, Andreas Simm, Rolf-Edgar Silber, Alexander Navarrete Santos

*Department of Cardiothoracic Surgery, Martin-Luther-University, Halle (Saale), Germany*
*E-mail address:* Kathleen.Jacobs@student.uni-halle.de (K. Jacobs)

Advanced Glycation Endproducts (AGEs) are posttranslational modifications resulting from non-enzymatic glycation of proteins. AGEs seem to be involved in ageing as well as in the development of cardiovascular diseases. One major cell population of vessels are smooth muscle cells (SMCs). Several studies suggests that binding of AGE-modified proteins modulate intimal SMC properties and is associated with increased proliferative activity. The aim of the study was to assess the effects of AGEs on the cell growth and ageing of primary culture SMCs.

Cultured SMCs were received from media explants isolated from residual bypass graft material (saphenous vein) from patients suffering from coronary heart disease. The proliferative activity was evaluated by alamarBlue® assay and cellular senescence was assessed measuring senescence-associated beta-galactosidase (SA-βgal) activity by cytochemical detection (X-gal). A flow cytometric method using 5-dodecanoylaminofluorescein di-β-D-galactopyranoside ($C_{12}$FDG), a fluorogenic substrate for SA-βgal activity, was established to quantify the increased lysosomal content of SA-βgal in senescent SMCs.

Primary culture of SMCs yields to a heterogenic population of non-senescent and senescent SMCs. In contrast to the published literature, our preliminary results show that AGE-BSA (100 – 500 μg/ml) seems to have no effect on the proliferation of the SMCs, on the activation of the pERK/MAP kinase pathway and also on the ageing features of the SMCs. A possible reason for this could be the fact that the SMCs are obtained from veins and not from arteries. Further experiments (e.g. analyzing the AGE receptors) are in progress and should explain the lack of effects.

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PP33

## The renal failure patient and hepatitis B virus, hepatitis C virus infection in Sobrata and Alzawia hospital in Libya

Khalifa Fatnasa

*Higher Institute of Conventional Studies, Tiji city, Libya*
*E-mail address:* KBF_2008@yahoo.com

Recently has been discovered that one of the risk factors to the patients of renal failure is to be infected by hepatitis B virus and hepatitis C virus, the infection may come through the dialysis processes in the dialysis center. A study to the patient of renal failure in Sobrata and Alzawia hospital has been made to check the fidelity of the information mentioned above. After the investigation has been made on 153 patients of renal failure in Sobrata and Alzawia hospital, we could realize that, 23 cases infected by HCV and one case infected by HBV in Sobrata hospital, and 21cases infected by HCV and one case by HBV in Alzawia hospital . The high percentage of infected people by HCV because of not enough sterilization time that is given to the machine used for dialysis process and careless of the staff during the dialysis process. The low percentage for HBV was because most of the patients have taken the hepatitis B virus vaccine.

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PP34

## Oxidized proteins in blood cells of patients with chronic kidney disease

D.A. Klyuyev, L.E. Muravlyova, V.B. Molotov-Luchanskiy

*State Medical University, Karaganda, Kazakhstan*
*E-mail address:* mythrandir79@mail.ru (D.A. Klyuyev)

The aim of the present studies was to detect reactive carbonyl derivates (RCD) of proteins in neutrophils and erythrocytes of patients with chronic kidney disease (CKD). The stadium of CKD was defined as 1-st (by criteria of US National Kidney Foundation and K/DOQI) and was presented in 2 nosological form: chronic pyelonephritis and chronic glomerulonephritis. There were 4 groups of subjects. Control subjects were healthy volunteers without any medication ($n=20$). 21 patients with chronic pyelonephritis were included in 2nd group. 20 patients with chronic glomerulonephritis (nephrotic form) were included in 3th group. 23 subjects with chronic glomerulonephritis (hypertonic form) were included in 4th group. The concentrations of RCD were detected in neutrophils and erythrocytes, following the protocol of Levine R., et al. (1990). The RCD levels were higher in red blood cells of CKD patients than in healthy controls ($p < 0.05$). Analyze the RCD distribution within each group of patients showed the presence of two trends. Based on those results all patients of each group were divided on two clusters. The RCD levels in red blood cells of 1st cluster patients were significantly higher than control ones ($p < 0.01$). The RCD levels in red blood cells of 2nd cluster patients were significantly lower than control ones ($p < 0.001$). That phenomenon did not observe in neutrophils. As compared to control ones in neutrophils of all groups CKD patients there was statistically significant increasing of RCD ($p < 0.001$). Future studies will be needed for understanding of significance of multidirectional alteration of RCD in neutrophils and erythrocytes of CKD patients. It will be interesting for discovery RCD participation in CKD progresses.

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PP35

## Role of mitophagy in lipofuscin formation

J. König, A. Höhn, T. Grune

*Institute of Nutrition, Department of Nutritional Toxicology, Friedrich Schiller University Jena, Germany*

According to the free radical theory of aging, reactive oxygen species (ROS) are responsible for cell damage and consequently for the aging of organisms. Mitochondria appear to play a key role in the aging process due to their substantial involvement in the production of ROS within the respiratory chain. Simultaneously,

mitochondrial proteins and mitochondrial DNA are susceptible to attacks by free radicals leading to enhanced macromolecular damage. Thus, mitochondrial quality control and clearance of damaged mitochondria via selective autophagy (mitophagy) seems to be of particular importance for cellular viability. It is postulated that there is a link between impaired mitochondrial degradation and the intracellular accumulation of the so called "aging pigment" lipofuscin. Lipofuscin consists mainly of heavily cross-linked and oxidized proteins as well as lipids, lesser amounts of carbohydrates and metals, as iron. Of interest is the fact that the major protein component in lipofuscin in neuronal ceroid lipofuscinosis, a neuronal degenerative disease, is formed by the subunit c of ATP-synthase, a mitochondrial protein. It also seems likely that iron-containing mitochondrial proteins may contribute substantially to the iron pool of lipofuscin. It is assumed that lipofuscinogenesis is supported by incomplete degradation of impaired mitochondrial proteins by the lysosomal system. Therefore, we examined the influence of autophagy induction and inhibition on the intracellular accumulation rate of lipofuscin.

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PP36

## Spinal muscular atrophy: An oxidative stress response counteracted with curcumin

A.S. Köstel-Bal [a,b], G. Bora-Tatar [a], H. Erdem-Yurter [a]

[a] *Department of Medical Biology, Faculty of Medicine, Hacettepe University, Sıhhiye, Ankara, Turkey*
[b] *Department of Pediatrics, Faculty of Medicine, Hacettepe University, Sıhhiye, Ankara, Turkey*

Spinal muscular atrophy (SMA), leading cause of infantile mortality, is an autosomal recessive neurodegenerative disorder with specific damage of motor neurons leading to progressive muscle weakness. The role of oxidative damage in the neurodegenerative process in SMA has been proposed. Polyphenols, with their antioxidant effects, may be used in SMA patients. In this study, the association between oxidative stress and SMA was examined in fibroblast cell lines derived from two different SMA type I patients, one SMA type I carrier and one healthy subject. Reactive oxygen species content of SMA type I, SMA type I carrier, and healthy fibroblasts was compared, and then the antioxidant effect of curcumin was analyzed. Expression differences of the antioxidant genes of SMA type I and healthy cell lines were evaluated with real-time PCR array before and after curcumin treatment. Collectively, our results led us to consider oxidative stress and neuronal development process together within the pathogenesis of SMA. The use of antioxidants, such as curcumin, as a supplement may be an alternative in SMA therapy.

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PP37

## Investigation of lipid peroxidation indices in sperm of men with chronic ascaridiasis

B.Zh. Kultanov, R.S. Dosmagambetova, V.N. Kislitskaya, S.A. Dzhangildinova

*Karaganda State Medical University, Karaganda, Kazakhstan*
*E-mail address:* kultanov.berik@mail.ru (B.Zh. Kultanov)

The purpose of our investigation was assessment of lipid peroxidation damage in sperm of men with chronic ascaridiasis.

Biological material was prepared in compliance with method of Dolgov V.V. (2006). For assessment of state of oxidative metabolism in gametes were determined content of primary, secondary and final products of lipid peroxidation: conjugated dienes, ketodienes, malonic dialdehyde, total primary and secondary products of lipid peroxidation, Shiff bases. Examined men were divided into following groups: men with chronic ascaridiasis, men with primary ascaridiasis and control group.

It was found that the content of primary products of lipid peroxidation (conjugated dienes and ketodienes) in sperm of men with chronic ascaridiasis was 1.5 times ($p < 0.05$) as much then indices of control group and was insignificant increased beside men with primary ascaridiasis. Also was revealed 1.3 times as much level of total primary and secondary products in sperm of man with chronic ascaridiasis in comparison with control group.

Men with chronic ascaridiasis had 1.6 times as much level ($p < 0.05$) of secondary products of lipid peroxidation (malonic dialdehyde) then current index of control group. In gametes of men with chronic and primary ascaridiasis was revealed increased content of final products of lipid peroxidation (Shiff bases) in comparison with control group.

Earlier we revealed damages of morphophysiological indices of spermatogenesis of men with ascaridiasis. We consider that probable causes of spermatogenesis damages are toxic catabolites of lipid peroxidation, which destroy cytoskeleton proteins. This conclusion corresponds with literary information, which indicates about immune and reproductive status damage, endogenous intoxication under helminthiasis.

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PP38

## Myosin in aging skeletal muscle: post-translational modifications and actomyosin interactions

M. Li [a], G. Renaud [a], K. Artemenko [b], J. Bergquist [b], L. Larsson [a]

[a] *Dept. of Neuroscience, Clinical Neurophysiology, Uppsala University, Uppsala, Sweden*
[b] *Dept. of Chemistry – Biomedical Center, Analytical Chemistry, Uppsala University, Uppsala, Sweden*
*E-mail address:* meishan.li@neuro.uu.se (M. Li)

Aging is associated with a decline of skeletal muscle mass and function, i.e., sarcopenia, but the underlying molecular mechanisms remain poorly understood. It is hypothesized that specific post-translational modifications (PTMs) of contractile proteins play an important role. In this study, mass spectrometry and a single fiber *in vitro* motility assay have been used to examine the aging effects on structure and function of the molecular motor protein myosin in human and rat. In humans, three specific PTMs were observed in MyHC IIx isoform only while five others occurred in all MyHC isoforms. Of specific interest are carbonylations found in the motor domain localized in the Src-homology domain 3 that has been suggested to influence essential light chain properties, subsequently influencing ATPase kinetics and contractile speed. In rats, one PTM was observed in MyHC I only while the remaining was independent of MyHC isoform. Of specific interest are PTMs (oxidation/carbonylation) observed in the myosin motor domain near the Swtich II loop, i.e., in a highly

flexible region that undergoes multiple conformational changes during the ATPase cycle. An aging-related slowing of the single fiber *in vitro* motility speed was observed in type I and IIa MyHC isoforms irrespective species. The force-generation capacity, on the other hand, was not affected by aging independent of MyHC isoform and species. The understanding of aging-related modifications of myosin is of fundamental importance for the designs of future intervention strategies aiming at reducing the negative effects of sarcopenia in clinical practice.

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PP39

## Oxidative damages in CHO-K1 cells treated with beauvericin

B. Mallebrera, G. Font, M.J. Ruiz

*Laboratory of Toxicology and Food Chemistry, Faculty of Pharmacy, University of Valencia, Burjassot, Valencia, (Spain*
*E-mail address:* bemasi@alumni.uv.es (B. Mallebrera)

BEA is a secondary metabolite of *Fusarium* fungus. It shows antiviral, antimicrobial, insecticidal, apoptotic and it is an inhibitor of cholesterol acyltransferase. However, BEA also has cytotoxic effect in mammalian cells and produce ROS generation. The aim of this study was to evaluate oxidative stress and antioxidant enzyme system in Chinese Hamster Ovary (CHO-K1) cells after BEA exposure. Moreover, for a characterization of the nature of BEA oxidative stress, cells were pre-treated before BEA exposure with D-L-buthionine-(S,R)-sulfoximine (BSO) a glutamate-cysteine ligase inhibitor, and N-acetyl-cysteine (NAC) a precursor to glutathione (GSH). The tested concentration of BEA selected in this study (0.1, 1 and 5μM) were below the $IC_{50}$ for CHO-K1 cells obtained after cytotoxicity studies. The results obtained demonstrated that BEA is cytotoxic to CHO-K1 cells ($IC_{50}$ 12.08 ± 1.1 after 24h of exposure by the MTT assay). It produces ROS generation (4-fold higher than control from 0-120 min by the DCFDA fluorescent probe). After addition of BSO in the medium, BEA decrease GSH levels in a dose dependent manner (from 15 to 125-folds respect to the control). While GSH levels increased from 15 to 20 after NAC exposure. The glutathione reductase (GR) activity was decreased (40% respect to control) after BEA exposure. However, higher decrease (55%) was observed when CHO-K1 cells were previously NAC treated. The glutathione peroxidasa (GP) activity increase in CHO-K1 cells in fresh medium and NAC exposed to BEA in 20 and 60%, respectively. The addition of BSO to the medium did not affect GP activity. The glutathione-*S*-transferase (GST) levels increases 100% respect to control in CHO-K1 cells. When BSO was added to the medium, the BEA exposure decreased from 25 to 50% the GST activity respect to control. Results suggested that GSH and related enzymes play an important role in antioxidant defence in CHO-K1 cells exposed to BEA.

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PP40

## An oxidant stimulus may induce both oxidative and reductive stress: the issue of redox individuality

Nikos V. Margaritelis [a], Antonios Kyparos [a], Vassilis Paschalis [b,c], Anastasios A. Theodorou [c], George Panayiotou [c], Gerasimos V. Grivas [a], Andreas Zafeiridis [a], Konstantina Dipla [a], Michalis G. Nikolaidis [a], Ioannis S. Vrabas [a]

[a] *Exercise Physiology and Biochemistry Laboratory, Department of Physical Education and Sports Science at Serres, Aristotle University of Thessaloniki, Serres, Greece*
[b] *Department of Physical Education and Sports Science, University of Thessaly, Karies, Trikala, Greece*
[c] *Laboratory of Exercise, Health and Human Performance, Research Center, European University of Cyprus, Nicosia, Cyprus*
*E-mail address:* nickmtelis@yahoo.gr (N.V. Margaritelis)

Several oxidant stimuli (including exercise) are frequently used in free radical biology to induce alterations in redox homeostasis. The majority of relevant studies have focused on group comparisons, overlooking individual differences within groups. Therefore, the aim of this study was to investigate the inter-individual variability of commonly used redox biomarkers in response to acute exercise. The eccentric exercise model was applied to produce extensive and long-lasting changes in redox biomarkers and to reveal more effectively the potential individual differences on redox homeostasis responses. Ninety eight young men performed an eccentric exercise session of the knee extensors on an isokinetic dynamometer. Blood and urine samples were collected before and 2 days after exercise. Muscle torque, creatine kinase (plasma), $F_2$-isoprostanes (urine), protein carbonyls (plasma) and glutathione (erythrocytes) were measured. Exercise significantly increased $F_2$-isoprostanes (+46%) and protein carbonyls (+61%) and decreased glutathione (−21%). A wide inter-individual variability in the biomarkers response (percent change from baseline) was observed, with a coefficient of variation equal to 71% for $F_2$-isoprostanes, 80% for protein carbonyls and 78% for glutathione. Interestingly, 13% of the participants exhibited a decrease in $F_2$-isoprostanes and protein carbonyls and 10% of the participants exhibited an increase in glutathione levels. Furthermore, 21% of the participants showed unexpected responses in one to three biomarkers. Non-linear regression analysis revealed moderate to large correlation between the baseline values and the post-exercise percent changes ($R=0.56$ for $F_2$-isoprostanes; $R=0.63$ for protein carbonyls; $R=0.37$ for glutathione). In contrast to the common belief, exercise does not necessarily induce oxidative stress in all individuals, but may even induce reductive stress in some of them. It needs to be emphasized that group means does not fully describe the responses of each group member.

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PP41

## Role of oxidative stress in hepatocyte mitosis

A.M. Tormos [a], R. Talens-Visconti [b], A. Bonora-Centelles [a], S. Perez-Garrido [a], J. Sastre [a]

[a] *Dpt. Physiology, Pharmacy, University of Valencia, Valencia, Spain*
[b] *Dpt. Pharmacology and Pharmacotechnology, Pharmacy, University of Valencia, Valencia, Spain*
*E-mail address:* ana.maria.martinez@uv.es (A.M. Tormos)

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 the effects of higher concentrations of superoxide and $H_2O_2$ may inhibit cell cycle or even have deleterious effects. The aim of this work was to study the role of oxidative stress during mitosis progression in three experimental models: primary hepatocytes culture as *in vitro* model, partial hepatectomy as *in vivo* acute process and biliary cirrhosis as *in vivo* chronic inflammation associated with hepatomegaly and hepatocyte proliferation. In the case of primary hepatocytes isolated by perfusion with collagenase, isolation with 5mM NAC diminished by14% the presence of polyploid cells, demonstrating that the oxidative stress generated during hepatocyte isolation seems to act as a proliferative agent that made hepatocytes go through mitosis but not through cytokinesis. We also tested the effect of oxidative stress in our model of liver specific p38α knock –out mice. p38α knock –out mice, which is characterized by higher levels of GSSG/GSH ratio and malondialdehyde than the control ones, underwent bile duct ligation and partial hepatectomy. Our experiments showed an increased rate of binucleated cells in the p38α knock –out livers, higher mitotic index and an increase in the GSSG/GSH ratio. Hence, p38α knock –out mice exhibited a blockade in the progression of mitosis due to cytokinesis failure, associated with an increase in oxidative stress.Thus, although p38 MAPK signalling has been mainly considered as an inhibitor of cell proliferation and a potential tumor suppressor, the absence of p38α may block or delay cell cycle progression at different stages, including cytokinesis, as observed by the high binucleation rate in liver specific p38α knock-out mice. According to our results, oxidative stress seems to be implicated in the regulation of the late mitosis, particularly cytokinesis.

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PP42

## RONS detoxification in the human protozoan parasite *Giardia intestinalis*

Daniela Mastronicola [a], Micol Falabella [b], Fabrizio Testa [b], Elena Forte [b], Marzia Arese [b], Leopoldo Paolo Pucillo [c], Paolo Sarti [a,b], Alessandro Giuffrè [a]

[a] *CNR Institute of Molecular Biology and Pathology, Rome, Italy*
[b] *Department of Biochemical Sciences and Istituto Pasteur–Fondazione Cenci Bolognetti, Sapienza University of Rome, Italy*
[c] *Clinical Chemistry and Microbiology Laboratory, National Institute for Infectious Diseases "Lazzaro Spallanzani", Rome, Italy*

*Giardia intestinalis* is the microaerophilic protozoon causing one of the most common human intestinal infectious diseases worldwide. The parasite, although highly vulnerable to $O_2$, preferentially colonizes the fairly $O_2$-rich mucosa of the proximal small intestine. This environment is particularly hostile for a parasite lacking most of the antioxidant systems and expressing ROS-releasing enzymes, such as DT-diaphorase. Novel genes putatively coding for antioxidant proteins have been recently identified in the parasite, probably acquired by lateral gene transfer from prokaryotes. *Giardia* is indeed the only pathogenic protist as yet identified encoding a flavohemoglobin (flavoHb) [*Mastronicola et al., Biochem Biophys Res Commun (2010)]* and among the very few eukaryotes encoding a superoxide reductase (SOR) [*Testa et al., Free Rad Biol Med (2011)]* and a flavodiiron protein (FDP) [*Mastronicola et al., IUBMB Life (2011)*]. Genes encoding these enzymes were cloned and expressed in *E. coli* as recombinant His-tagged proteins, then purified by affinity column. Proteins were functionally characterized both as isolated and in living parasites by high-resolution respirometry, NO-amperometry and time-resolved spectroscopy. Expression in parasitic cells and modulation in stress conditions were assayed by immunoblotting and RT-PCR. We found that the newly identified proteins are expressed in *Giardia* as residential scavengers ready to face oxidative and nitrosative stress. In particular, $O_2$ is reduced to $H_2O$ by FDP, NO is efficiently metabolized to nitrate by flavoHb in aerobic conditions, whereas superoxide anion is detoxified to $H_2O_2$ by SOR, avoiding $O_2^{\bullet-}$ (superoxide) persistence in the cell environment . We also identified, for the first time in *Giardia*, new enzymatic systems able to cope with peroxides. It is suggested that all together these novel set of enzymes, by enabling survival of *Giardia* to oxidative/nitrosative stress in the human intestine, may contribute to parasite pathogenicity, thereby representing potential targets for new antigiardial therapeutic strategies.

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PP43

## Genome sequencing reveals mitochondrial thiol systems are essential for antioxidant defence in human adrenal glands

E. Meimaridou, R. Prasad, J.C. Kowalczyk, A.J.L. Clark, H.L. Storr, L.A. Metherell

*Centre for Endocrinology, William Harvey Research Institute, Queen Mary University of London, London, UK*

Familial Glucocorticoid Deficiency (FGD) results from the inability of the adrenal cortex to produce cortisol in response to ACTH stimulation and can be fatal if unrecognised. The disease manifests clinically with increased ACTH and reduced cortisol levels. Our group has recently demonstrated that oxidative stress is implicated in the pathogenesis of this disorder. We previously identified mutations in nicotinamide nucleotide transhydrogenase (NNT) in patients with FGD by targeted exome sequencing. NNT supplies the high concentrations of NADPH needed for the glutathione and thioredoxin pathways to detoxify mitochondrial $H_2O_2$. Recently whole exome sequencing of FGD patients with unknown aetiology identified a novel homozygous mutation in the mitochondrial selenoprotein, thioredoxin reductase 2 (TXNRD2) and two further homozygous mutations in glutathione peroxidase 1 (GPX) and peroxiredoxin 3 (PRDX3) that act synergistically to induce oxidative damage. RT-PCR revealed that NNT and selenoproteins GPX1, PRDX3 and TXNRD2 are highly expressed in human adrenals and knockdown of these genes in adrenocortical cell lines showed perturbation in redox homeostasis. Oxidative stress impedes steroidogenesis but paradoxically steroidogenesis itself induces oxidative stress as a result of electron leak throughout the steroidogenic pathway. In fact the final step of cortisol production, catalysed by CYP11B1 within the mitochondria, accounts for approximately 40% of the total electron flow from NAPDH directed at ROS production during cortisol synthesis. An efficient ROS removal network is therefore of particular importance for the adrenal cortex and may explain why FGD patients with TXNRD2, NNT, GPX1 and PRDX3 mutations present with adrenal insufficiency. Our results suggest that both glutathione and thioredoxin antioxidant systems are critical for ROS detoxification in adrenocortical cells, with their loss leading to defective oxidative stress responses, an impairment of steroidogenesis and hence adrenal insensitivity to ACTH.

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PP44

## Development of a comprehensive analytical method for lipophlic reactive carbonyl compounds in biological samples

Susumu Tomono, Noriyuki Miyoshi, Hiroshi Ohshima

*University of Shizuoka, Shizuoka, Japan*
*E-mail address:* miyoshin@u-shizuoka-ken.ac.jp (N. Miyoshi)

Lipid peroxidation products formed by oxidation of polyunsaturated fatty acids include a wide variety of reactive carbonyls (RCs) (aldehydes and ketones). Increased formation of RCs has been associated with several oxidative stress-related diseases and some RCs have been measured as biomarkers for such diseases. In this study, we have developed a global analytical method for comprehensive profiling of numerous lipophilic RCs present in biological samples using LC/ESI-MS/MS with selected reaction monitoring (SRM). The method consists of (1) extraction of lipophilic RCs with a chloroform/methanol mixture, (2) derivatization of RCs with dansyl hydrazine (DH) and (3) SRM detection of the characteristic product ion of a 5-dimethylaminonaphthalene-1-sulfonyl moiety (*m/z* 236.1) yielded from positively ionized RCs-DH derivatives using 675 SRM channels to monitor the *m/z* range from transition *m/z* 275 > 236.1 to 949 > 236.1. The analytical results were summarized in RCs maps which facilitated to visualize the occurrence and levels of various lipophilic RCs. Initially the method was validated using 27 authentic RCs added to mice plasma. The method was found to be sensitive, reproducible, accurate and specific for RCs. We then applied it to analyze RCs formed by *in vitro* lipid peroxidation of linoleic acid in the presence of $Cu^{2+}$ and ascorbic acid, and detected totally 51 RCs including 4-hydroxy-2-nonenal and 4-oxo-2-nonenal. This developed method was also applied to analyze RCs present in plasma samples of obese/diabetes *ob/ob* mice and normal C57BL/6J mice. It was found that more than 500 RCs were detected in the plasma samples of both normal and obese mice and some RCs including acrolein and glyoxal were significantly elevated in the obese mice. The approach to detect RCs profiling would be useful to study the roles of lipid peroxidation in oxidative stress-related disorders and to discover new biomarkers for early diagnosis of such diseases.

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PP45

## Regulation of cell death signaling by sorafenib in hepatocellular carcinoma. role of p53 gene family members

A.J. De la Rosa [a], A. Lovas [b], M. Müller [b], F.J. Padillo [c,d], J. Muntané [c,d]

[a] *Institute of Biomedicine of Seville, University Hospital "Virgen del Rocío"/IBiS/CSIC/University of Seville, Seville, Spain*
[b] *Department of Internal Medicine I, University Hospital Regensburg, Regensburg, Germany*
[c] *Department of General Surgery, University Hospital "Virgen del Rocío"/IBiS/CSIC/University of Seville, Seville, Spain*
[d] *National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Spain*

Background: Sorafenib, a multi-tyrosine kinase inhibitor, induces cell death in hepatoma cells. Different p53 gene family members have been shown to regulate the expression of cell death receptors during oxidative/nitrosative stress, chemo- and radio-therapy. Objectives: The aim of the study was the identification of the role of p53 during the regulation of cell death signaling in sorafenib-treated hepatoma cells. Methods: Liver tumor sections from patients suffering of hepatocarcinoma (Alcohol, VHB and VHC), and hepatoma cell lines with different p53 genetic profile (HepG2, Huh7 and Hep3B) were included. Different cell death parameters, and the expression of p53/p63/p73 isoforms, CD95, TNF-R1 and TRAIL-R1 were assessed. Results: The survival of patients with hepatocarcinoma was related to increased expression of cell death receptors, and reduced expression of p63ΔN and p73ΔN in tumors. Sorafenib increases caspase-8 activation in Huh7 > HepG2 > Hep3B cells. Sorafenib increased p63TA, p73TA and all cell death receptor expression, as well as decreased p63ΔN expression in Huh7 and Hep3B. Sorafenib increased p53 and TNF-R1, and reduced p63ΔN expression in HepG2. The ligand-induced cell death susceptibility in HepG2 and Hep3B was related to TNF-R1, as well as to CD95, TNF-R1 and TRAIL-R1, respectively. However, TNF-α and Trail, but not CD95L, increased cell death in sorafenib-treated Huh7 cells. Conclusions: 1) The survival of patients with hepatocarcinoma was associated with reduced p63ΔN and p73ΔN, and increased cell death receptor expression in tumor. 2) The induction of cell death receptor expression and apoptosis by sorafenib were related to increased p53/p63TA/p73TA, and reduced p63ΔN expression in hepatoma cells.

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PP46

## Oxidized proteins in blood of patients with very severe chronic obstructive pulmonary disease

L.E. Muravlyova, V.B. Molotov-Luchanskiy, D.A. Kluev, E.A. Kolesnikova

*State Medical University, Karaganda, Kazakhstan*
*E-mail address:* lem2403@mail.ru (L.E. Muravlyova)

The aim of the present studies was to detect reactive carbonyl derivates of proteins and advanced oxidation protein products (AOPP) in blood of patients with severity chronic obstructive pulmonary disease (COPD). There were 2 groups of subjects. Control subjects were healthy volunteers without any medication (n=20). 10 patients with COPD, severity bronchial form, exacerbation, respiratory insufficiency of grade 2-3 were included in 2nd group. The concentrations of reactive carbonyl derivates of proteins were detected in plasma, neutrophils and erythrocytes, following the protocol of Levine R., et al. (1990). The levels of AOPP were detected in plasma and neutrophils following the protocol of Witko-Sarsat et al. (1996).

Severe COPD patients had higher level of AOPP in plasma ($p < 0.001$). The carbonyls were significantly lower in plasma of COPD patients than in healthy controls (by 83%, $p < 0.001$). In neutrophils of COPD patients there was decreasing of the carbonyls (by 22%, $p < 0.05$) and AOPP (by 67%, $p < 0.001$). As compared to control ones in erythrocytes of COPD patients there was statistically significant increasing of carbonyls by 2-2.2 times ($p < 0.001$). The negative correlation between carbonyls in plasma and AOPP in neutrophils (-0.97) and between carbonyls in plasma and neutrophils (-0.82) was observed.

The key finding to emerge from this study is multidirectional alteration of different types of oxidized proteins in plasma, neutrophils and erythrocytes of very severe COPD patients that may contribute to the concept of COPD as a disease network. Future studies will be needed to clear the ways of oxidized

proteins participation in disease progresses and its possible utility in predicting COPD exacerbations.

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PP47

## The association between red blood cell membrane fatty acids, oxidative stress and inflammatory response after coronary angioplasty

V. Muzakova [a], T. Cermak [a], J. Matejka [b], J. Skalicky [c], X. Stramova [a], P. Lastovicka [a], L. Koukalova [a], A. Cegan [a]

[a] *University of Pardubice, Faculty of Chemical Technology, Dept. of Biological and Biochemical Sciences, Pardubice, Czech Republic*
[b] *Regional Hospital of Pardubice, Dept. of Cardiology, Pardubice, Czech Republic*
[c] *Regional Hospital of Pardubice, Dept. of Clinical Biochemistry and Diagnostics, Pardubice, Czech Republic*
*E-mail address:* vladimira.muzakova@upce.cz (V. Muzakova)

Background: Treatment of advanced coronary artery disease (CAD) with significant coronary stenosis by percutaneous transluminal coronary angioplasty (PTCA) with stent implantation is accompanied with inflammatory response, negatively influencing patient prognosis. The study was aimed to elucidate whether red blood cell (RBC) membrane fatty acid profile influences inflammation and oxidative stress after PTCA. Methods: Studied parameters were determined in patients with CAD undergoing PTCA before, 24 and 48 hours after stent implantation (n=20). RBC membrane fatty acids were measured by GC, plasma levels of malondialdehyde, beta-carotene, lycopene and alpha-tocopherol were determined by HPLC. HDL, LDL-cholesterol, triacylglycerol and hs C-reactive protein were determined by standard procedures using analytical system VISTA (Siemens). Statistical analysis and analyses of correlation matrix were done with software STATISTICA v. 10.0. Results: Patients after PTCA exhibited significant increase of inflammation (hs-C-reactive protein 1.75 mg/l ± 1.13, after 24 h: 3.65 mg/l ± 2.36, 48 h: 5.16 mg/l ± 6.22) as well as oxidative stress (malondialdehyde: 1.08 μmol/l ± 0.37, 24 h: 1.49 μmol/l ± 0.23, 48 h: 1.69 μmol/l ± 0,26.) with maximum after 48 h. Considering RBC membrane fatty acids, inverse association with increase of inflammation was found for oleic acid (r=-0.46) and 9-desaturase activity (r=-0.33). In case of oxidative stress, n-6 PUFA docosatetraenoic (r=0.51), linoleic acid (r=0.30) and SFA arachidic acid (r=0.50) were positively related, while palmitoleic acid (r=-0.42) and oleic acid were in negative relation to oxidative stress. Plasma levels of lycopene and beta-carotene were inversely related to oxidative stress, while vitamin E correlated positively. Conclusion: Inflammatory response after PTCA was negatively associated with RBC membrane oleic acid; oxidative stress was inversely related to RBC palmitoleic and oleic acid and positively to arachidic and docosatetraenoic acid. Plasma levels of lycopene and beta-carotene confirmed their antioxidant function, while vitamin E acts as prooxidant.

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PP48

## β-Amyloid 1-42 and RAGE play a key role in RA-induced neuronal differentiation

S. Piras [a], A.L. Furfaro [a], A. Piccini [a], M. Passalacqua [a], R. Borghi [b], A. Garuti [b], M.A. Pronzato [a], U.M. Marinari [a], M. Tabaton [a], M. Nitti [a]

[a] *Department of Experimental Medicine, University of Genoa, Genoa, Italy*
[b] *Department of Internal Medicine and Medical Specialities, University of Genoa, Genoa, Italy*
*E-mail address:* piras.sabri@tiscali.it (M. Nitti)

β-Amyloid (Aβ) 1-42 accumulation is crucial in the pathogenesis of Alzheimer disease. Aβ toxicity depends on its aggregation state and the aggregated forms show a prooxidant, neurotoxic activity. However, it is widely accepted that the non aggregated form of Aβ 1-42 has a physiological, but still not completely understood, role in the brain. Therefore, we investigated the involvement of Aβ 1-42 in retinoic acid (RA)-induced neuronal differentiation. RA has been shown to antagonize the oligomerization of Aβ, reducing its toxicity, and significantly increase the membrane expression of RAGE, a recognized receptor for Aβ.

Using SHSY5Y cells, we showed that 4 days RA treatment increased APP protein level (+40% vs. control, Western Blot analysis), BACE mRNA expression (+ 7 fold vs. control, Real Time PCR analysis) and Aβ 1-42 release in cell medium (+60% vs. control, ELISA test). Moreover, RAGE membrane expression was increased by RA, as showed by immunofluorescence and Western blot analyses. The addition of Aβ 1-42 antibody or RAGE blocking antibody to the cells during RA differentiation strongly impaired neurite elongation and cell adhesion.

Furthermore, preliminary data let us to hypothesise a role of the Amphoterin-induced gene and ORF-1 (AMIGO-1), a newly characterised adhesion molecule involved in promoting neurite extension, as a target of Aβ-RAGE signalling.

Our data point out a physiological role of Aβ in cell differentiation: retinoic acid induces Aβ 1-42 production which, through the binding of RAGE on cell surface, favours neurite elongation.

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PP49

## Oxidative modification of collagen and their effect on proteolytic susceptibility

K. Nowotny, T. Grune

*Institute of Nutrition, Department of Nutritional Toxicology, Friedrich-Schiller-University Jena, Jena, Germany*
*E-mail address:* kerstin.nowotny@uni-jena.de (K. Nowotny)

Collagen is the most abundant protein in the human body and as a structural element it is part of the extracellular matrix of bones, tendons, cartilage, blood vessels and the skin. Currently, more than 20 different types of collagen have been described. Thereby the fibrillary types I, II and III are the most dominant in humans.

During the aging process and as a result of various inflammatory diseases collagen is increasingly oxidized and cross-linked. In general, oxidative modifications of proteins are associated with an increased susceptibility to proteolytic degradation. If proteins are cross-linked, protein degradation is limited. Studies that investigated the degradation of oxidized and cross-linked proteins, focused mainly on the proteolysis of intracellular and globular proteins. However, data for extracellular, fibrillary proteins such as collagen are scarce. In this regard, in vitro experiments were conducted in which first a suitable oxidizing system for collagen type I was established. Furthermore, collagen was cross-linked using glyoxal and methylglyoxal. The proteolytic susceptibility of native and modified collagen was tested. A dramatic influence of collagen modification on its proteolytic degradation was observed.

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PP50

## Study of the effects of natural compounds in the lifespan of the nematode *Caenorhabditis elegans*

Nikoletta Papaevgeniou [1], Marianthi Sakellari [1], Efstathios S. Gonos, Niki Chondrogianni

*Institute of Biology, Medicinal Chemistry and Biotechnology, Athens, Greece*
*E-mail address:* me_nikole@hotmail.com (N. Papaevgeniou)

Senescence is the process of cumulative changes and damage to molecular and cellular structure that disrupts metabolism with the passage of time, resulting in homeostasis collapse and death. Senescence occurs both on the organismal level as well as on the level of its individual cells. Some of the changes observed are related to the increase of oxidative stress and the reduced function of the basic cellular proteolytic mechanism, the proteasome. Proteasomes are protein complexes responsible for the degradation of normal or damaged proteins. Therefore, proteasomes are part of a major mechanism by which cells regulate the concentration and therefore the function of particular proteins and degrade misfolded or otherwise damaged proteins. The objective of the present work is the study of the effects of natural compounds on the lifespan and healthspan of the nematode *Caenorhabditis elegans*, which is used as a model organism for aging. Main direction of the study is the investigation of the effects of these compounds on the functionality of the proteasome and other signaling pathways associated with the aging process (e.g. dietary restriction signaling pathway, insulin/IGF-1-like signaling pathway), by conducting lifespan assays in wild type and several mutant strains, protein activities assay, immunoblot analysis and Real-time PCR analysis, in order to indentify anti-aging compounds with possible proteasome activating properties. These results will show us, for the first time, if the activation of the proteasome in a multicellular organism is achievable through natural compounds that exist in the Greek flora and are part of the Mediterranean diet.

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[1] Equal contribution.

PP51

## Silencing of mitochondrial $NADP^+$-dependent isocitrate dehydrogenase gene enhances glioma radiosensitivity

S.Y. Kim, J.W. Park

*School of Life Sciences and Biotechnology, College of Natural Sciences, Kyungpook National University, Taegu, Korea*

Reactive oxygen species (ROS) levels are elevated in organisms that have been exposed to ionizing radiation and are protagonists in the induction of cell death. Recently, we demonstrated that the control of mitochondrial redox balance and the cellular defense against oxidative damage are primary functions of mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDPm) via the supply of NADPH for antioxidant systems. In the present study, we report an autophagic response to ionizing radiation in A172 glioma cells transfected with small interfering RNA (siRNA) targeting the IDPm gene. Autophagy in A172 transfectant cells was associated with enhanced autophagolysosome formation and GFP-LC3 puncuation/aggregation. Furthermore, we found that the inhibition of autophagy by chloroquine augmented apoptotic cell death of irradiated A172 cells transfected with IDPm siRNA. Taken together, our data suggest that autophagy functions as a survival mechanism in A172 cells against ionizing radiation-induced apoptosis and the sensitizing effect of IDPm siRNA and autophagy inhibitor on the ionizing radiation-induced apoptotic cell death of glioma cells offers a novel redox-active therapeutic strategy for the treatment of cancer.

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PP52

## Detection of mitochondrial tryparedoxin peroxidase in the mitochondrial membrane fraction of *Trypanosoma cruzi*

E.F. Peloso, F.R. Gadelha

*Departamento de Bioquímica, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil*
*E-mail address:* eduardofpeloso@yahoo.com.br (E.F. Peloso)

*Trypanosoma cruzi* is the etiologic agent of the Chagas disease, a parasitosis with great clinical importance and until now there is no vaccine and effective treatment. Thus, research aim at the search for new therapeutic targets in order to develop a more specific therapy. Among these targets, the antioxidant system of the parasite has emerged, and key enzymes of this system as cytosolic and mitochondrial tryparedoxin peroxidases (TcCPx and TcMPx, respectively), has increasingly shown its importance in parasite survival. The cytosolic pathway in which TcCPx participates have already been characterized, however, the mitochondrial one not yet. The objective of this study was to determine the exact location of TcMPx and thus be able to suggest to which proteins this enzyme interacts. Therefore, we obtained mitochondrial membrane fraction (MMF) of *T. cruzi* Y strain, and from this fraction, experiments of oxygen consumption, citrate synthase activity and detection of the expression of this protein were performed. TMPD / Ascorbate supported oxygen consumption, indicating that MMF successfully obtained. In order to prove that MMF was free from mitochondrial matrix enzymes, citrate synthase activity was performed. This activity was observed in

intact cells but not in MMF. Thereafter, western blotting was performed for detection of TcMPx expression in MMF, and the result showed the presence of this protein not only in the whole-cell and supernatant of cellular lysis proteic extracts, but also in the MMF. The detection of this enzyme in the mitochondrial membrane opened new perspectives in relation to TcMPx interatome, and experiments are being carried out to finalize the characterization of the mitochondrial antioxidant system of *T. cruzi*.

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PP53

## Nitrite administration ameliorates mitochondrial bioenergetics and is neuroprotective in cellular and vertebrate models of Parkinson's disease

Chiara Milanese [a,b], Mark Gladwin [c], Pier G. Mastroberardino [a]

[92] *Department of Genetics, Erasmus Medical Center Rotterdam, The Netherlands*
[93] *RiMED Foundation, Palermo, Italy*
[94] *Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA, USA*

Nitrite oral administration has been proposed as a therapeutic avenue in cardiac ischemia due to its ability to decrease mitochondrial production of reactive oxygen species (ROS), via transient inhibition of respiratory complex I (C-I). Augmented ROS and mitochondrial C-I dysfunction are also hallmarks of Parkinson's disease (PD), a chronic neurodegenerative disorder. Indeed, pro-oxidant inhibitors of C-I have been associated with PD by epidemiological studies and investigations on animal models.

Here we explored the neuroprotective capacity of nitrite administration in cellular and animal models of PD. In a dopaminergic (DA) neuronal cell line (SH-SY5Y), nitrite administration ameliorates $MPP^+$ induced cell death. Bioenergetics analysis indicates that $MPP^+$ perturbs, as expected, mitochondrial respiration and this defect is reversed by nitrite administration. In zebrafish embryos, nitrite pre-treatment ameliorates the locomotor activity after $MPP^+$ exposure, improves viability, and reduces DA neuron loss in the brain. On a molecular level, the physiological effects of nitrite are mediated by nitrosation of cysteine residues in proteins, as evidenced by specific labeling with fluorescent maleimide derivatives after $Cu^{2+}$/ascorbate reduction of nitrosothiols. Finally, nitrite administration improves mitochondrial respiration efficiency in human fibroblasts derived from genetic PD patients harboring mutations in the LRRK2 gene, by increasing mitochondrial membrane potential and by reducing proton leakage.

In summary, nitrite administration might constitute an amenable neuroprotective strategy in PD. We provide evidence that nitrite mitigates neurodegeneration in PD models and that ameliorates the mitochondrial respiratory profile in primary fibroblasts from genetic PD cases.

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PP54

## Identification of gliadin as an advanced glycation end product-modified compound in bread crust extract and their effect on mouse macrophage activation

S. Pötzsch [a], M. Dalgalarrondo [b], B. Bakan [b], D. Marion [b], V. Somoza [c], R.-E. Silber [a], A. Simm [a], A.Navarrete Santos [a]

[a] *Department of Cardiothoracic Surgery, University Hospital Halle (Saale), Germany*
[b] *Unité Biopolymères, Interactions, Assemblages, Institut National de la Recherche Agronomique, Nantes, France*
[c] *Department of Nutritional and Physiological Chemistry, University of Vienna, Austria*
*E-mail address:* sandy.poetzsch@student.uni-halle.de (S. Pötzsch)

Advanced glycation end products (AGEs) represent non-enzymatic posttranslational modification-derived products, which are thought to play a role in age-related diseases, like diabetes and Morbus Alzheimer. AGEs accumulate endogenously or exogenously by food intake. The dietary AGEs significantly increase the AGE pool in the body. Therefore nutrition seemed to be a very strong factor to influence the rate of aging. Previous studies reported an oxidative as well as antioxidative capacity for food derived AGEs. Our group used bread crust (BCE) as an AGE-rich dietary extract, which induced a moderate elevation of ROS production causing an activation of $p42/p44^{MAPK}$, $p38^{MAPK}$ and NF-κB in cardiac fibroblasts. However it is still unclear which protein/peptide is the active compound as well as what kind of modification leads to their formation.

The present work focuses on the identification of the bioactive compounds in bread crust extract. The amino acid analysis by RP-HPLC and LC-MS/MS by Orbitrap Velo was used to determine these compounds. We identified some gliadines/secalines in the bread crust extract and by means of boronate affinity chromatography the majority of these proteins in the BCE seemed to by glycated. The soluble BCE was fractionated by use of a reversed Phase-HPLC with a Zorbax 300SB-C18 column. 31 fractions were collected and analyzed regarding their typical AGE fluorescence at 360/440 nm as well as 330/405 nm. The results were checked by immunoblotting with specific antibodies against AGE's and ω5- and γ-gliadin. Fractions no. 17 till 31 showed a very strong signal for ω5- and γ-gliadin as well as for Carboxymethyllysine (CML), Pentosidine and Imidazolone. Preliminary results showed that gliadin stimulate macrophages due to mechanisms including activation of the MAPKinases ($p42/p44^{MAPK}$, $p38^{MAPK}$). Further experiments are still in progress to elucidate the results.

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PP55

## Effects of Omega-3 fatty acids supplements on vascular dysfunction biomarkers, in childhood obesity

L.A. Popescu [a], B. Virgolici [a], O. Timnea [b], A.E. Ranetti [a], D. Oraseanu [a], L. Zagrean [a]

[a] *"Carol Davila" University of Medicine and Pharmacy, Department of Fundamental Sciences, Bucharest, Romania*
[b] *Ecologic University of Bucharest, Faculty of Physical Education and Sports, Bucharest, Romania*

Childhood obesity is associated with increased risk for atherosclerosis. This study aimed to determine the effects of omega-3 fatty acids supplements on vascular dysfunction biomarkers in obese children and to find correlations between these markers.

Forthy eight obese children (9-16 years old) and thirty lean children were involved. Each day, for three months, obese children took omega-3 fatty acids (DHA 130 mg and EPA 25 mg) and vitamins (A 200 μg, D 1.25 μg, E 2.5 mg and C 30 mg). The measured variables were: erythrocyte superoxid dismutase (SOD) and glutathione peroxidase (GPx) activities as blood antioxidants, leptin and adiponectin as adipocytokines with antagonic effects on vascular function and serum pancreatic elastase and cortisol as markers of inflammatory status. ELISA methods were used.

All the measured parameters were modified in the obese children versus the lean subjects. In the obese children, the treatment lowered the rise of erythrocyte SOD ($p < 0.001$) and serum pancreatic elastase ($p < 0.001$) activities, decreased levels for leptin ($p < 0.001$) and increased levels for adiponectin ($p < 0.001$). Erythrocyte GPx activity and serum cortisol levels were not modified significantly. Serum pancreatic elastase was positively correlated with cortisol ($r = 0.34$, $p < 0.05$) and GPx activity ($r = 0.31$, $p < 0.05$) and negatively with SOD activity ($r = -0.30$, $p < 0.05$). Adiponectin was positively correlated with GPx ($r = 0.31$, $p < 0.05$) activity and negatively with SOD activity ($r = -0.31$, $p < 0.05$).

In conclusion, omega 3 fatty acids supplements have beneficial effects in childhood obesity by improving the blood levels of some important vascular dysfunction biomarkers.

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PP56

## Correlation between oxidative stress and fatty acids profiles in plasma phospholipids after fish oil treatment in aged male rats

Tamara Popović [a], Sunčica Borozan [b], Aleksandra Arsić [a], Jasmina Debeljak Martačić [a], Silvio de Luka [c], Marija Glibetić [a]

[a] *Institute for Medical Research, University of Belgrade, Serbia*
[b] *Faculty of Veterinary Medicine, University of Belgrade, Serbia*
[c] *Institute of Pathophysiology, Faculty of Medicine, University of Belgrade*

Unsaturated fatty acids are important in prevention and protection in many diseases. In all these patologies role of redox active species (oxigen and nitric) is crucial. Supplementation as a modern pattern of adding deficient supstances is quite present nowdays.

Our aim was to correlate parameters of oxidative stress and fatty acids profiles in phospholipides of plasma and liver in aged male rats.

Experiments were performed on male Wistar rats (22 months, b.w.370g). Animals were devided in two groups. Control group ($n = 10$) was received standard laboratory food and water ad libitum. Treated group ($n = 10$) was received n-3 PUFA (Natural Wealth,

Nordvik, Serbia), for six weeks (45mg EPA + 30mg DHA a day). All parameters were measured at the end of experiment after sacrificing. Parameters of oxidative stress (MDA, CAT, SOD, nitrites, SH groups) were measured with standard laboratory kits on UV/VIS spectrophotometer and ELISA in erythrocytes and plasma. Fatty acids profiles were determined by GC chromatography after samples preparation standard procedure.

In the control group of rats there was positive correlation between MDA and DHA acid ($r = 0.830$) and activity of SOD and oleic acid ($r = 0.888$). Negative correlation was between SOD and DHA ($r = -0.877$). In treated group there was positive correlation between SH groups and activity of SOD ($r = 0.841$) and between CAT activity and plasma nitrites concentration ($r = 0.884$). Negative correlation were found between SOD and linoleic acid ($r = -0.829$).

After treatment of fish oil there was increased activity of SOD, CAT and decreased lipid peroxidation (MDA), plasma nitrites concentration and SH group concentration. Treatment increased percentage of linoleic acid and dihomo-γ-linoleic acid. Correlations confirm the antioxidative treatment of fish oil in aged male rats.

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PP57

## Spectroelectrochemistry of 1,4-naphthoquinones and 5-nitroindazoles: Reduction mechanisms and free radical stability

B. Pozo [a], B. Aguilera-Venegas [a,b], C. Olea-Azar [a], V.J. Arán [c], C.O. Salas [d], M.C. Zúñiga [a]

[a] *Laboratory of Free Radicals, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile*
[b] *Laboratory of Antioxidants, Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile*
[c] *Medicinal Chemistry Institute (IQM), Superior Council of Scientific Research (CSIC), Madrid, Spain*
[d] *Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile*
*E-mail address:* ganjamin@gmail.com (B. Pozo)

A comprehensive electrochemical and ESR study is conducted here aiming to assess the reduction mechanisms for a series of 1,4-naphthoquinone (NQ) and 5-nitroindazole (NI) derivatives: For NI derivatives, cyclic voltammetry (CV) experiments exhibit a quasi-reversible wave ($E_{1/2} \sim -1.02$ V) corresponding to the nitro reduction. Additionally, depending on the 1-substituent, an additional shoulder (at lower potential) may arise as a consequence of a self-protonation process between the nitro radical and acidic moieties from the 1-substituent. For NQ derivatives, CV experiments exhibit two quasi-reversible waves ($E_{1/2} \sim -0.66$ V and -1.12 V respectively) corresponding to the semiquinone radical formation and the subsequent reduction of the additional CO group to a di-anion specie. Afterwards, aiming to establish the hyperfine splitting patterns, NI and NQ derivatives were electrolyzed (at the reduction potential for the nitro radical and semiquinone, respectively) and the ESR spectrum was recorded. NI radicals display an ESR spectrum of 25 thin and well-defined lines whose splitting pattern corresponds to the hyperfine interactions derived from two triplets and three doublets (main pattern) suggesting that the spin density takes place mainly over the benzene ring and part on the 5-membered ring. Contrariwise, semiquinone radical exhibits an ESR spectrum composed by a main single line which splits into low-intensity peaks throughout the main signal. The simulation of the ESR spectra for NQ radicals clearly suggests that the unpaired electron is highly unlocalized through the quinone ring, stabilizing the radical by resonance effects that confer a

superior stability to the semiquinone radical over the nitro respectively. Indeed, in agreement with their reduction potentials.

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PP58

## Preventive effect of Ellagic acid on blood pressure, oxidative stress and cardiac remodelling in L-NAME induced hypertensive rats

P. Prachaney [a], P. Boonprom [a], T. Berkban [a], S. Bunbupha [b], J.U. Welbat [a], P. Pakdeechote [b], V. Kukongviriyapan [c], U. Kukongviriyapan [b]

[107] *Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khoen Kaen, Thailand*
[108] *Department of Physiology, Faculty of Medicine, Khon Kaen University, Khoen Kaen, Thailand*
[109] *Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khoen Kaen, Thailand*

The aim of this study was to investigate the preventive effect of Ellagic acid (EA), a potent antioxidant agent, on blood pressure, oxidative markers and cardiac wall remodelling in L-NAME induced hypertensive rats. Male Sprague-Dawley rats were given L-NAME (40 mg/kg/day) to induce hypertension, and simultaneously treated with EA 15 mg/kg/day for 4 weeks (L-NAME + EA group), or a vehicle (L-NAME group). Age-matched rats served as a control group. Systolic blood pressure (SBP) was monitored using a tail cuff method once a week throughout the experiment. After 4 weeks of treatment, the rats were weighed, anaesthetized with peritoneal injection of pentobarbital-sodium (60 mg/kg) and scarified by exsanguinations. The heart was isolated. The left ventricular weight (LVW), heart weight (HW), relative heart weight (LVW/BW), cardiac wall thickness and ventricular cross-sectional area were determined as ventricular hypertrophy index. Plasma malonyldialdehyde (MDA) and vascular superoxide production were also analysed. EA significantly reduced SBP of L-NAME treated rats when compared to those of the L-NAME group ($167.96 \pm 1.21$ vs. $197.91 \pm 7.95$ mmHg; $p < 0.05$). The prevention of increase in SBP of L-NAME + EA was associated with a decrease in superoxide production in carotid arteries ($65.67 \pm 4.48$ vs. $109.91 \pm 8.45$ counts/min/mg dry weight; $p < 0.05$) and MDA ($6.74 \pm 0.4$ vs. $9.56 \pm 1.01$ μM; $p < 0.05$). However, there were no significant difference of rat body weight, HW, LVW and LVW/BW ratio among groups. The left ventricular wall thickness of L-NAME treated with EA was slightly smaller than those in L-NAME rats but not reach a statistically significant level. The cross-sectional area of left ventricle in L-NAME and L-NAME treated with EA were significantly greater than those in control group, but there were no significant difference in cross-sectional area between these two groups. In conclusion, EA has antihypertensive and antioxidant properties in nitric oxide deficiency model, but has no effect on a cardiac wall remodelling.

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PP59

## Ability of silybin and its derivatives to prevent protein oxidation in different model systems

K. Purchartová [a], C.P. Baron [b], V. Křen [c]

[a] *Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic*
[b] *National Food Institute, Technical University of Denmark, Lyngby, Denmark*
*E-mail address:* k.a.m.i.k@centrum.cz (K. Purchartová)

Flavonolignan silybin is a major component of silymarin isolated from seeds of the milk thistle (*Silybum marianum*). Natural silybin is a mixture of two diastereoisomers - silybin A and silybin B. Besides hepatoprotective effects, silybin was lately reported as anticancer, chemoprotective, dermatoprotective and hypocholesterolemic agent. Silybin plays an important role as antioxidant and free radical scavenger as well. Therefore, the antioxidant activity of silybin, dehydrosilybin, 23-*O*-butanoyl and 23-*O*-palmitoyl esters of silybin (respectively C4 and C16) was investigated. Especially their ability to prevent activation of hemoglobin (Hb) to highly reactive hypervalent heme protein species (ferrylHb and perferrylHb) was examined. Indeed, Hb cytotoxicity has been associated with the generation of protein radicals, which are formed when the ferric iron of Hb ($Fe^{3+}$) is oxidised by $H_2O_2$ to ($Fe^{4+}$) to form perferrylHb and ferrylHb, with the later also bearing a radical on its protein. The relationship between the structural properties of silybin and its derivatives and their ability to prevent oxidation of Hb was investigated in model system in the presence or the absence of lipids. The antioxidant activities of silybin, dehydrosilybin, 23-*O*-butanoyl and 23-*O*-palmitoyl silybin derivatives were correlated with their interaction with Hb species. Results are discussed in relation to the potential of dehydrosilybin, silybin and C4 and C16 derivates to prevent activation of Hb to hypevalent heme protein species.

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PP60

## Disruption in energy metabolism and mitochondrial function in a cellular model of inflammation-induced acute kidney injury

C. Quoilin C. [a], A. Mouithys-Mickalad [b], S. Lécart [c], B. Gallez [d], M.-P. Fontaine-Aupart [e], M. Hoebeke [a]

[a] *Laboratory of Biomedical Spectroscopy, Department of Physics, University of Liège, Liège, Belgium*
[b] *Center of Oxygen Research and Development, Department of Chemistry, University of Liège, Liège, Belgium*
[c] *Centre de Photonique Biomédicale, Fédération LUMAT, University Paris Sud, Orsay, France*
[d] *Laboratory of Biomedical Magnetic Resonance, Louvain Drug Research Institute, Catholic University of Louvain, Brussels, Belgium*
[e] *Institut des Sciences Moléculaires d'Orsay, CNRS and University Paris Sud, Orsay, France*
*E-mail address:* cquoilin@ulg.ac.be (C. Quoilin C.)

Sepsis is a very complex clinical condition characterized by stimulation of a systemic inflammatory response due to an infection. It has a profound deleterious effect on kidney functions leading to sepsis-induced acute kidney injury (AKI). This failure seems to occur

through complex mechanisms involving the immune system response, inflammatory pathways, cellular dysfunction and hemodynamic instability. To study the role of cellular energetic metabolism dysfunction and mitochondrial impairment in the occurrence of AKI during sepsis, we developed an inflammation-induced *in vitro* model using proximal tubular epithelial cells (HK-2) exposed to a bacterial endotoxin (lipopolysaccharide, LPS). This investigation has provided key features on the relationship between endotoxic stress and mitochondrial respiratory chain assembly defects. Firstly, we have shown that renal cells subjected to LPS are no longer capable to use adequately the available oxygen to maintain their metabolic functions. One hypothesis of this down-regulation suggests that impairment in mitochondria oxidative phosphorylation could prevent cells from using oxygen for adenosine triphosphate (ATP) production and potentially could cause sepsis-induced organ failure. Our study has then investigated this possible mitochondrial impairment to explain the decreased $O_2$ consumption rate observed in LPS-treated HK-2 cells. After exposure to LPS, functionality of mitochondria was affected without any disturbance in their spatial organization. LPS seemed rather to interrupt mitochondrial oxidative phosphorylation by blocking cytochrome c oxidase activity. As a consequence, disruptions in the electron transport and the proton pumping across the system occurred, leading to a decrease of the mitochondrial membrane potential, an electron leakage as the form of superoxide anion, a release of cytochrome c in the cytosol and a decrease in ATP production. This irreversible defect in the production of cellular energy would support the concept that kidney failure in sepsis may occur on the basis of cytopathic hypoxia.

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PP61

## Role of glyoxalases system in skin aging and in response to dicarbonyl mediated stress

Radjei Sabrina, Bertrand Figuet, Isabelle Petropoulos, Carine Nizard

*Laboratory of Aging, Stress and Inflammation UR4 UPMC - LVMH Research, Paris, France*
*E-mail addresses:* sabrina.radjei@snv.jussieu.fr, sabrinaradjei@hotmail.fr (R. Sabrina)

During aging and in certain pathologies linked to oxidative stress, the proteins irreversibly modified by carbonylation, conjugation with lipid peroxidation products or glycation, accumulate due to a failure of the protein maintenance systems. Glycation occurs when glucose reacts with amines of proteins leading to the formation of advanced glycation end products (AGE) but AGE can also be produced when the dicarbonyl compounds glyoxal (GO) and methylglyoxal (MG) react with proteins.

The most important detoxification system of these compounds is the glyoxalases system composed of two intracellular enzymes glyoxalase 1 (Glo1) and glyoxalase 2 (Glo2) [1]. The involvement of these two enzymes in the aging process has been studied. Indeed, Glo1 activity is reduced during replicative senescence [2] and its overexpression increases longevity in several animal models [3,4]. However, their role in human skin remains poorly studied.

The aim of this work is to better understand the role of Glyoxalases in skin, in particular in the detoxification of compounds GO and MG in response to oxidative stress, and to study their role in the protection of proteins during skin aging.

To first analyse the regulation of Glyoxalases in skin during aging and UV exposition, an immunohistochemistry study was performed on photoprotected or photoexposed skin sections of 10 young donors and 10 old donors. Our results show that Glo1 is expressed exclusively in the undifferentiated keratinocytes of the epidermis basal layer and is increased in aged skins compared to young skins. Regarding protein glycation, we found that AGE accumulate with age and UV exposition in the dermis but carboxymethyllysine modified proteins are more present in the epidermis of young compared to old subjects.

The transcription factor NF-E2-related factor 2 (Nrf2), which is a key factor in the cellular response to stress, has been shown to regulate the expression of Glo1 [5]. Expression of Nrf2 analysed by IHC on the same samples show a decrease in aged keratinocytes suggesting that Glo1 is regulated by a different pathway in the epidermis progenitors. To better understand the role of glyoxalase enzymes during stress, HaCaT cells were subjected to sublethal and lethal concentrations of GO and MG for 24 hours.

A significant increase of glycated proteins was observed immediatly after stress, together with a decreased activity of Glo1 with no change of its expression. A 24 h recovery leads to a reactivation of the enzyme.

We have currently isolated cellular clones with overexpression or inhibition of Glyoxalases which will allow the identification of preferential protein targets of glycation through proteomic studies.

We expect that our study may contribute to decipher the role of glyoxalases in protein maintenance which is a key element of cellular homeostasis and to identify whether these enzymes could be targets for future anti-aging strategies.

### References

[1] Glyoxalase in ageing, Xue M *et al.*, Seminars in Cell and Developmental Biology, 2010.
[2] Protein modification and replicative senescence of WI-38 human embryonic fibroblasts. Ahmed et *al.*, Aging Cell, 2010.
[3] Glyoxalase-1 prevents mitochondrial protein modification and enhances lifespan in Caenorhabditis elegans. *Morcos et al.*, Aging cell, 2008.
[4] Modulation of the glyoxalase system in the aging model Podospora anserina: effects on growth and lifespan. *Scheckhuber et al.*, Aging, 2010.
[5] Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. *Xue M et al.*, Biochem J, 2012.

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PP62

## In Vitro and In Vivo UV Light Skin Protection by an Antioxidant Derivative of NSAID Tolfenamic Acid

V. Skiadopoulos [a], D. Skea [a], E. Aggelakopoulou [a], I. Simati [a], M. Kyriazi [a], G.Th. Papaio[a], A. Kourounakis [b], M. Rallis [a]

[a] *Dept of Pharmaceutical Technology, School of Pharmacy, National and Kapodistrian University of Athens*
[b] *Department of Pharmaceutical Chemistry, School of Pharmacy, National and Kapodistrian University of Athens*
*E-mail address:* rallis@pharm.uoa.gr (M. Rallis)

Moderate doses of UV light are beneficial for the skin, with anti-rachitic, anti-depressive effects, while they may also assist the treatment of skin diseases such as psoriasis. In contrast, elevated doses induce erythema, ageing and carcinogenesis. The common ground for all these skin diseases/ageing is initially inflammation.

The first line of therapy for skin inflammation is the class of corticosteroids, with their known side effects, while non-steroidal anti-inflammatory drugs (NSAIDs) are mainly used as analgesic and secondarily against skin inflammation. Some substances or mixtures with antioxidant properties like resveratrol and curcumin seem to

prevent skin inflammation against toxic environmental factors such as UV light. In this study we evaluate an active agent combining anti-inflammatory and antioxidant properties. The design and synthesis of this NSAID derivative was accomplished by combining in its molecular structure the anti-inflammatory drug tolfenamic acid and the anti-oxidant cysteine ethyl ester (AK) . AK was evaluated in vitro and compared to the parent drug tolfenamic acid at a dose of 1 μg/ml, using mouse keratinocyte primary cultures. Cells in 96 well plates were exposed to a 5mJ/cm$^2$ dose of UVB and UVA light followed by a cell viability (MTT) test and measurement of the intracellular oxidative stress (CM-$H_2$DCFDA fluorescein). The comparative anti-inflammatory effect of AK was estimated in vivo, on the back skin of hairless mice, after 3 or 7 Minimal Erythemal Dose exposures of UVB + UVA light and topical application of the agents. The effect was evaluated based on macroscopic/photograph observation, transepidermal water loss measurements, skin redness and in the case of the 3 MED dose measuring lipid peroxidation levels.In vitro, cell viability was preserved by AK almost at control levels (i.e. viability of cells without UV irradiation) while oxidative stress was decreased in the case of treatment with AK. It is noteworthy that the parent tolfenamic acid did not show any protection in either cases. In vivo, and at both MED doses, the tolfenamic AK derivative efficiently reduced skin inflammation, while the parent tolfenamic acid molecule did not show any significant activity.

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PP63

## Protein alkylation by the α,β-unsaturated aldehyde acrolein: A reversible mechanism of electrophile signaling in the pulmonary epithelium?

Matthew J. Randall, Milena Hristova, Page C. Spiess, Albert van der Vliet

*University of Vermont, Burlington, Vermont, USA*

Chronic obstructive pulmonary disease, an inflammatory lung disease, is caused mainly by cigarette smoking. Sputum and exhaled breath condensates of COPD patients were identified to contain increased levels of reactive aldehydes. Acrolein, one critical reactive aldehyde found in cigarette smoke, has a high affinity for adduction to cysteine thiols. Such alkylation by Michael addition is considered irreversible, although previous literature has suggested that 1,4-Michael adducts can undergo retro-Michael cleavage *in vitro*. We tested whether protein adducts of acrolein could be reversed in human bronchiolar epithelial HBE1 cells, by cell exposure to 30 μM acrolein for 30 minutes, followed by a "recovery" period up to 24 hrs. Acrolein exposure rapidly and reversibly depletes cellular GSH and rapidly inhibits thioredoxin reductase (TrxR) activity, a major enzyme in redox regulation, coinciding with protein-acrolein adduction. Recovery of TrxR activity occurred after 4-8 hrs, consistent with gradual decreases of total protein-acrolein adducts and TrxR1-acrolein adduct. Control experiments with the 26S proteasome inhibitor MG132 and the protein synthesis inhibitor cycloheximide indicated that recovery of TrxR activity occurred largely independent of *de novo* protein synthesis. To assess a possible role for GSH in the repair of TrxR activity we prevented GSH regeneration with buthionine sulfoximine which attenuated TrxR recovery and also slightly attenuated time-dependent decreases in protein-acrolein adducts within c-jun N-terminal kinase and thioredoxin 1 (Trx1). Using an siRNA approach, we also identified a role for Trx1 in the repair of TrxR after inactivation by acrolein. In conclusion, our findings indicate that acrolein-induced protein alkylation is not necessarily a feature of irreversible protein damage but may reflect a reversible signaling mechanism that is regulated by

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## Combination of antioxidant structures in one molecule can yield potent anti-inflammatory agents

P. Theodosis-Nobelos, G. Papagiouvannis, K. Tsiakitzis, A. Gavalas, P. Kourounakis, E. Rekka

*Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotelian University of Thessaloniki, Thessaloniki, Greece*
*E-mail address:* rekka@pharm.auth.gr (E. Rekka)

It is known that oxidative stress and active oxygen species are involved in the inflammatory processes. They contribute to the formation of chemotactic agents, neutrophil accumulation at the inflamed area, lipid and protein oxidation and DNA damage. In addition, cycloxygenase activation generates free radicals as intermediates. Consequently, compounds with antioxidant activity are expected to interfere with the inflammatory process. In this study, a number of new compounds are synthesised, containing one or more antioxidant moieties, without any of the characteristics found in the known non steroidal anti-inflammatory drugs. The aim of this work is the investigation of the hypothesis that compounds with antioxidant structure could possess potent anti-inflammatory activity. Thus, not only the most serious side effect of the non steroidal anti-inflammatory agents, gastrointestinal toxicity, could be avoided, but also a protective action against oxidative insult could be offered. The compounds are synthesised from trolox, containing the chromane part of alpha-tocopherol, mainly responsible for the antioxidant activity of vitamin E, 3,5-di-*tert*-butyl-4-hydroxy-benzoic acid or 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) acrylic acid, derivatives of established antioxidants with low toxicity. These acids react with cysteamine, cysteine ethyl ester or γ-aminobutyric acid to give the corresponding amides using carbonyl diimidazole. In the last case, the carboxylic group of GABA is protected with trimethyl-silyl-chloride. Determination of their antioxidant activity, expressed as peroxidation of rat hepatic microsomal membrane lipids and interaction with DPPH is performed. It is found that all compounds are potent antioxidants, with $IC_{50}$ values as low as 1.6μM. Compounds are tested for anti-inflammatory activity, as inhibition of rat paw oedema caused by carrageenan administration. The tested compounds are very potent anti-inflammatory agents inhibiting oedema down to 87% at a relatively low dose. So far, these results support our initial suggestion that compounds with antioxidant activity can affect very significantly conditions like inflammation, which involve oxidative insult.

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PP65

## Carotenoids inhibit peroxyl radical-induced oxidation of hemoglobin and lipids in human erythrocytes

Renan Campos Chisté [a,b], Marisa Freitas [a], Adriana Zerlotti Mercadante [b], Eduarda Fernandes [a]

[a] *REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto (UP), Porto, Portugal*
[b] *Department of Food Science, Faculty of Food Engineering, University of Campinas (UNICAMP), Campinas-SP, Brazil*
*E-mail address:* renanchiste@gmail.com (R.C. Chisté)

Human erythrocytes are known to be highly susceptible to oxidative damage. Here, we evaluated the protective potential of carotenoids usually detected in human blood plasma, namely β-carotene, zeaxanthin, lutein, β-cryptoxanthin and lycopene, against hemoglobin and lipid oxidation in erythrocytes caused by peroxyl radicals ($ROO^{\bullet}$) generated by AAPH (α,α'-azodiisobutyramidine dihydrochloride). Human erythrocytes were isolated from fresh blood from healthy volunteers (n=4) and diluted to a known density ($500x10^6$ cells/mL). Erythrocytes were then exposed to carotenoids (0.09-3 μM), for 30 min, at 37 °C, in a water bath, followed by the addition of 50 mM AAPH. The percentage of hemoglobin oxidation was spectrophotometrically determined at 630 nm, after exposing to AAPH for 4 h. Lipid peroxidation was measured by the thiobarbituric acid-reactive substance (TBARS) assay, after exposing to AAPH for 3 h. Due to the presence of an interfering peak at 453 nm, which distorts the Vis-spectra of TBARS, the percentage of inhibition at 532 nm was obtained after performing the second derivative in its recorded spectra (400–600 nm). Among the tested carotenoids, β-carotene and zeaxanthin were the most efficient in preventing the oxidation of hemoglobin ($IC_{50}=2.9\pm0.3$ μM and $2.9\pm0.1$ μM, respectively), whilst lutein presented 44.7% of inhibition at the highest tested concentration. These carotenoids were more efficient than the positive controls: quercetin ($IC_{50}=10.2\pm0.4$ μM), trolox ($IC_{50}=60.4\pm3.7$ μM) and ascorbic acid ($IC_{50}=108.8\pm5.0$ μM). Regarding lipid peroxidation, lutein was more efficient ($IC_{50}=2.53\pm0.7$ μM) than zeaxanthin ($2.78\pm1.1$ μM) and β-carotene ($3.0\pm0.5$ μM). Again, these carotenoids presented higher efficiency as compared to quercetin ($IC_{50}=17.4\pm1.7$ μM), trolox ($44.1\pm7.3$ μM) and ascorbic acid ($123.7\pm10.5$ μM). It is interesting to notice that β-cryptoxanthin and lycopene did not inhibit hemoglobin oxidation or TBARS formation even at the highest tested concentration. These findings indicate that lutein, zeaxanthin and β-carotene may be useful in preventing $ROO^{\bullet}$-induced oxidative damage to erythrocytes.

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PP66

## A structure-activity relationship study of flavonoids as dual inhibitors of LOX and COX

D. Ribeiro [a], M. Freitas [a], S.M. Tomé [b], A.M.S. Silva [b], G. Porto [c], E. Fernandes [a]

[a] *REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal*
[b] *Departamento de Química & QOPNA, Universidade de Aveiro, Aveiro, Portugal*
[c] *Hematologia Clínica, Hospital Geral de Santo António, Porto, Portugal*
*E-mail address:* dcf10009@ff.up.pt (D. Ribeiro)

Flavonoids have been proving to be excellent antioxidants and a promising alternative in the treatment of inflammatory processes where the traditional molecules are not reaching all the requisites in terms of effectiveness and safety. Notwithstanding, their mechanism of action is not fully disclosed yet and therefore it is of high importance to screen structure/activity relationships. In this sense, lipoxygenases (LOX) and ciclooxygenases (COX) are enzymes of preponderant importance in the arachidonic acid cascade, playing a major role in the production of leukotrienes and prostagladins, well known players in the inflammatory process. [1] The aim of this study was to evaluate the ability of a broad series of flavonoids to inhibit human 5-LOX, through a study of the inhibition of LTB4 formation in human neutrophils, and COX-1 and 2, by measuring the inhibition of the production of PGE2, in a whole blood assay, as previously reported [2]. Overall, the studied flavonoids proved to have encouraging features to make them effective modulators of the inflammatory processes. Additionally, the obtained results evidenced the pivotal role of the catechol group in the B-ring of flavonoids, as it has been already proved to be important in their antioxidant activity, underlying the relationship between the scavenging capacity of flavonoids and their inhibition of these enzymes.

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**References**
[1] Ana Gomes, et al., Current Medicinal Chemistry 15 (2008) 1586; 2008.
[2] Methods in Molecular Biology 644 (2010) 91; 2010.

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PP67

## Cellular redox status by alternariol in Caco-2 cells

C. Fernández-Blanco, G. Font, M.J. Ruiz

*Laboratory of Toxicology, Faculty of Pharmacy, University of Valencia, Burjassot, Valencia, Spain*
*E-mail address:* M.Jose.Ruiz@uv.es (M.J. Ruiz)

Alternariol (AOH) is a secondary fungal metabolite that can produce toxic effects on animal and human health. Its toxicity on different mammalian cell lines has been demonstrated, but the mechanisms have not been fully clarified. The induction of oxidative stress, as a possible mechanism of toxicity, has been assumed. Oxidative stress occurs, as a consequence of an inequity between the prooxidant/antioxidant systems, causing an increase of intracellular generation of reactive oxygen species (ROS). Moreover, Glutathione (GSH) and some enzymes are important antioxidant defense lines in cells. AOH can alter the action of the enzymes implicated in the glutathione redox system: GSH, glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione transferase (GST). The aim of this study is demonstrated if AOH produces toxicity affecting cell viability, generation of ROS species and lipid peroxidation (LPO). And to determine the GSH role in the production of oxidative stress in Caco-2 cells derived from adenocarcinoma human colon. Cytotoxicity was carried out using the MTT and NR assays in the concentration range from

3.125 to 100 μM for 24, 48 and 72 h of exposure. The $IC_{50}$ value for AOH was 25μM with MTT assay. No $IC_{50}$ values were obtained by NR assay. The early intracellular production of ROS was determined with H2-DCFDA fluorescent probe. Caco-2 cells were exposed to 15, 30 and 60μM of AOH from 0 to 120 min. LPO was determined by the TBARS method in cells exposed to 15, 30 and 60 μM for 24 h. ROS generation and LPO ranged from 108% to 122% and from 144% to 255%, respectively compared to the control cells. Regarding GSH and the enzymatic activity, significant differences were observed in the GR, GPx and totally GSH at 60 μM of AOH. However, significant increase of GST was observed up to 15 μM of AOH.

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PP68

## Accelerated age-related loss of muscle mass in homozygotic SOD1 knockout mice is not associated with neuronal oxidative damage

Giorgos K. Sakellariou [a], Adam P. Lightfoot [a], Aphrodite Vasilaki [a], Gareth Nye [a], Holly Van Remmen [b], Susan Brooks [c], Anne McArdle [a], Malcolm J. Jackson [a]

[a] *MRC-Arthritis Research UK Centre for Integrated Research into Musculoskeletal Ageing (CIMA), Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK,*
[b] *University of Texas Health Center at San Antonio and the Barshop Institute for Longevity and Aging Studies, San Antonio, TX, USA,*
[c] *Institute of Gerontology, University of Michigan, MI,USA*
*E-mail address:* giorgos@liv.ac.uk (G.K. Sakellariou)

Introduction: Recent evidence suggests that lack of CuZn superoxide dismutase (SOD1) in muscle-specific SOD1 knockout mice [1] did not lead to the accelerated muscle ageing phenotype seen in the homozygotic-whole body SOD1KO strain [2,3]. Skeletal muscle and motor neurons are implicated in the atrophy process and these studies indicate that muscle degeneration observed in SOD1KO mice may be related to a failure of redox homeostasis in the motor neurons. Methods*:* To assess whether alterations in the redox environment of motor neurons are the primary initiating factor for the observed atrophy, sciatic nerves and skeletal muscle tissues from SOD1KO and age-matched wild type (WT) mice were dissected and analysed for potential changes is redox status and adaptive responses. Results*:* Overt phenotypic changes of accelerated ageing were observed in the *anterior tibialis* and *gastrocnemius* muscles of SOD1KO mice. Other tissues/organs including the heart, liver, kidneys, spleen, brain and lungs were unaffected indicating that removal of SOD1 induces specific effects in skeletal muscle. Muscles from SOD1KO mice showed increased oxidative damage indicated by increased protein oxidation, lipid peroxidation and DNA damage in comparison with muscles from WT mice. Surprisingly, no differences in redox markers or protein expression of protective enzymes were observed in the sciatic nerves from the two strains. Discussion*:* These data imply that the accelerated loss of muscle mass shown in SOD1KO mice is not associated with neuronal oxidative damage, although oxidative modification of specific proteins or small localized transient changes in redox homeostasis may not be detected by current approaches.

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**References**

[1] Zhang, Y., *et al.* (2013). *FASEB J.* In press.
[2] Sakellariou, GK., et al., Aging Cell 10 (2011) 749-760; 2011.
[3] Vasilaki, A., et al., Aging Cell 9 (2010) 979-990; 2010.

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PP69

## Arginine catabolism is driven mainly towards NO synthesis in erythrocytes of patients with type 2 diabetes at first clinical onset

O. Savu [a], O.-M. Bradescu [a], C. Serafinceanu [a], L. Iosif [b], I.-V. Grajdeanu [c], I. Stoian [b,c]

[a] *"N.C. Paulescu" National Institute for Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania*
[b] *R&D IRIST LABMED, Bucharest, Romania*
[c] *Internal Medicine Department, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[d] *Biochemistry Department, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

There is a lack of information related to the involvement of erythrocytes (RBCs) in arginine (Arg) metabolic pathways in diabetes. In the current study, we investigated the Arg/NO metabolic pathway in RBCs of 26 patients with type 2 diabetes at first clinical onset and 19 age-matched non-diabetes control subjects. Arg content and arginase activity were assayed in RBCs of all subjects by capillary electrophoresis and urea production in the sample, respectively. Nitrate and nitrite analysis was performed spectophotometrically in plasma of all subjects by an assay that combines reduction of nitrate with vanadium (III) and measurement of nitrite in a single step. HbA1c was measured by standardized immunoturbidimetry. Patients with type 2 diabetes exhibited similar levels of Arg, compared with non-diabetes controls. Interestingly, arginase activity was significantly lower in patients with diabetes. Higher levels of NO production were observed in RBCs from patients with type 2 diabetes compared with non-diabetes subjects. In contrast, we found no difference in NO production in plasma of our subjects. Our data show that Arg catabolism is driven mainly towards NO synthesis in RBCs of patients with type 2 diabetes at first clinical onset. The decreased arginase activity could be considered a potential mechanism of increased RBCs NO production in early diabetes. Therefore, RBCs pool would represent a potentially compensatory intravascular compartment for endothelial dysfunction under diabetic milieu.

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PP70

## Enhancement of oxidative stress by lipoperoxides in two human cancer cell lines leads to death by different modulation of redox-response

C. Scanarotti [a], S. Vernazza [a], J. Danailova [a], M. Caratelli [b], E.L. Iorio [c], S. Penco [a], M.A. Pronzato [a], A.M. Bassi [a]

[a] *Dept. Experimental Medicine General Pathology, Genoa, Italy*

[b] *Diacron International, Italy*
[c] *International Observatory of Oxidative Stress, Italy*
*E-mail address:* chiara_scanarotti@yahoo.it (C. Scanarotti)

Redox status play a role in cell homeostasis, e.g. ROS overproduction leads to severe cellular damage and death. Oxidation therapy is an innovative anticancer approach to modulate the redox status of tumor cells. One way to achieve this goal is to deliver excess ROS into tumor tissue, directly in cancer cells.

The aim of our in vitro study was to analyze the mechanisms by which the lipoperoxides (LOOHs), increasing oxidative stress, modulate proliferation and angiogenesis, in two human cancer cell lines (bladder carcinoma, *mtp53*-5637, and colon adenocarcinoma, *wtp53*-DLD-1). To mimic the environment surrounding tumor, HECV normal endothelial cells, were exposed directly to LOOH and to conditioned medium (CM), derived from LOOH pretreated cancer cells.

Several markers related to redox status, proliferation/death and angiogenesis were investigated on DLD-1, 5637 and HECV cells, during exposure to 0.1-1% LOOH.

Comparative analysis of baseline parameters (Nrf2, HSP70, iNOS), between normal and cancer cells confirms an imbalance in DLD-1 and in 5637, in spite of endothelial cells. This underlies that among cancer and normal cells there are biological differences, which are necessary for the survival of the tumor. In DLD-1 and 5637, LOOHs inhibited cell growth by decrease of APRIL, VEGF and other proangiogenic factors (iNOS and Hsp70). This negative modulation, more or less marked, depending on the tumor line, seems to depend by redox-mediated mechanisms. In both cell lines, oxidative markers ($O_2^{\bullet}$, TBARs) increase with change in GSH/GSSG ratio and Nrf2 expression. During exposure to CM, HECV proliferation index increases markedly and conversely drop in CM-LOOH cultures.

Analysis of the apoptotic markers showed that cancer cells exhibit different susceptibility to apoptosis, for a different expression of p53; while HECV are refractory to the triggering of apoptosis.

So, LOOHs, by reducing pro-proliferative and proangiogenic factors can be proposed as co-adjuvant in cancer therapy.

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PP71

## The oral bioavailability of curcumin from a micronized powder and micelles is 9- respectively 200-fold higher than that of native curcumin in healthy young women and men

Christina Schiborr [a], Alexa Kocher [a], Jan Frank [a,b]

[a] *Institute of Biological Chemistry and Nutrition, University of Hohenheim, Stuttgart, Germany*
[b] *Institute of Nutrition and Food Science, University of Bonn, Bonn, Germany*
*E-mail address:* christina.schiborr@nutrition-research.de (C. Schiborr)

Curcumin is a phenolic compound isolated from the plant turmeric with antioxidant, anti-inflammatory and other disease-preventive biological activities. The intestinal uptake of curcumin is low and it is quickly metabolised and rapidly excreted from the body. Considering its potent health-beneficial properties, researchers have tried to increase the uptake and retention of curcumin in order to enhance its biological activities. The aim of our project was to develop novel curcumin preparations that enhance the oral bioavailability, retention and, thus, the biopotency of curcumin and to study their safety in humans. Twenty-three subjects participated in this trial, which followed a single-blind crossover design with the three study arms separated by $\geq$1-week washout periods. All participants orally ingested in random order a single dose of 500 mg curcumin as native powder, micronized powder, or liquid micelles. Blood samples were collected at nine different time points after the curcumin dose. Urine was collected during the 24-hour period of the intervention day. Oral administration of curcumin micronisate and curcumin micelles, compared to native curcumin, resulted in more than 9-fold and 200-fold higher bioavailability (based on AUC), respectively. With a dose of 500 mg of curcumin micelles, we observed maximum plasma curcumin concentrations (2622 nmol/L) comparable to those observed after the intake of gram doses of native curcumin in previously published studies. Even 24 h after the intake of curcumin micelles, curcumin plasma concentrations were ~17-times higher (77 nmol/L) than after intake of native curcumin (4 nmol/L). Thus, the bioactive compound is not only absorbed more efficiently from micelles, but also remains present in the organism at high concentrations for much longer. All safety parameters remained within the reference ranges following the consumption of all three curcumin formulations. Curcumin micelles may be promising new tools to safely deliver the nutraceutical in clinical trials.

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PP72

## Peroxidative metabolism of fatty acids in the course of tick-borne encephalitis

I. Jarocka, A. Moniuszko, K. Bielawska, S. Pancewicz, W. Łuczaj, E. Skrzydlewska

*Medical University of Białystok, Bialystok, Poland*
*E-mail address:* skrzydle@umb.edu.pl (E. Skrzydlewska)

Tick-borne encephalitis (TBE) is a tick-borne illness caused by three viruses from family Flaviviridae, genus Flavivirus. The disease is characterized by a biphasic illness and neurological manifestations hallmark the second phase. The diagnosis of TBE can be made by demonstration of IgM and IgG antibody as well as by MRI or EEG abnormalities but all ways demonstrate only non-specific findings. Because this disease belongs to inflammatory diseases its course may be accompanied by an increase in reactive oxygen species generation and non-saturated fatty acids peroxidation. Therefore the aim of this study was to estimate the peroxidative metabolism of PUFA's in the course of TBE. Changes in the free and phospholipid fatty acids level and in the level of isoprostanes and neuroprostanes were determined in the plasma and cerebrospinal fluid of patients with TBE (n=44) and of healthy controls (n=30). The level of MDA, HNE, HHE, acrolein and croton aldehyde as well as the activity of phospholipase A2 and PAF acetylhydrolase were assayed in the plasma. The plasma phospholipid PUFA's level was decreased while free PUFA's level was slightly increased. Almost 5-fold and 4-fold higher level of the total plasma isoprostanes and neuroprostanes, respectively were observed in plasma TBE patients compared to the controls, while in the cerebrospinal fluid they increased over 2-fold. The plasma level of MDA, HNE, HHE, acrolein and croton aldehyde was enhanced by about 3-, 2-, 2-, 4, 2-fold, respectively. The plasma PLA2 and PAF acetylhydrolase activity was over 2- fold higher in TBE patients than in the healthy subjects. It suggests that in the course of TBE the metabolism of PUFA's

is significantly enhanced. The products of this metabolism may be useful in TBE diagnosis.

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PP73

## Influence of free fatty acids and quercetin on ROS generation and other mitochondrial function of human preadipocytes

Agnieszka Śliwa, Joanna Góralska, Anna Polus, Anna Gruca, Aldona Dembińska-Kieć

*Department of Clinical Biochemistry; Jagiellonian University Medical College, Kraków, Poland*
*E-mail address:* a.sliwa@uj.edu.pl (A. Śliwa)

One of the reasons for complication of obesity, such as diabetes type 2, could be mitochondrial dysfunction, increase ROS generation and decrease antioxidant activity. FFAs increase mitochondrial generation of ROS by depolarization of the mitochondrial inner membrane due to the uncoupling effect. Quercetin is one of the most common flavonoids which exert antioxidant activities. The effect of these nutrients on metabolism of human preadipocytes is still not well recognized.

The aim of the study was investigated the effect of selected free fatty acids and quercetin on mitochondrial function human preadipocytes.

The human immortalized preadipocytes (Chub-S7) were incubated for 24h with 30uM of FFA (PA, OA, AA, EPA, TTA) or with quercetin (10uM, 30uM, 50uM, 70uM, 100uM). Mitochondrial function was monitored by measurements of the mitochondrial oxygen consumption (high-resolution respirometry), ATP content (Parkin Elmer), mitochondrial membrane potential [flow cytometry (BD) and high thoroughput fluorescent microscopy (BD Bioimager 855)], activity of the caspase-9 (R&D Systems). ROS generation was monitored by use fluorescent method (DCFH-DA).

Palmitic acid impaired mitochondrial function in preadipocytes, manifested by decreased intracellular ATP content and increased ROS generation. Arachidonic and oleic acid increased ROS generation and caspase-9 activity related with the mitochondrial apoptosis pathway. Eicosapentaenoic and tetradecylthioacetic acid decreased mitochondrial respiration and ATP content. Quercetin inhibited ROS generation and increased mitochondrial membrane potential.

Selected nutrients: free fatty acids and flavonoid- quercetin affect mitochondrial function of human preadipocytes which could be especially interesting in the light of recent data, indicating that regulation of mitochondrial activity may be the target pathway for treating obesity.

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PP74

## 2-Chloro-1,4-naphthoquinone derivative of quercetin as an efficient inhibitor of AKR1B1 and AKR1B10. Implications for diabetic complications, inflammatory disorders and cancer

Marta Soltesova-Prnova [a], Ivana Milackova [a], Magdalena Majekova [a], Ruzena Sotnikova [a], Lucia Kovacikova [a], Lucia Rackova [a], Beatriz Díez-Dacal [b], Dolores Perez-Sala [b], Shabnam Enayat [c], Sreeparna Banerjee [c], Miroslav Veverka [d], Milan Stefek [a]

[a] *Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia*
[b] *Department of Chemical and Physical Biology, Centro de Investigaciones Biológicas, CSIC, Madrid, Spain*
[c] *Department of Biological Sciences, Middle East Technical University, Ankara, Turkey*
[d] *EUROFINS Bel/Novamann International, s.r.o., Bratislava, Slovakia*
*E-mail address:* marta.prnova@gmail.com (M. Soltesova-Prnova)

Lipid peroxidation-derived aldehydes, such as 4-hydroxy-trans-2-nonenal (HNE) and their glutathione conjugates (e.g. GS-HNE), were found to be efficiently reduced by aldo-keto reductase AKR1B1 to the corresponding alcohols, DHN (1,4-dihydroxy-nonene) and GS-DHN (glutathionyl-1,4-dihydroxynonene), which mediate inflammatory signals. The reduced aldehyde-glutathione conjugate GS-DHN is considered a novel signaling intermediate in the transduction of reactive oxygen species-initiated cell signals, leading eventually to an inflammatory response. A closely related aldo-keto reductase, AKR1B10, has been implicated in various types of cancer. AKR1B10 was found to be significantly over-expressed in cancers of the lung and liver. Pharmacological inhibition or genetic ablation of AKR1B1 and AKR1B10 has been shown to prevent cytokine and growth factor induced inflammatory signals and proliferation of cancer cells. Recently 21 novel derivatives of the flavonoid quercetin have been synthesized and screened for inhibition of aldose reductase and for antioxidant action. Among the compounds studied, 2-chloro-1,4-naphthoquinone derivative of quercetin (CHNQ) exhibited the highest biological activities, surpassing those of the parent quercetin. In this study, the inhibition of human aldo-keto reductases AKR1B1 and AKR1B10 by CHNQ was studied in greater detail. The inhibitory activity of CHNQ was characterized by $IC_{50}$ values in low micromolar region. To identify crucial interactions within the enzyme binding site, molecular modeling studies were performed. Selectivity in relation to rat aldehyde reductase was recorded. In addition, implications for treatment of diabetic complications, inflammatory disorders and cancer were indicated, based on CHNQ studies performed in appropriate models comprising model of diabetic cataract in vitro, rat model of ulcerative colitis in vivo and model of proliferating colon cancer cell lines in culture. At CHNQ concentrations below 25 μM, no cytotoxicity was observed in cultured fibroblasts.The results, along with the physicochemical properties not violating Lipinski's rules, point to a good bioavailability of CHNQ and to its prospective pharmacological use.

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PP75

## Determining the impact of oxidation on the motility of single muscle-fibres expressing different myosin isoforms

D. Spanos [a], M. Li [b], C.P. Baron [a], L. Larsson [b]

[a] *National Food Institute, Technical University of Denmark, Lyngby, Denmark*
[b] *Dept. of Neuroscience, Clinical Neurophysiology, Uppsala University, Uppsala, Sweden*
*E-mail address:* disp@food.dtu.dk (D. Spanos)

Under oxidative stress, myosin has been shown to be one of the muscle proteins that are extensively modified, leading to carbonylation and cross-linking. However, how oxidation affects the actomyosin interaction in muscle fibres with different metabolic profiles and expressing different myosin heavy chain (MyHC) isoforms has not been previously investigated. Oxidation of myosin isolated from muscle fibres originating from various porcine muscles with a different metabolic profile was studied using a single muscle fibre in-vitro motility assay, allowing measurements of catalytic properties (motility speed) and force-generation capacity of specific MyHC isoforms. In the experimental procedure, single muscle fibres were split in different segments and each segment was exposed to a different concentration of hydrogen peroxide. Speed and force measurements were recorded and compared, to assess the effect of myosin oxidation on motility and force. The MyHC isoform expression in the single muscle fibre was subsequently determined on silver-stained gel SDS-PAGE. Preliminary results indicate a decrease of directionality and speed of the in-vitro motility as a result of an oxidative environment, and the successful use of the assay in determining fibre-specific responses to oxidation. Subsequent analyses will focus on the location of protein modifications on the myosin molecule and on how these modifications induce changes in speed and force.

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PP76

## Cu(II)-disulfide complexes display simultaneous superoxide dismutase- and catalase-like activities

Margarita E. Aliaga [a], Daniela Andrade-Acuña [a,b], Camilo López-Alarcón [a], Cristián Sandoval-Acuña [b], Hernán Speisky [e,d]

[a] *Facultad de Quimica, Pontificia Universidad Católica de Chile, Santiago, Chile*
[b] *Nutrition and Food Technology Institute, University of Chile*
[c] *Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile*
*E-mail address:* hspeisky@inta.uchile.cl (H. Speisky)

Superoxide is a potentially toxic by-product of cellular metabolism. Since this species can contribute to the development of biological damage, preventing its accumulation *via* its removal from cells is of interest. Here we addressed the *in vitro* ability of complexes formed between copper(II) ions and various biologically-occurring disulfides (RSSR: oxidized glutathione, cystine, homocystine and α-lipoic acid) to react with superoxide. Results show that each of the studied complexes is able to react with superoxide (generated by a xanthine/xanthine oxidase system) at rate constants (*k*Cu(II)-RSSR) close to 106 M-1 s-1, a value which is three orders of magnitude lower than that reported for superoxide dismutase (SOD) but comparable to that of several other copper-containing complexes reported as SOD mimetics. The interaction between the tested Cu(II)-RSSR and superoxide, led to the generation and recovery of concentrations of hydrogen peroxide and oxygen that were, respectively, below and above those theoretically-expected from a sole SOD mimetic action. Interestingly, molecular oxygen was generated when the Cu(II)-RSSR complexes were directly incubated with hydrogen peroxide. Taken together, these results reveal that the Cu(II)-RSSR complexes not only have the capacity to dismutate superoxide but also to simultaneously act like catalase mimetic molecules. When added to superoxide-overproducing mitochondria (condition attained by inhibiting its complex I), three of the tested complexes were able (2-4 μM), not only to totally restore, but also to lower below the basal level the mitochondrial production of superoxide. The present study is first in reporting on the potential of Cu(II)-disulfides complexes to act as SOD and catalase like molecules, suggesting a potential for these type of compounds to act as such under both, physiological and/or oxidative-stress conditions.

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PP77

## Placental antioxidant systems –maternal age influence

L. Gaman [a,b], D. Lixandru [a], M. Gilca [a,b], I. Gheorgiu [a], C. Muscurel [a], E. Panait [a], I. Horhoianu [a], V. Atanasiu [a], I. Stoian [a,b]

[a] *University of Medicine and Pharmacy Carol Davila Bucharest, Romania*
[b] *R&D Irist Labmed, Bucharest, Romania*

Maternal age has increased during last years due to social and economical factors. Because ageing is associated with increased oxidative stress we have considered of interest to sudy placental antioxidant systems in relation with mother age.183 women presented at term at Municipal Universitary Hospital have been enrolled in the study.Five age groups have been considered: : group 1- less then 20 years( n=19), group 2 – age between 20-25 years (n=32), group 3- age between 25-30 years (n = 70), group 4 -age between 30-35 years (n=47), and group 5 –age more then 35 years (n=15). The activity of catalase(CAT), superoxidismutase (SOD), glutathione reductase(GRed), glutathione peroxidise,(GPx), glutathione trasferase(GT) and the concentrations of free and total thiols groups have been measured. Catalase, glutathione transferase, glutathione peroxidase and total thiols were significantly lower in groups 1 and 2 compared with groups 4 and 5. No significant change has been observed in SOD activity in all the groups. Significaly increased total thiols levels were observed in groups 4 and 5 compared with groups 1 and 2.Our results are sustaining increased oxidative stress at placental level in mothers aged more then 30 years

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PP78

## A proof of concept study to model a tiered oxidative stress response in lung epithelial cells exposed to cigarette smoke aqueous extract

M. Taylor, I. Pentland, T. Carr, N. Cockcroft, F. Lowe

*British American Tobacco, Group R&D, Southampton, U.K*
*E-mail address:* markr_taylor@bat.com (M. Taylor)

Cigarette smoke exposure is associated with inflammation and oxidative stress, which plays a pivotal role in the pathogenesis of many diseases and has been linked to the development of atherosclerosis, lung disease and cancer. Responses in lung epithelial cells to oxidative stress have been reported to follow a “tiered” structure, consisting of distinct responses that occur sequentially

with increasing oxidant exposure. Exposure of lung epithelial cells to increasing concentrations of oxidants causes rapid antioxidant depletion with an associated lowering of the GSH:GSSG ratio. This leads to activation of the Antioxidant Response Elements (ARE) mediated through the transcription factor Nrf2, followed by the activation of NF-κB which initiates a pro-inflammatory response at intermediary oxidative stress levels and finally culminates in cytotoxicity in the form of apoptosis or necrosis. Here we show how three *in vitro* techniques may be utilised in combination to characterise these three distinct tiers, following exposure to cigarette smoke aqueous extract (CSEaq). Intracellular reduced glutathione levels were depleted and pro-inflammatory cytokines interleukin 1α and interleukin 6 were elevated in a dose dependent manner. At higher doses of CSEaq, apoptosis and necrosis occurred. The induction of each tier occurred sequentially with increasing CSEaq dose, confirming literature observations. Following further development work to characterise the tier boundaries, this approach could be potentially useful for future assessment of combustible and non-combustible tobacco products, and other products which may induce oxidative stress in epithelial cells.

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PP79

## Glycogen and aging: an in vitro model of a new relationship

G. Testoni [a,b], V. Vanhooren [a,b], C. Libert [b], J. Guinovart [a]

[a] *Institute for Research in Biomedicine, Barcelona, Spain*
[b] *Department for Molecular Biomedical Research, VIB, Gent, Belgium*
*E-mail address:* giorgia.testoni@irbbarcelona.org (G. Testoni)

Glycogen is a branched polymer of glucose that serves to store glucose in cells in times of nutritional abundance for usage in times of need. It is present in organisms as diverse as bacteria and man. Glycogen biogenesis has often been implicated in neurodegenerative and age-related diseases. However, a clear mechanism underlying this relationship remains unclear.

Some pathological conditions are characterized by an accumulation of abnormal and undegradable glycogen called Polyglucosan Bodies (PGBs). These deposits occur in Glycogen Storage Diseases (GSDs) such as Andersen, Cori and Pompe Diseases, and in Lafora disease, where the PGBs are called Lafora Bodies (LBs). These aggregates show an excessive amount of poorly branched glycogen bound by proteins, thus resulting in resistance to degradation and cell toxicity. During aging in humans and mice, similar but less abundant undegradable glycogen deposits called Corpora Amylacea (CAs) are observed. Although CAs and LBs are similar in composition, it remains unclear whether they are formed through the same mechanisms.

Here we sought to reproduce an *in vitro* model of PGB formation mimicking the process that occurs in GSDs and aging in order to study the mechanism of PGB origin in detail and to determine whether abnormal glycogen accumulation is a cause or a consequence of aging. For this purpose, we established a stressed-induced premature cellular senescence system using Mouse Embryonic Fibroblasts (MEFs) chronically treated with low concentrations of $H_2O_2$.

Wild-type (WT) MEFs became senescent (positive for B-Gal, p21, p16 markers) after 10 days of treatment with low concentrations of $H_2O_2$ and showed a change in glycogen content, which was easily observed by Periodic Acid Schiff staining (PAS), immunofluorescence, and biochemical quantification.

These effects were compared to a glycogen-free system, namely in MEFs obtained from Glycogen Synthesis Knockout (GS KO) mice. The reaction of GS KO MEFs to the treatment differed to that shown by GS WT MEFs, thereby demonstrating that glycogen is involved in stress-induced premature senescence. We show that glycogen and glycogen metabolism-related proteins undergo a progressive change during senescence. Our results indicate that augmented glycogenesis is directly linked to cellular senescence, thus providing new insights into the metabolic background of aging and aging-related disease.

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PP80

## Differential regulation of the proteasome and of antioxidant responses during aging in the somatic tissues and the gonads of *Drosophila*

Eleni N. Tsakiri [a], Gerasimos P. Sykiotis [b], Issidora S. Papassideri [a], Evangelos Terpos [c], Meletios A. Dimopoulos [c], Vassilis G. Gorgoulis [d], Dirk Bohmann [e], Ioannis P. Trougakos [a]

[a] *Department of Cell Biology and Biophysics, Faculty of Biology, University of Athens, Panepistimiopolis, Athens, Greece*
[b] *Division of Endocrinology, Department of Internal Medicine, University of Patras Medical School, Patras, Greece*
[c] *Department of Clinical Therapeutics, School of Medicine, University of Athens, Athens, Greece*
[d] *Department of Histology and Embryology, School of Medicine, University of Athens, Athens, Greece*
[e] *Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY, USA*

The ubiquitin-proteasome system is central to the regulation of proteostasis as it is involved in the degradation of a multitude of protein substrates including damaged proteins. Nevertheless, the proteasome regulation and the impact of *in vivo* impaired proteasome functionality on the proteostasis networks and aging processes remain poorly understood. We addressed these issues at the model organism *Drosophila melanogaster*. We found a decline of proteasome activity and expression and an accumulation of ubiquitinated and carbonylated proteins in somatic tissues during aging; on the contrary gonads retain youthful phenotypes and are resistant, independently of age, to oxidative challenge. Moreover, we found that RNAi-mediated knock down of 20S proteasome subunits resulted in larval lethality. We therefore studied the molecular effects of proteasome dysfunction in adult flies by developing a pharmacological model of dose-dependent inhibition of peptidase activities in structurally intact proteasomes. We observed that impaired proteasome function promoted several "old-age" phenotypes and a reduction of flies' lifespan. Also, loss-of proteasome activity induced in the young somatic tissues, and independently of age in the gonads, higher expression levels and assembly rates of proteasome subunits. Proteasome dysfunction was signaled to the proteostasis network of the responsive tissues by reactive oxygen species that originated from malfunctioning mitochondria and triggered an NFE2-related factor 2 (Nrf2)-dependent upregulation of the proteasome subunits. We further show that RNAi-mediated Nrf2 knock down reduced proteasome activities and longevity, while activation of the Nrf2 in transgenic flies upregulated basal proteasome expression and peptidase activities independently of flies' age. Our *in vivo* findings exemplify the molecular cross-talk of proteasome functionality and Nrf2-

mediated antioxidant responses towards the maintenance of organismal homeostasis.

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PP81

## Leptin improves mitochondrial quality control in MCF-7 breast cancer cells

M. del Mar Blanquer-Rosselló, J. Sastre-Serra, J. Oliver, P. Roca, A. Valle

*Grupo Multidisciplinar de Oncología Traslacional, Institut Universitari d'Investigació en Ciencies de la Salut, Universitat de les Illes Balears, Ciber Fisiopatología Obesidad y Nutrición (CB06/03) Instituto Salud Carlos III, Palma de Mallorca, Spain*
*E-mail address:* adamo.valle@uib.es (A. Valle)

Mitochondria are highly dynamic organelles and undergo constant fusion and fission that are essential for maintaining physiological functions of cells. Mitochondrial dynamics are involved in oxidative stress control, migration and cell invasion in breast cancer cells. Obesity is a known risk factor for the development and poor prognosis of breast cancer in postmenopusal women. Among the many factors associated with obesity, leptin has emerged as a potential link. Although this adipokine has many roles in breast cancer cell growth and invasion, little is known about the role of leptin in mitochondrial dynamics and oxidative stress. Thus, our goal was to test whether leptin is able to alter mitochondria dynamics and oxidative stress in breast cancer cells. Toward this end, we analyzed the expression signature of mitochondrial biogenesis (PGC-1alpha, NRF1, NRF2, TFAM, MTSSB, OXPHOS) and dynamics (Drp1, Fis1, Mnf1, Mnf2, Opa1) genes in leptin-treated MCF-7 by RT-PCR and western blot. Mitochondrial network dynamics and mitophagy were monitored by confocal microscopy and oxidative stress status was estimated by $H_2O_2$ production, antioxidant enzyme levels and carbonyl protein content. We observed that leptin increased the expression of the biogenesis program without a net increase in OXPHOS system. Mitochondrial fusion and fission genes were up-regulated, suggesting a more frequent pattern of fusion-fision cycles. Confocal microscopy confirmed the ability of leptin to increase the mitochondrial quality control by balanced biogenesis, dynamics and mitophagy. These changes in mitochondria network were accompanied by an improved oxidative stress status. Altogether, these results suggest a new mechanism based on changes in mitochondria quality control for the effects of leptin in breast cancer cell behavior.

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PP82

## Does manipulation of environmental oxygen modify the Reactive Oxygen Species generation during myogenesis?

Aphrodite Vasilaki, Rachel McCormick, Anne McArdle, Malcolm J. Jackson

*MRC-Arthritis Research UK Centre for Integrated Research into Musculoskeletal Ageing (CIMA), Faculty of Health and Life Sciences, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK*
*E-mail address:* vasilaki@liv.ac.uk (A. Vasilaki)

Studies have indicated that regulated changes in ROS formation are important in maintaining the normal sequence and development of myogenesis. Both excessive formation and a reduction in ROS have been shown to affect muscle differentiation in a negative way. Cultured cells are typically grown in 20% oxygen however this is not physiological for a number of cell types, including skeletal muscle. A number of studies have demonstrated modifications in ROS generation in cultured skeletal muscle cells but these were carried out under non-physiological conditions (20% oxygen). The aim was therefore to examine the generation of ROS in cultured skeletal muscle cells under more physiological conditions (6% oxygen) and determine whether this affects muscle myogenesis. Primary muscle cells were cultured in 35mm gelatin coated tissue culture plates in DMEM containing 20% (v/v) FCS. To induce myotube formation the medium was replaced with DMEM containing 2% horse serum. To assess whether manipulation of extracellular oxygen in cultured muscle cells modifies myoblast proliferation and myotube formation, cultures were grown in 20% or 6% oxygen environments and ROS activities were measured at different stages of myogenesis using fluorescent probes e.g. dihydroethidium (DHE). Data demonstrated that proliferation of satellite cells was increased when cells were grown in 6% oxygen compared with 20% oxygen. Myoblasts grown in 20% oxygen showed an increase in DHE oxidation compared with myoblasts grown in 6% oxygen ($1764.1 \pm 141.9$ vs $1003.7 \pm 124.6$). Myotubes grown in 20% oxygen also showed an increase in DHE oxidation compared with myotubes grown in 6% oxygen ($2337.6 \pm 155.5$ vs $1712.9 \pm 110.0$). These data indicate that the oxidation of DHE in resting skeletal muscle myoblasts and myotubes is influenced by changes in environmental oxygen concentration and that increased formation of ROS during skeletal muscle differentiation may influence myogenesis in a negative manner.

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PP83

## Increased lipoperoxidation levels could not reflect oxidative damage in mollusks

C. Ornelas-Gómez [a], D. Domínguez-Ojeda [a], A.L. Rendón-Ramírez [b], A.B. Martínez-Rizo [a], J.B. Velázquez-Fernández [a]

[a] *Autonomous University of Nayarit, Tepic, México*
[b] *Unidad de Biofísica, CSIC-Universidad del País Vasco, Leioa, Spain*
*E-mail address:* jesusbvf@gmail.com (J.B. Velázquez-Fernández)

In recent years, oxidative damage (OD) has been used as a environmental biomarker to monitor damage by pollutants in aquatic ecosystems or organisms. Traditionally, OD is believed to be caused by an imbalance of redox state of cells, either by an increase in oxidative or pro-oxidant agents or by a decrease in antioxidant dell defenses. Either way, the changes in OD biomarkers has been interpreted as a damage in metabolism of cells, i.e., an increase in OD has been interpreted as a damage, and an decrease in LPO as a result of a good antioxidant defense. Some pollutants can generate OD. The use of a positive control would shed light on interpretation of the extent of OD. We have used a oxidative challenge to make positive controls of mollusks. We measured thiobarbituric-acid reactive speciest to evaluate

lipoperoxidation. In some species, the oxidative challenge generated OD measurable in the same tissue, in others, the lipoperoxidation evaluated in the tissue was significantly lower than the negative control. It seems that these organisms are able to get rid of lipoperoxidation products by releasing them to the environment so its lipoperoxides are kept at minimum level.

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PP84

## Enhanced levels of myeloperoxidase in the ROS-induced vascular damage is related to nephropathy in Type 2 Diabetes

S. Rovira-Llopis, M. Rocha, M. Guzman-Fulgencio, F. Iannantuomi, R. Falcon, A. Jover, A. Hernandez-Mijares, V.M. Victor

*Fundacion para la Investigacion Sanitaria y Biomedica de la Comunidad Valenciana FISABIO, Endocrinology Service, University Hospital Doctor Peset , Valencia, Spain*
*E-mail address:* vmvictor@gmail.com (V.M. Victor)

It is still unclear whether microvascular complications of type 2 diabetes correlate with leukocyte-endothelium interactions and/or myeloperoxidase (MPO) levels. In the present study, we found that serum levels of glucose, the rate of ROS and MPO concentration were higher in type 2 diabetic patients. Patients with nephropathy (39.6%) presented higher MPO levels that correlate positively with the albumin/creatinine ratio ($r=0.59$, $p<0.05$). In addition, nephropatic patients showed increased leukocyte-endothelium interactions due to an undermining of polymorphonuclear leukocytes (PMN) rolling velocity and increased rolling flux and adhesion, which was accompanied by a rise in levels of the proinflammatory cytokine tumour necrosis factor alpha (TNFα) and the adhesion molecule E-selectin. Furthermore, MPO levels were positively correlated with PMN rolling flux ($r=0.855$, $p<0.01$) and adhesion ($r=0.682$, $p<0.05$). Our results lead to the hypothesis that type 2 diabetes induces oxidative stress and an increase in MPO levels and leukocyte-endothelium interactions, and that these effects correlate with the development of nephropathy.

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PP85

## Enhancing photodynamic therapy of refractory solid cancers: combining second-generation photosensitizers with multi-targeted liposomal delivery

Ruud Weijer [a], Mans Broekgaarden [a,b], Remko van Vught [b], Eefjan Breukink [b], Thomas M. van Gulik [a], Michal Heger [a,b]

[a] *Department of Experimental Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*
[b] *Membrane Biochemistry and Biophysics, Institute of Biomembranes, University of Utrecht, Utrecht, The Netherlands*
*E-mail address:* r.weijer@amc.nl (R. Weijer)

Photodynamic therapy (PDT) for the treatment of solid cancers entails the application of a photosensitizer (PS), its subsequent accumulation in target tissue, followed by local illumination. This results in PS activation and generation of deleterious reactive oxygen species (ROS), culminating in photochemical destruction of cancerous tissue. However, PDT with currently approved PSs is associated with phototoxicity and generally limited efficacy, as: (1) the PSs possess unfavorable photophysical and photochemical properties, (2) the route of administration is suboptimal, and (3) the upregulation of survival pathways in tumor cells may impede cell death after PDT. To circumvent these issues, we developed a comprehensive PDT modality based on liposomal encapsulation of a 2nd-generation PS, zinc phthalocyanine (ZnPC), which is directed to pharmacologically relevant tumor sites: tumor cells, tumor endothelium, and tumor interstitial spaces. Interstitially-targeted liposomes (ITLs) were composed of DPPC and DSPE-$PEG_{2000}$ (96:4 mol%). Endothelial cell-targeting liposomes (ETLs) contained DPPC, DC-cholesterol, cholesterol, and DSPE-$PEG_{2000}$ (66:25:5:4 mol%). Tumor cell-targeting liposomes (TTLs) were comprised of DPPC, cholesterol, and DSPE-$PEG_{2000}$-maleimide (66:30:4 mol%) coupled to specific single-domain antibodies. All liposomal formulations contained ZnPC at a ZnPC-to-lipid molar ratio of 0.003. We characterized ZnPC-liposomes in terms of spectral properties and substrate oxidation potential. PDT efficacy and dark toxicity were examined in human umbilical vein endothelial cells (HUVECs) and A431 and Sk-Cha1 cancer cell lines. Our preliminary studies revealed that ZnPC can be efficiently encapsulated in liposomes that, upon irradiation, generate ROS that oxidize proteins, cell membrane constituents such as phospholipids, and a ROS-sensitive fluorescent probe ($DCFH_2$). In addition, ZnPC-liposomes appear to be non-toxic in dark conditions in vitro, but upon exposure to 671-nm light induce mainly necrosis in a lipid dose-dependent fashion. If successfully implemented in vivo, the multi-targeting strategy is expected to augment therapeutic efficacy at lower systemic PS concentrations and reduce phototoxicity.

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PP86

## Identification of 6-$NO_2$Trp containing proteins in SHRSP – possible biomarker and relation to hypertension

H. Kawasaki [a], M. Ikeda [b], K. Sakai [c], A. Matsumoto [d], K. Iizumi [a], M. Uda [a], T. Baba [a], K. Takamori [a], F. Yamakura [e]

[a] *School of Medicine, Juntendo University, Chiba, Japan*
[b] *Faculty of Social Environment,Tokoha University, Shizuoka, Japan*
[c] *JaICA, Nikken Seil Co Ltd. Shizuoka, Japan*
[d] *School of Health and Sports Science, Juntendo University, Chiba, Japan*
[e] *School of Health Care and Nursing, Juntendo University, Chiba, Japan*
*E-mail address:* yamakura@sakura.juntendo.ac.jp (F. Yamakura)

Purpose: Protein nitration is a post-translational modification that is induced by reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^{\bullet}$) and nitrogen dioxide ($NO_2$). Nitration of tyrosine residues in proteins has been studied extensively and found in many diseases and also in physiological processes. We have found a novel type of protein nitration, formation of 6-nitrotryptophan (6-$NO_2$Trp) residues, developed its specific antibody, and constructed a method to identify the position of 6-$NO_2$Trp in proteins by using LC-ESI MS-MS. In this study, we applied this method for stroke-prone spontaneously hypertensive rat, SHRSP, which is known to cause stroke almost 80% after 25 weeks of age in male.

Methods: SHRSP (male, 18.5 weeks of age, n=8) were used in this study. Liver, heart, and kidney were dissected and serum was also prepared. 6-$NO_2$Trp-containing proteins were detected by western blotting using anti-6-$NO_2$Trp antibody after SDS-PAGE and 2D-PAGE. The protein bands, which were observed specifically in SHRSP as anti-6-$NO_2$Trp-positive bands in the western blotting, were digested with trypsin and subjected to LC-ESI-MS-MS analysis. WKY rat was used as a control.

Results: Although several similar immuno-reactive bands were observed in serum from SHRSP and WSK rats, we found a few bands that appeared only in SHRSP or more nitration in SHRSP than in WKY. We successfully identified one of them as apolipoprotein E and determined the position of 6-$NO_2$Trp in the amino acid sequence, which is Trp274, with the sequence coverage of 71%. We found no nitrotyrosine residue. Trp274 is known to have an important role to form high-density lipoprotein. We also found other proteins containing 6-$NO_2$Trp specifically in the extracts from the organs of SHRSP.

Conclusion: Formation of 6-$NO_2$Trp in the specific protein could be a biomarker for hypertension. This modification could also have some relation for the development of hypertension.

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PP87

## The antioxidant effects of minocycline and tetracycline on 3-nitropropionic acid induced experimental Huntington's disease model

Meral Yüksel, Hülya Şahin

*Department of Medical Laboratory Techniques, Marmara University Vocational School of Health Related Services, Haydarpasa-Istanbul, Turkey*
*E-mail address:* meralyuksel@gmail.com (M. Yüksel)

3- nitropropionic acid is a fungal toxin, which inhibits succinate dehydrogease activity in both of Krebs cycle and electron-transport chain. Systemic administration of 3-NP to rats results with choreiform movements and lesions in striata, such as Huntington's disease (HD). Minocycline is a second generation tetracycline compound with known neuroprotective activity in animal models. In this study we investigated the effect of minocycline (MIN) and tetracycline (TET) on oxidative stress parameters in an experimental HD model mediated by 3-NP in rats.

Sprague-Dawley rats (12 weeks old, female, n=32) were included in the study. HD group received 3-NP at a dose of 20 mg/kg/day and the controls become SF for 1 week. TET (30 mg/kg/day) and MIN (45 mg/kg/day) are given before 3-NP injections for 3 days and additionally for 1 week. After 10 days, rats were sacrificed, their brains were removed, striatal parts were dissected for tissue glutathione (GSH), malondialdehyde (MDA), luminol and lucigenin enhanced chemiluminescence (CL) measurements. Statistical analysis was assessed using one-way ANOVA, followed by Tukey-Kramer post hoc test, $p < 0.05$ was set as significant. Our results have shown that striatal MDA levels in 3-NP ($63.6 \pm 20.7$ nmol/g tissue) induced rats are higher than controls ($25.3 \pm 5.1$ nmol/g tissue; $p < 0.001$). MIN and TET injections are reduced MDA levels significantly ($p < 0.01$). Tissue GSH levels are lower in 3-NP group ($1.63 \pm 0.42$ umol/g tissue) with respect to the control rats ($3.92 \pm 1.57$ umol/g tissue; $p < 0.01$). MIN and TET applications increased striatal GSH levels significantly ($p < 0.01$ and $p < 0.05$). Luminol and lucigenin enhanced CL levels were higher in 3-NP group ($p < 0.001$); whereas MIN and TET administration reduced CL measurements significantly ($p < 0.01$). In conclusion our results have suggested that MIN and TET injections are effective against 3-NP induced rats. Reduced lipid peroxidation, increased GSH levels and reduced luminol and lucigenin enhanced CL measurements show that MIN and TET have antioxidant effects.

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PP88

## ESR study on the hyperfine structure of free radicals derived from 1,3-disubstituted-5-nitroindazoles and 1,4-disubstituted-7-nitroquinoxalin-2-ones

I. Zárate [a], B. Aguilera-Venegas [a,b], C. Olea-Azar [a], V.J. Arán [c]

[a] *Laboratory of Free Radicals, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile*
[b] *Laboratory of Antioxidants, Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile*
[c] *Medicinal Chemistry Institute (IQM), Superior Council of Scientific Research (CSIC), Madrid, Spain*
*E-mail address:* zarate.utem@gmail.com (I. Zárate)

Nitro compounds derived from the indazole(NI) and quinoxaline(NQ) structures are currently emerging as potential drugs against various human pathologies. *In vitro* studies reveal antimicrobial, antifungal and antiangiogenic features. Moreover, current studies have disclosed a potent activity against African and American trypanosomiasis. For the latter, it is believed that these drugs exert their biological properties through the initial bioreduction via putative nitroreductases which leads to the formation of a nitro radical and the subsequent formation of highly reactive oxygen species (ROS) by interaction with molecular oxygen. However, the ROS production depends on the stability of the nitro radical and therefore, its electronic structure must be assessed. Our results reveal that NI and NQ are prone to be electrochemically reduced, exhibiting a quasi-reversible wave at around -1.10 V corresponding to the nitro radical formation. At this reduction potential, NI and NQ exhibit ESR spectra differenced by the number of the lines (and widths thereof). The results from the ESR simulation suggest that the differences observed between NI and NQ spectra are due to the relaxation phenomena between the free radical and the environment (free radical generated in organic sample-DMSO). On the contrary, hyperfine patterns were comparable at all: easily described in terms of two triplets from nitrogen corresponding to coupling of the unpaired electron with the nitro group and N-1, and three doublets corresponding to the hydrogens belonging to benzene ring. Accordingly, the electronic distribution of the unpaired electron suggests that the reduction of both NI and NQ leads to resonance-stabilized free radicals with a quite similar behavior as regards to their stability. Unsurprisingly, *T.cruzi* cultures incubated with NI (or NQ) showed a similar ROS production.

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PP89

## Evaluation of the antioxidant capacity in natural products: Propolis and chia plant

C. Castro [b], P. Guzman [b], C. Delporte [b], C. Olea-Azar [a], M.C. Zúñiga [a]

[a] *Laboratory of Free Radicals, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile*
[b] *Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Chile*
*E-mail address:* mczuniga@ciq.uchile.cl (M.C. Zúñiga)

The current trend in the developed world as far as food is concerned, indicates a clear increase by consumers for foods that are nutritionally balanced and safe. This change in the tastes, preferences and the demands of modern consumers creates a new area of development and challenges in food and nutritional science. Within this context, the food industry needs to meet such demands by, for example, incorporating additional ingredients in the manufacture and development of new products. One alternative that has great potential within the food industry is the use of natural products, such as ancestral seeds and propolis. Based on reports to date and in relation to the high nutritional and functional value of chia seeds, it follows that some of these components are also present in the plant. These components, commonly treated as waste, could be a source antioxidant with a greatly unexplored industrial use. Furthermore, the most active hive product regarding its antioxidant properties is the propolis. In this work, we determined the antioxidant activity of chia Plant and propolis extracts. The antioxidant properties were evaluated using ORAC (Oxygen Radical Absorbance Capacity-Fluorescein), Using two different target molecule: (i) Fluorescein (ORAC-FL) and (ii) Pyrogallol red (ORAC-PGR). The rate of reaction between the PGR and AAPH is much faster than FL, so the second method gives information on the reactivity of antioxidants more than stoichiometric factors as fluorescein. ORAC-PGR is a recent method developed to determine the reactivity of the antioxidants, independently of the amount of antioxidant present in the sampleThe propolis extract showed antioxidant activity in vitro, when was evaluated by the ORAC-FL and ORAC-PGR, unlike of the plant chia extracts showed lower values.

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PP90

## Lymphocyte peroxiredoxin-2 levels are depleted one week after ultra-endurance exercise

J.E. Turner [a,3], S.J. Bennett [b,1], J.A. Bosch [c,d], S. Aldred [e], H.R. Griffiths [b]

[a] *School of Cancer Sciences, University of Birmingham, Birmingham, UK*
[b] *Life and Health Sciences, Aston University, Birmingham, UK*
[c] *University of Amsterdam, Amsterdam, The Netherlands*
[d] *Mannheim Institute of Public Health, Social and Preventive Medicine, University of Heidelberg, Germany*
[e] *School of Sport and Exercise Sciences, University of Birmingham, UK*
*E-mail address:* s.j.bennett1@aston.ac.uk (S.J. Bennett)

Peroxiredoxin-2 (PRDX-2) belongs to a family of thiol containing proteins and is important for antioxidant defense, redox signaling and cell function. This study examined whether lymphocyte PRDX-2 levels are altered over one month following ultra-endurance exercise. Nine middle-aged men participated in a 145 mile ultra-endurance running race event. Blood drawing was undertaken immediately before, upon completion/retirement, and at one, seven and twenty eight-days following the race. PRDX-2 levels were examined at each time-point, for all participants (n=9) by reducing SDS-PAGE and western blotting. Further analysis using non-reducing SDS-PAGE and western blotting was undertaken in a sub-group of men who completed the race ($n = 4$) to investigate PRDX-2 oligomeric state (indicative of oxidation state). Ultra-endurance exercise caused a significant alteration in lymphocyte PRDX-2 levels ($F_{(4,32)}$ 3.409, $p=0.020$, $\eta^2 = 0.299$): seven-days after the race PRDX-2 levels fell by 70% ($p=0.013$) and at twenty eight-days after the race returned to near-normal levels. PRDX-2 dimers (intracellular reduced PRDX-2 monomers) in three of the four participants, who finished the race, were increased upon race completion. Furthermore, PRDX-2 monomers (intracellular over-oxidized PRDX-2 monomers) in two of these four participants were present upon race completion, but absent seven-days after the race. This study found that PRDX-2 levels in lymphocytes were reduced below normal levels seven-days after an ultra-endurance running race. We suggest that excessive reactive oxygen species production, induced by ultra-endurance exercise may, in part, explain the depletion of lymphocyte PRDX-2 by triggering its turnover after oxidation.

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[3] These authors contributed equally to this work