3ème Symposium International
Nutrition, Biologie de l'Oxygène et Médecine

8 -10 Avril 2009 - PARIS – France

CAMPUS DES CORDELIERS – 15 RUE DE L'ECOLE DE MEDECINE, PARIS 6EME

MICRONUTRIMENTS, EXERCICE, DESEQUILIBRE ENERGETIQUE ET MALADIES DU VIEILLISSEMENT

MICRONUTRIENTS, EXERCISE, ENERGY AND AGING DISORDERS

ORGANISE PAR LA SOCIETE FRANCAISE DE RECHERCHE SUR LES RADICAUX LIBRES ET L'OXYGEN CLUB OF CALIFORNIA

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MICRONUTRIMENTS, EXERCICE, DESEQUILIBRE ENERGETIQUE ET MALADIES DU VIEILLISSEMENT

MICRONUTRIENTS, EXERCISE, ENERGY AND AGING DISORDERS

Mercredi 8 avril

14h – 16h **Enregistrement**

16h – 16h 15 **Ouverture du Congrès**
Josiane Cillard, Présidente de la SFRR-France,

CONFERENCES PLENIERES
Keynote Lectures

Modérateurs **Helmut Sies (Düsseldorf) - Lester Packer (Los Angeles)**

16h 15 – 17h **Modulation nutritionnelle des antioxydants et des gènes myogéniques au cours de l'exercice**
Nutritional modulation of antioxidant and myogenic genes in exercise
José Viña (Valence)

17h – 17h 45 **Lactate, ERO et dynamique et biogénèse mitochondriale musculaire**
Lactate, ROS and muscle mitochondrial dynamics and biogenesis
George A. Brooks (Berkeley)

17h 45 – 18h 30 **Les micronutriments: mise à jour sur les flavonoïdes et le sélénium**
Micronutrients: an update on flavonoids and on selenium
Helmut Sies (Düsseldorf)

18h 30 – 19h 30 **Cocktail de bienvenue**
Welcome reception

Jeudi 9 avril

SESSION 1 : **EXERCICE, UTILISATION DE L'ENERGIE, STRESS OXYDANT ET ANTIOXYDANTS**
EXERCISE, ENERGY UTILIZATION, OXIDATIVE STRESS, AND ANTIOXIDANTS

Modérateurs **Joao Laranjinha (Coimbra) - Hélène Rouach (Paris)**

9h – 9h 35 **Histoire de l'étude du stress oxydant et des radicaux libres dans l'exercice physique**
A short history of the study of oxidative stress and free radicals in exercise
Kelvin J. Davies (Los Angeles)

9h 35 – 10h **Exercice physique et dommages oxydatifs générés dans l'ADN**
Physical exercise and oxidatively generated damage to DNA
Jean Cadet (Grenoble)

10h – 10h 25 **Stress oxydant et fatigue du muscle squelettique**
Oxidative stress and skeletal muscle fatigue
Michael B. Reid (Lexington)

10h 25 – 10h 50 **Pause café – Coffee break**

Modérateurs **Giuseppe Poli (Turin) – Enrique Cadenas (Los Angeles)**

10h 50 – 11h 15 **Etude de la modulation alimentaire des fonctions endothéliales par combinaison des modèles in vivo et un vitro: application au stress hyperoxique**
Studying the dietary modulation of endothelial functions by combining in vivo and in vitro models: application to hyperoxic stress
Fabio Virgili (Rome)

11h 15 – 11h 40 **Vitamines E et C dans les dommages du ligament croisé antérieur**
Vitamins E and C in ACL (Anterior Cruciate Ligament) injury
Maret G. Traber (Corvallis)

11h 40 – 12h 40 **Posters**

12h 45 – 13h **Photo du groupe–Group photo**

13h – 14h **Déjeuner - Lunch**

SESSION 2 : **EXERCICE ET ADAPTATION AU STRESS OXYDANT**
EXERCISE AND ADAPTATION TO OXIDATIVE STRESS

Modérateurs **Alberto Boveris (Buenos Aires) - Bertrand Rihn (Nancy)**

14h – 14h 25 **Régulation redox de la perte de la masse et des fonctions musculaires chez les personnes âgées**
Redox regulation of loss of muscle mass and function in the elderly
Malcolm Jackson (Liverpool)

14h 25 – 14h 50 **Signalisation redox dans l'atrophie musculaire liée au vieillissement**
Redox signaling in disuse muscle atrophy
Scott K. Powers (Gainsville)

14h 50 – 15h 15 **La conséquence de l'inactivité physique dans la régulation redox**
The consequence of physical inactivity on redox regulation
Zsolt Radak (Budapest)

15h 15 – 15h 40 **Régulation de l'inflammation et de la signalisation redox des anti-oxydants par**

l'exercice
Regulation of inflammation and antioxidant redox signaling by exercise
Young-Joon Surh (Séoul)

15h 40 – 16h **Pause café – Coffee break**

Communications orales sur sélection des résumés
Oral communications after abstracts selection (Young Investigator Award)

Modérateurs **Leopold Flohé (Magdeburg) - Giuseppe Valacchi (Sienne)**

16h – 16h 20 **L'entraînement de type endurance ne stimule pas la biogenèse mitochondriale dans le muscle squelettique de rats âgés**
Endurance training failed to stimulate mitochondrial biogenesis in aged skeletal muscle rats
Derbré F. (Valencia)

16h 20 – 16h 40 **Effets d'un exercice isolé sur la production d'espèces réactives de l'oxygène dans les mitochondries isolées de muscle et de foie.**
Effects of a single bout of exercise on the production of reactive oxygen species in muscle and liver isolated mitochondria.
Dubouchaud H. (Grenoble)

16h 40 – 17h **Un concentré inhibiteur Bowman-Birk protège le muscle soléaire de l'atrophie, de la faiblesse musculaire et du stress-oxydant induits par une décharge prolongée.**
Bowman-Birk inhibitor concentrate protects soleus muscle from atrophy, weakness and oxidative stress induced by prolonged unloading.
Arbogast S. (Paris)

17h – 17h 20 **Effets de la supplémentation en polyphénols de raisin sur l'inflammation et la résistance à l'insuline : résultats préliminaires sur la respiration mitochondriale.**
Effect of supplementation with grape polyphenols on inflammation and insulin resistance (IR) : preliminary results on mitochondrial respiration.
Lambert K.(Montpellier)

17h 20 – 17h 40 **Le Resveratrol régule l'activité de la DDAH après un stress-oxydant induit par le glucose dans les cellules endothéliales d'aorte bovine.**
Resveratrol regulates DDAH activity after glucose-induced oxidative stress in bovine aortic endothelial cells.
Frombaum M. (Paris)

17h 40 – 18 h **Intérêt de l'approche nutrigénomique pour l'étude de l'effet des polyphénols de plantes sur le transcriptome inflammatoire de la maladie coronarienne.**
A nutrigenomic approach to study the effect of herbal polyphenols on inflammatory transcriptome in coronary artery disease.
Dhawan V. (Chandigarh)

19h 30 **Banquet :** *Restaurant de l'UNESCO*
7, place Fontenoy – Paris 7ème
Prix « Jeune Chercheur », *Young Investigator Award,* **(Prix OCC)**
Jury : John Maguire (Président,), Pierre Cillard, Tilman Grune, Chantal Houée-Levin, Okezie Aruoma.
Prix « Posters », *Poster Awards :*

Prix SFRR-France, SFRR-Europe, Exon, Société de Physiologie

Jury : Denis Blache (Président), Paul Delamarche, Théo Efstathiou, Cesar Fraga, André Gorenflot, Jean-Pierre Iliou, Malcolm Jackson,

Vendredi 10 avril

SESSION 3 : **DESEQUILIBRE ENERGETIQUE ET MALADIES DU VIEILLISSEMENT: ACTIVITE PHYSIQUE ET INTERVENTION NUTRITIONNELLE**
ENERGY AND AGING DISORDERS : NUTRITION AND EXERCISE INTERVENTIONS

Modérateurs **Dominique Bonnefont-Rousselot (Paris) - Nesrin Kartal-Ozer (Istambul)**

9h 00 – 9h 25 **Comment la mitochondrie protège du stress oxydant pendant l'exercice**
How mitochondria protect against oxidative stress during exercise
Li L. Ji (Madison)

9h 25 – 9h 50 **Facteurs de croissance et modulation de l'apoptose induite par les ERO dans les cellules myogéniques**
Growth factors and modulation of apoptosis induced by ROS in myogenic cells
Daniela Caporossi (Rome)

9h 50 – 10h 20 **Pause café – Coffee break**

Modérateurs **Françoise Beaugé (Paris) - Cecilia Carreras (Buenos Aires)**

10h 20 – 10h 40 **Cardioprotection et longévité avec les vins et leurs composants cardioprotecteurs resvératrol, tyrosol et hydroxy tyrosol**
Cardioprotection and longevity with wines and their cardioprotective components, resveratrol, tyrosol and hydroxytyrosol
Dipak K. Das (Farmington)

10h 40 – 11h **Quel est l'effet de l'augmentation de consommation de fruits et légumes sur 9 marqueurs de la peroxydation lipidique chez des patients diabétiques de type 2 ?**
Which effect of increased dietary intake of fruits and vegetables on nine markers of lipid peroxidation in type 2 diabetes patients ?
Joël Pincemail (Liège)

11h – 11h 25 **Les acides gras polyinsaturés n-3 à longue chaîne: un outil nutritionnel pour la prévention du syndrome métabolique**
N-3 long chain polyunsaturated fatty acids : a nutritional tool for prevention of metabolic syndrome
Jacques Delarue (Brest)

11h 25 – 11h 50 **Effets antihypertenseurs des flavonoides**
Antihypertensive effects of flavonoids
Cesar G. Fraga (Buenos Aires-Davis)

11h 50 – 12h 15 **Exercice , Stress oxydant et diabète**
Exercise, oxidative stress and diabetes
Arlette Gratas-Delamarche (Rennes)

12h 15 – 13h 15 **Déjeuner - Lunch**

Modérateurs **Juan Poderoso (Buenos Aires) - Tilman Grune (Stuttgart)**

13h 15 – 13h 35 **Effet d'un neutraceutique fermenté sur le taux de thiorédoxine et la signalisation du TNF-α chez les patients cirrhotiques.**
Effect of a fermented nutraceutical on thioredoxin level and TNF-α signalling in cirrhotic patients
Francesco Marotta (Milan)

13h 35 – 14h **L'axe redox énergétique dans le vieillissement et la neurodégénérescence**
The energy-redox axis in aging and neurodegeneration
Lipeng Yap (Los Angeles)

14h – 14h 25 **Rôle du zinc dans les fonctionnements et dysfonctionnements du cerveau**
Zinc in brain function and dysfunction
Patricia Oteiza (Davis)

14h 25 – 14h 50 **Restriction calorique et activité physique dans la prévention du vieillissement**
Caloric restriction and exercise as an anti-aging intervention
Christiaan Leeuwenburgh (Gainsville)

14h 50 – 15h 15 **Dégradation et réparation des protéines oxydées au cours du vieillissement et du stress oxydant**
Oxidized protein degradation and repair in aging and oxidative stress
Bertrand Friguet (Paris)

15h 15 – 15h 40 **La néoptérine – nouveau biomarqueur de malnutrition et d'inflammation chez les personnes âgées**
Neopterin - new bioindicator of malnutrition and inflammation in the elderly
Zdenek Zadak (Hradec Kalové)

15h 40 – 16h *Relations complexes entre le tabagisme, l'activité physique et le stress oxydant : le tabagisme annule l'effet bénéfique de l'entraînement chez l'homme*
Complex relationships between smoking, physical activity and oxidant stress : Smoking negates the beneficial effects of exercise training in human.
Denis Blache (Dijon)

16h – 16h 45 **Table ronde: Exercice, Obésité, Syndrome Métabolique : Modulation nutritionnelle**
Round table : Exercise, Obesity, Metabolic Syndrome : Nutrient Modulation

Coordinateurs : Maret Traber (Corvalis) – Regina Brigelius-Flohe (Nuthetal)
Discussion : George Brooks (Berkeley) - Arlette Gratas-Delamarche (Rennes)
Malcolm Jackson (Liverpool) - Young-Joon Surh (Séoul)
Kelvin Davies (Los Angeles) – Giuseppe Poli (Turin)

16h 45 – 16h 55 **Synthèse et conclusion**
Synthesis and concluding remarks
Lester Packer (Los Angeles)

16h 55 – 17h **Clôture du congrès**
Closing of the meeting

Abstracts

Oral Presentations

PL1

Nutritional modulation of antioxidant and myogenic genes in exercise

Jose Vina, Carmen Gomez- Cabrera
Departamento de Fisiologia, Facultad de Medicina,Valencia

A major aim of our laboratory is to identify longevity-associated genes and then to find ways of up-regulating them by nutritional or physiological manipulations, chiefly physical exercise.
It has been known for some years now that training induces the expression of antioxidant enzymes such as superoxide dismutase, catalase, or glutathione peroxidase. In fact, we recently proposed that exercise itself can be considered as an antioxidant as it increases the endogenous antioxidant defences (Gomez-Cabrera *et al.*, 2008b). In this regard, exercise may be an even more effective antioxidant than supplementation with antioxidant vitamins.
The concept of hormesis as applied to exercise was put forward by Radak's group (Radak *et al.*, 2005) and by our own group (Ji *et al.*, 2006; Vina *et al.*, 2006). We proposed that moderate exercise causes small increases in the level of oxidants which result in an up-regulation of the antioxidant-related genes.
Moreover, treatment of animals (or humans) with high doses of vitamin C hampers the efficiency of training. This is based on the fact that vitamin C lowers the expression of critical factors controlling the mitochondriogenic pathway such as PGC-1α, NRF1 and TFAM (Gomez-Cabrera *et al.*, 2008a). Details of this work will be discussed.
We have recently been concerned with the role of the cancer protection pathway of p53 and p16/ARF with the promotion of longevity (Matheu *et al.*, 2007). Thus our critical finding was that cancer protection and increases in longevity (independent of cancer) may be related. The fact that double-transgenic s-Arf/p53 mice live longer than controls is due to the fact that p53 becomes an antioxidant as it up-regulates a new class of antioxidant enzymes, this is sestrins (Sesn)1 and Sesn2 (Matheu *et al.*, 2007).
We have recently observed that moderate exercise up-regulates p53 and this results in a significant protection against oxidants like paraquat. Up-regulating p53 by molecular means or by exercise significantly protects against death caused by paraquat. These results will also be discussed.
Finally, recent work from our laboratory has shown that animals knocked out for PGC1α (the co-activator of PPARγ) respond to exercise in a very similar fashion as that observed in old animals. Experiments will be discussed showing that in fact, the mechanisms to prevent senile sarcopenia may be similar in old animals as those which occur in young ones, but that are knocked-out for PGC1.

REFERENCES:

Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borras C, Pallardo FV, Sastre J & Vina J. (2008a). Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. *Am J Clin Nutr* **87,** 142-149.

Gomez-Cabrera MC, Domenech E & Vina J. (2008b). Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free Radic Biol Med* **44,** 126-131.

Ji LL, Gomez-Cabrera MC & Vina J. (2006). Exercise and hormesis: activation of cellular antioxidant signaling pathway. *Ann N Y Acad Sci* **1067,** 425-435.

Matheu A, Maraver A, Klatt P, Flores I, Garcia-Cao I, Borras C, Flores JM, Vina J, Blasco MA & Serrano M. (2007). Delayed ageing through damage protection by the Arf/p53 pathway. *Nature* **448,** 375-379.

Radak Z, Chung HY & Goto S. (2005). Exercise and hormesis: oxidative stress-related adaptation for successful aging. *Biogerontology* **6,** 71-75.

Vina J, Borras C, Gomez-Cabrera MC & Orr WC. (2006). Part of the series: from dietary antioxidants to regulators in cellular signalling and gene expression. Role of reactive oxygen species and (phyto)oestrogens in the modulation of adaptive response to stress. *Free Radic Res* **40,** 111-119.

Lactate, ROS and muscle mitochondrial dynamics and biogenesis

George A. Brooks (Berkeley)
Integrative Biology, University of California, Berkeley.

Once thought to be the consequence of oxygen lack in contracting skeletal muscle, the glycolytic product lactate is formed and utilized continuously under fully aerobic conditions. “Cell-cell” and “Intracellular Lactate Shuttle” concepts describe the roles of lactate in delivery of oxidative and gluconeogenic substrates as well as cell signaling. Examples of the cell-cell shuttles include lactate exchanges between working skeletal muscle and heart, and between muscle and liver. Lactate exchange between astrocytes and neurons that is linked to glutamatergic signaling in the brain is an example of a lactate shuttle supporting cell-cell signaling. The Mitochondrial Lactate Oxidation Complex (mLOC) consists, minimally, of the lactate/pyruvate transporter MCT1, LDH and cytochrome oxidase. Lactate regulates MCT1 protein expression at the level of transcription as part of a vast ROS-sensitive signaling network, a “transcriptome”, that regulates muscle mitochondrial biogenesis and many other adaptations know to occur in response to exercise training. As well, lactate affects dynamics of the mitochondrial reticulum (i.e., the continuous fusion and fission of reticular fragments), likely through the GTPase Drp-1. Rather than purely a stress response, a more appropriate view may be that lactate production is part of a stress-strain mechanism. The stress to maintain ATP homeostasis activates glycolysis and lactate production. In turn, the transient rises in [lactate] fuels cell respiration, changes redox and induces ROS generation that activates a transcriptional network signaling adaptive cell responses.

Micronutrients: an update on flavonoids and on selenium

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Micronutrients include vitamins, minerals, trace elements and secondary plant metabolites. Among the latter, the group of polyphenols has attracted attention in recent years, notably the flavonoids. Our diet encompasses a variety of flavonoids, and the epidemiologically identified inverse association between flavonoid intake and mortality from cardiovascular diseases has sparked chemical, biological and medical research (1). Current efforts focus, on the one hand, on the molecular mechanisms of action of biologically interesting flavonoids, and on nutritional and medical applications on the other hand. Recent progress and methodological pitfalls will be presented.
Selenium as an essential trace element is part of the active center of selenoproteins, where it is present in the form of selenocysteine (2). Selenoprotein P is released from the liver into the bloodstream, ultimately supplying selenocysteine to peripheral tissues. Regulation of hydrogen peroxide levels depends, in part, on the activity of selenoproteins, e.g. glutathione peroxidases. The expression of selenoprotein P is under the control of the FoxO1a transcription factor, interacting with the coactivator PGC-1alpha and hepatocyte nuclear factor HNF-4alpha (3).
The contribution of micronutrients to the overall oxidant/antioxidant balance will be discussed as a contribution to the current debate of the role of oxidative stress in health and disease.

(1) Terao, J., ed. (2008) *Arch.Biochem.Biophys*. **476**, 101-215 (Highlight Issue: Polyphenols and Health)
(2) Steinbrenner, H., Sies, H.. (2009) *Biochim. Biophys. Acta*, in press (Highlight Issue)
(3) Speckmann, B. *et al* (2008) *Hepatology* **48**, 1998-2006

A short history of the study of oxidative stress and free radicals in exercise

Kelvin J. A. Davies,
Ethel Percy Andrus Gerontology Center, and Division of Molecular & Computational Biology, the University of Southern California, Los Angeles, California- 90089-0191. USA.

In 1982 we first demonstrated, by electron spin resonance spectroscopy, that exercise can increase free radical production in muscle and liver. Our further studies showed that moderate endurance-type exercise represents a mild oxidative stress that is not experienced in short duration, high intensity (i.e. sprint-type) exercise. Interestingly, only exercise of the endurance-type elicits mitochondrial adaptive responses. Endurance-trained animals and humans exhibit marked increases in mitochondrial biogenesis. In contrast, sprint-type exercise training does not involve repeated mild oxidative stress and does not induce mitochondrial biogenesis, but does increase the glycolytic capacity of skeletal muscles and maximum oxygen consumption capacity (VO_{2max}). These results, and others, led us to propose that adaptive responses to oxidative stress are a major stimulus to mitochondrial biogenesis in endurance exercise training. We further showed that increased mitochondrial mass is largely responsible for increased endurance capacity following endurance training. Thus, we propose that free radicals, generated during the mild oxidative stress of moderate endurance exercise training, induce a series of adaptive responses in skeletal muscles that induce dramatic increases in mitochondrial biogenesis. In contrast, endurance exercise at extreme intensity elicits severe oxidative stress and widespread muscle oxidative damage. Such effects are similar to those seen only in the most extreme competitive endurance activities. Moderate endurance exercise training stimulates the expression of shock and stress genes (*e.g. hsp70*) in untrained animals and humans. Adaptive responses to long-term training include the loss of shock and stress gene expression during training sessions, and only high intensity exercise elicits a shock/stress gene response in trained individuals.

Physical exercise and oxidatively generated damage to DNA

Jean Cadet, Thierry Douki and Jean-Luc Ravanat,
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It is well documented that high intensity physical exercise in humans leads to an increase, particularly in muscles, in the formation of reactive oxygen species. These include unreactive superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2 that are the likely precursors of highly oxidizing hydroxyl radical ($^{\bullet}OH$). This may be accompanied by inflammation processes giving rise to nitrogen reactive species such as nitric oxide and peroxynitrite that are able to induce nitrosative and oxidative reactions with biomolecules. Detailed information at least for the reactions of $^{\bullet}OH$ and one-electron oxidants with purine and pyrimidine DNA nucleobases have been gained in cells allowing the utilization of several dedicated degradation products including 8-oxo-7,8-dihydroguanine (8-oxoGua) and 5,6-dihydroxy-5,6-dihydrothymine as relevant biomarkers of exposure to oxidative stress agents. Evidence has been provided in the literature that exercise induces an increase in the steady-state levels of oxidatively generated damage to DNA of circulating lymphocytes. Thus it was shown that the yields of strand breaks and 8-oxoGua that were assessed using the comet assay and a relevant modified version that requires an enzymic incubation step with formamidopyrimidine DNA *N*-glycosylase, a repair protein, are significantly more elevated upon completion of physical activity. It has to be pointed out that the enzymic detection of purine lesions which can be extended to oxidized pyrimidine bases using endonuclease III as the repair enzyme is more appropriate despite its lack of specificity than the highly informative HPLC-tandem mass spectrometry. This is due to the fact that the latter analytical method requires a DNA isolation step that may lead to artefactual increase in the level of 8-oxoGua whose extent is inversely proportional to the amount of DNA used in the measurement. A non-invasive assay relying on the HPLC-MS/MS measurement of urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine as a potential repair product of related triphosphate derivative in the nucleotide pools has been also used to monitor the intensity of oxidative reactions upon physical exercise. However caution has to be taken for the interpretation of the data in terms of oxidative stress reactions that is not straightforward and still lacking of biological validation.

Oxidative stress and skeletal muscle fatigue

Michael B. Reid (Lexington)
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Over a half century ago, electron spin resonance (ESR) technology demonstrated that skeletal muscle generates free radicals. However, the physiological significance of muscle-derived oxidants was not recognized until the early 1980s, when free radicals were detected by ESR in intact human muscles. This signal was found to increase during strenuous exercise, stimulating a decade of research in which fatiguing exercise was shown to increase markers of oxidative stress – oxidized glutathione, protein carbonyls, lipid hydroperoxides, etc. – in circulating blood and muscles of otherwise-healthy humans. The functional importance was less clear since nutritional antioxidants blunted biochemical changes without altering exercise performance. A breakthrough came in 1990 when pharmacologic antioxidant administration was shown to inhibit muscle fatigue. This indicated that oxidative stress plays a causal role in fatigue and is not simply a by-product. Subsequently, researchers have made substantial progress in defining cellular mechanism. Reactive oxygen species (ROS), rather than nitric oxide derivatives, appear to be the dominant stimulus for fatigue. The contribution of oxidative stress to fatigue is temperature-dependent, substantial at 37^{o}C but less evident at cooler temperatures. In intact cells, the molecular site of ROS action appears to be proteins of the myofibrillar lattice. Translational research has shown the physiological relevance of oxidative stress in humans by use of N-acetylcysteine (NAC) as an experimental probe. Multiple laboratories have shown that NAC delays fatigue, both in isolated muscle groups and in whole-body exercise. These findings identify oxidative stress as a mediator of fatigue in humans and a potential site for therapeutic intervention in future clinical studies. *Supported by the National Space Biomedical Research Institute via NASA grant NCC 9-58.*

Studying the dietary modulation of endothelial functions by combining *in vivo* and *in vitro* models : application to hyperoxic stress

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* Università di Messina, Italy
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Diet-health relationship is very complex and a wide spectrum of components of ingested food items act on cellular, tissue and organ responses through multiple pathways. The isolation of a specific role of a single component often appears essentially impossible.
We have recently proposed a novel experimental method based on the combination of *in vivo* supplementations and cell culturing to study the effect of complex matrices on the cellular response of different cell lines. According to this model, healthy volunteers are fed with the "test food" and, at appropriate times, blood is withdrawn and serum utilized to enrich the culture medium of specific cell types, in particular primary human endothelial cells (HUVEC).
Using this methodological approach we have addressed the effect of polyphenol rich food administration on the consequences of a mild sub-lethal hyperoxic stress on endothelial cells functions at molecular level.
Data obtained utilizing cultured cell model have been paired with observation taken from human subjects undergoing a standardized hyperoxic/hyperbaric stress by underwater diving (25 minutes at 30 mt dept) also receiving a pre-dive administration of a polyphenol rich food.
Our results suggest the need to reconsider some hypothesis on the mechanisms of action of nutritional molecules on the endothelial functions, taking into account the complexity of ingested food and its metabolization.

Vitamins E and C in ACL (Anterior Cruciate Ligament) Injury

Maret G. Traber[1] and Tyler Barker[2],
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[2]The Orthopedic Specialty Hospital, Murray, UT 84107, USA

Muscle dysfunction follows anterior cruciate ligament (ACL) injury and surgery. Ischemia-reperfusion during surgery can increase oxidative stress; moreover, released pro-inflammatory cytokines can induce and exacerbate oxidative injury. The purpose of this study was to evaluate prior antioxidant (AO) supplementation on recovery following ACL surgery. A randomized, double-blind, placebo-controlled trial was conducted in men (18-45 y) who received either AO (400 IU vitamin E & 1000 mg vitamin C) or placebo (PL) from ~2-wks prior to surgery (Bsl) through 3-mo post-surgery. Lower limb strength was used to assess muscle dysfunction; circulating biomarkers of oxidative stress and inflammation were also measured. AO supplementation increased plasma α-tocopherol and ascorbic acid (AA). At 90-min (post-surgery), elevated AA *decreased* in the AO group and an inverse correlation between AA and (interleukin) IL-8 (r^2 = 0.50, P< 0.05) was observed. IL-10 was elevated at 90-min and 72-h in the PL group. AO supplementation prevented a decrease in the pro-to-anti-inflammatory cytokine ratios of IL-6:IL-10, IFN-γ:IL-10 and IL-2:IL-10 observed in PL subjects at 90 min. Thus, short-term AO supplementation ameliorated the depression in the pro- to anti-inflammatory responses and the Th1-type cytokines to IL-10 immune responses observed during reperfusion post-surgery. Recovery of the injured limb's maximum isometric force correlated with higher AA status at baseline (BSL) in all subjects, and with elevated plasma AA in the AO group. A higher IL-6:IL-10 ratio correlated with the force recovery in the AO, but not the PL group. Although there were no significant differences in peak isometric force and power output between treatment groups, our findings suggest that higher plasma AA concentrations are associated with greater strength gains. The recovery also inversely correlated with plasma F_2-isoprostanes suggesting that lower oxidative stress was associated with improved repair. In conclusion, AO supplementation provided protection against surgery-induced immuno-suppression. Recovery of limb strength correlated with plasma AA, with normalized pro-to-anti-inflammatory cytokine ratios, and was inversely associated with oxidative stress. Thus, improved antioxidant status is associated with a better prognostic outcome following ACL surgery.

Redox regulation of loss of muscle mass and function in the elderly

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Ageing leads inevitably to a loss of approximately 40% of muscle mass between the ages of 50 and 70 years old but the processes leading to this loss of mass and function are incompletely understood. Although increased generation of reactive oxygen species (ROS) through mitochondrial generation of superoxide leading to increased oxidative damage has been considered to be a potential cause of age-related functional deficits for a number of years, direct evidence to support this hypothesis has been lacking. Many studies have attempted to correlate markers of oxidative damage with age-related deterioration in tissue functions, but recent data suggest that age-related changes in redox-regulated physiological processes may play an important role. One potentially important redox-regulated process in skeletal muscle is the up-regulation of protective systems following unaccustomed or excessive exercise. Our studies indicate i) that skeletal muscle ageing is accompanied by an increase in the activity of ROS in single muscle fibres, ii) that the ability of mucle to respond to the stress of excessive contractile activity is attenuated in ageing models and these changes can be accelerated in mice with a deletion of a specific regulatory mechanism for ROS (knockout of copper, zinc superoxide dismutase) and iii) that age-related loss of the ability to respond to contractile activity can be prevented by upregulation of the stress protein, HSP70 that also leads to a slowing of age-related loss of muscle function. Current studies are examining whether the age-related changes in redox regulation are related to a gross oxidation of regulatory thiols in muscle or to site-specific loss of thiol homeostasis.

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Redox signaling in disuse muscle atrophy

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Prolonged periods of skeletal muscle inactivity is associated muscle fiber atrophy. This disuse-induced muscle atrophy results from increased proteolysis and decreased protein synthesis. Investigations of the cell signaling pathways that promote disuse muscle atrophy have increased our understanding of this complex process. In this regard, growing evidence implicates disturbances in redox signaling as an important regulator of the cell signaling pathways that promote protease activation in skeletal muscle during prolonged disuse. This tutorial lecture will discuss the roles of reactive oxygen species in the control of inactivity-induced skeletal muscle atrophy. The specific objectives of this lecture are to identify the intracelluar sources of reactive oxygen species in muscle fibers and to discuss the evidence that connects redox disturbances to signaling pathways that activate proteases and promote skeletal muscle atrophy.

The consequences of physical inactivity on redox regulation

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Physical inactivity is a risk factor of number of diseases including, cardiovascular diseases, diabetes II, breast and colon cancer as well as Alzheimer disease. Reactive oxygen species (ROS) derived reactions are indicated as causative and/or associative factor of these diseases. Organs and cells are exposed to intermitted exposure to increased generation of ROS during regular physical activity, which actually can serve a powerful preconditioning against oxidative stress. The activity of antioxidant and oxidative damage repairing enzymes is readily regulated by ROS, which changes intermittently by exercise training. In addition, redox sensitive transcription factors and other regulatory proteins are also trained by regular to-and-back shift of redox milieu. On the other hand, physical inactivity with a relatively constant level of ROS and maintained redox cellular condition does no able to provide a challenging condition to the stimulation of antioxidant/oxidative damage repairing systems. As a result, when the organism are exposed to oxidative challenge cells from physically untrained subjects are in significantly greater risk to suffer from the damaging effects of ROS than cells trained by preconditioning physical exercise. Cells from exercise trained cells are able to slow down the cell cycle to provide longer time to efficient DNA damage repair and by this significantly reduce the rate of apoptosis as a response to oxidative stress. The function of sirtuins is also dependent upon the redox state, and sirtuin-dependent pathways are implicated in cell metabolism, tumor suppression, DNA repair, apoptosis, inflammation and mitochondrial biogenesis among others. Physical inactivity or activity significantly effects the function of sirtuins.

Regulation of Inflammation and Antioxidant Redox Signaling by Exercise

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Exercise has been associated with a dramatic increase in oxygen uptake by whole body. Although numerous studies have highlighted the health beneficial effects of exercise, there are multiple lines of compelling evidence supporting that excessive exercise may evoke detrimental effects. In particular, exhaustive or intense exercise causes oxidative and inflammatory tissue injury. Abnormal upregulation of cyclooxygenase-2 (COX-2), a rate-limiting enzyme in the prostaglandin biosynthesis, has been implicated in many inflammation-associated chronic disorders. Nuclear factor-kappaB (NF-κB) is a major transcription factor involved in regulation of COX-2 gene expression. When cells are subjected to oxidative stress, they tend to initiate defense mechanisms to minimize damage and ensure survival. Cellular protection against oxidative stress includes expression of antioxidant enzymes. Of particular interest is heme oxygenase-1 (HO-1), a novel stress-response enzyme with potent anti-inflammatory and anti-oxidant properties. Induction of HO-1 expression is known to be mediated by stress response or anti-oxidant response elements which are under the control of the redox sensitive transcription factor NF-E2-related factor-2 (Nrf2). To determine whether induction of the inflammatory and anti-oxidant enzymes is dependent on intensity and duration of exercise, expression of COX-2 and HO-1 as well as activation of their respective regulators, NF-κB and Nrf2 was examined in human peripheral blood mononuclear cells (PBMCs). Volunteers who participated in the exercise program were subject to four exercise intensities [40, 60, 80, and 100% of heart rate reserve (HRR)] and exhaustive exercise (40~100% HRR) on the treadmill as well as resting conditions. Isolated human PBMCs were collected immediately and 1 h after exercise and subjected to the the gel shift assay and Western blot analysis. As exercise intensity increased, expression of COX-2 and HO-1 was enhanced, which was associated with elevated DNA binding of NF-κB and Nrf2, respectively. During the 1 h recovery after the exhaustive exercise, Nrf2-ARE binding activity and HO-1 expression returned to the basal levels. In follow-up studies with mice, HO-1 expression in muscle analyzed 1 h after exhaustive exercise was dramatically increased. The present study demonstrate the coordinated modulation of inflammatory and antioxidant redox signaling pathways in response to exercise.

Endurance training failed to stimulate mitochondrial biogenesis in aged skeletal muscle rats

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OBJECTIVE: This study was designed to determine the effect of endurance training on the mitochondrial biogenesis pathway in skeletal muscle of aged rats.

DESIGN: 24 young (5 months) and aged (22 months) male Wistar rats were exercised for 3 weeks following endurance training. (75% $VO_{2max.}$). Expression analyses were realized in soleus (fiber type I) and gastrocnemius (intermediate fiber type) muscles.

RESULTS: Expression of key transcription factors involved in mitochondrial biogenesis (PGC-1α, NRF-1) and of cytochrome c (a marker of mitochondrial content) increased significantly in response to training in young rat soleus muscle but remained unchanged in aged rats. Aging is associated with a decrease of protein content of cytochrome c oxidase subunit II (COX II) and an increase of mitochondrial transcription factor A (TFAM) in soleus muscle suggesting a mitochondrial dysfunction. Endurance training didn't modify COX II and TFAM in both young and aged rats. Neither age nor training altered all theses protein expressions in gastrocnemius muscle.

CONCLUSION: Our study confirmed that fiber type I muscle appears more affected by age-related mitochondrial alterations than intermediate fiber type muscle. Short-term endurance training failed to stimulate mitochondrial biogenesis and to reduce mitochondrial dysfunction in skeletal muscle of aged rats.

Effects of a single bout of exercise on the production of reactive oxygen species in muscle and liver isolated mitochondria.

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Acute exercise is often associated with tissue damages from oxidative stress due to limitations in antioxidant capacities or to enhanced reactive oxygen species (ROS) production. In addition, ROS could also have a regulatory role as signaling molecules. In most cells, mitochondria are the major source of ROS but the effect of exercise on such mitochondrial ROS production has not been studied in details. The aim of this study was therefore to measure both the mitochondrial oxygen consumption (JO_2) and the mitochondrial H_20_2 production, as an index of ROS production, after a single bout of treadmill exercise in rats sacrificed immediately after a 1-hour exercise bout (E^0) or after a 2-hour recovery (E^2) compared to controls (C). We observe differences between liver and muscle mitochondria in response to acute exercise. While there is no change in mitochondrial JO_2 in liver, we show that mitochondrial JO_2 is increased in muscle right after exercise when the respiratory chain is energized with both complex I and II substrates. These changes disappear after a 2-hour recovery period. In muscle, mitochondrial H_20_2 production is increased after exercise (from 50 % to several times, depending on the conditions tested). These changes remain significant after a 2-hour recovery period in muscle, whatever the substrate used to energize the respiratory chain. In liver, there is also an increase in H_20_2 production (+ 20%) in response to exercise that is limited to non-phosphorylating conditions. The use of specific combinations of substrates and inhibitors of the respiratory chain suggests that a single bout of exercise alters ROS production at the complex III level in muscle but at the complex I level in liver. Future studies should focus now on the physiological role of such ROS as signals for muscle and liver adaptations to exercise.

Bowman-Birk inhibitor concentrate protects soleus muscle from atrophy, weakness and oxidative stress induced by prolonged unloading.

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Muscle atrophy and weakness is caused by prolonged mechanical unloading induced among others by bedrest or space flight. Unloading also induces oxidative stress in muscle, a putative cause of weakness. We hypothesized that dietary supplementation with Bowman- Birk Inhibitor Concentrate (BBIC), a soy protein extract, would protect soleus, an antigravity muscle, against atrophy and weakness. Adult mice were fed a diet supplemented with 1% BBIC during hindlimb unloading. After 12 days unloading, we measured a significant decrease in soleus muscle mass. Force per cross-sectional area and total force were significantly lower in unloaded solei relative to controls. BBIC supplementation had a protective effect, blunting decrements in soleus weight and force.

Cytosolic oxidant activity, assessed using 2',7'-dichlorofluorescin diacetate, was significantly increased in unloaded muscle, peaking at day 3 and remaining elevated through day 12. Increases in oxidant activity correlated directly with loss of muscle mass and were abolished by BBIC supplementation. *In vitro* assays established that BBIC has direct antioxidant properties and also inhibits serine protease activity. We conclude that dietary supplementation with BBIC protects skeletal muscle during prolonged unloading, promoting redox homeostasis in muscle fibers and blunting atrophy-induced weakness. *Supported by National Aeronautics and Space Administration Grants*

Key words: skeletal muscle, cachexia, free radicals, oxidative stress, antioxidant, microgravity, nutrition

OP13

Effect of supplementation with grape polyphenols on inflammation and insulin resistance (IR) : preliminary results on mitochondrial respiration.

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The mechanisms responsible for skeletal muscle IR remain incompletely understood. However, low-grade chronic inflammation, oxidative stress and mitochondrial alteration have been suggested to take part in the development of IR. Recent studies have found that polyphenolic (PP) compounds found in red grape have interesting properties against IR. Thus, the aim of this study was to investigate the impact of a supplementation with phenolic compounds from red grape marc in a genetically modified mouse presenting a chronic low-grade inflammation (CD4dnTGFbRII mouse (TGF)) compared to C57 (CTL). We have 4 groups of mouse: control + placebo, control + PP, TGF + placebo and TGF + PP. PP were dissolved in drinking water for 4 weeks. Our data were analysed by two-way ANOVA with animal and treatment as factors. Our preliminary results are on isolated mitochondria from skeletal muscle with pyruvate/malate as substrate. We found a significant decrease in basal respiration (without ADP) in TGF. ADP-stimulated respiration, state 3, showed a significant effect both with animals and treatment: TGF mouse had a significant decrease in state 3 (228 ± 28 vs 332 ± 25 nmol d'O/min/mg prot) compared to CTL and PP supplemented animals presented also a significant decrease in state 3. However, there was no interaction. The respiratory control ratio was significantly decreased in TGF as well as in PP groups. These data could be explained by a decrease in oxidative stress with PP supplementation due to the decrease in mitochondrial respiration which needs further investigation.

Resveratrol regulates DDAH activity after glucose-induced oxidative stress in bovine aortic endothelial cells

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OBJECTIVE: Altered NO biosynthesis is thought to play a role in the initiation and progression of atherosclerosis Decreased NO bioavailability may result from an increase in the asymmetric dimethylarginine (ADMA), an endogenous NO-synthase (NOS) inhibitor normally metabolized by dimethylarginine dimethyl-aminohydrolase (DDAH). This study aimed at investigating if trans-resveratrol (RES), a naturally occurring polyphenol, prevents ADMA formation under high glucose-mediated oxidative stress conditions.

METHODS: Bovine aortic endothelial cells (BAECs) were pre-incubated in the presence of RES and then co-incubated with normal (7mM) and high concentration (25mM) of D-glucose for 24 hours in the presence or the absence of RES (0.1 to 10 µM). Mannose (16 mM) was used as osmotic control. In cell lysates, ADMA concentrations were determined by HPLC, DDAH activity by spectrophotometry and its expression by immunoblot.

RESULTS: Exposure to high glucose concentrations increased intracellular ADMA concentration to 80% and reduced DDAH activity to 20% activity as compared to control cells. RES (0.1-10 µM) dose-dependently inhibited glucose-stimulated ADMA accumulation; at 10 µM, it restored DDAH activity as in control cells.

CONCLUSION: ADMA accumulation in BAECs is influenced by glucose-induced oxidative stress, and RES counteracts ADMA accumulation through the DDAH pathway. This provides a new mechanism for the cardioprotective effects of RES and may contribute to the development of novel therapeutics.

A nutrigenomic approach to study the effect of herbal polyphenols on inflammatory transcriptome in coronary artery disease.

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Background: Atherosclerosis has a multifactorial etiology and inflammation plays a pivotal role in the process of atherogenesis. In traditional Ayurvedic system of medicine, certain herbal preparations have been identified to have beneficial effects on cardiovascular patho-physiology. However, in the context of modern medicine it becomes imperative to have scientific validation of the claim made in Ayurvedic medicine. *Terminalia arjuna* is an important cardiotonic plant described in the ancient Indian medical science. Evidence in literature indicates that it has potent hypolipidemic, antioxidative and anti-inflammatory properties, but scientific validation and proof for the same is lacking. Therefore, the present study was conducted to investigate the effect of this medicinal herb on expression of inflammatory genes in subjects with coronary artery disease.

Methods : In the present study 40 subjects with angiographically proven coronary artery disease (Group I) and 40 age and sex-matched healthy controls (Group II) were enrolled at special cardiology clinic in PGIMER. The polyphenols were extracted from the bark of *Terminalia arjuna* (TA) and were isolated using standard methods. The isolated polyphenols were further purified and fractionated by HPLC. The peak fractions (Fraction I to V) were collected and dialysed against normal saline and subsequently used for culture experiments. Time and dose-dependent studies were carried out to evaluate the effects of the bark extract of *Terminalia arjuna* (TAE) on cell viability, cell proliferation and on LPS-induced expression of inflammatory genes - IL-18, COX-2, RAGE and MMP-2 in PBMCs of study subjects by semi-quantitative RT-PCR and Western blot analysis. Further, PBMCs from the study subjects were cultured in presence of HPLC purified sub-fractions of the bark extract of *Terminalia arjuna* **(**0-150ug/ml; 0 = control**).**

Results: Trypan blue exclusion and BrdU cell proliferation assay showed that upto 200 µg of bark extract/ml/10^6 cells had no cytotoxic effects as it did not affect the viability and proliferation of PBMCs upto 48 hours. Exposure of mononuclear cells to both TAE and HPLC isolated Fractions II and IV revealed a dose-dependent down regulation of genes coding for IL-18, COX-2, MMP-2 and RAGE both at the transcriptional and translational level.

Conclusion : Our observations clearly indicate that this medicinal herb attenuates the expression of inflammatory genes and thereby, possesses anti inflammatory properties and does not demonstrate cytotoxic properties Our data validates that this medicinal herb has cardioprotective properties by virtue of its inflammatory potential and provides scientific basis for its use in future clinical trials for the prevention and management of CAD.

How mitochondria protect against oxidative stress during exercise

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Generation of reactive oxygen species (ROS) is one of the most prominent biological events during contractile activity of skeletal muscle. Mitochondria are the principle organelle in the cell subject to increased oxidative stress during physical exercise. Thus, it is of grant importance to elucidate the mechanisms and pathways to enhance antioxidant defense and maintain its functionality. The current communication will present available experimental data to demonstrate that mitochondria can develop at least three strategies to copy with increased oxidative stress during acute exercise. These are (1) to increase the gene expression of uncoupling protein-3 (UCP3) to dissipate the electron motive force so as to reduce the generation of superoxide radicals from the electron transport chain; (2) to induce manganese superoxide dismutase (MnSOD) through redox-sensitive signal transduction pathways of nuclear factor (NF) κB and mitogen-activated protein kinase (MAPK); and (3) to use the peroxisome proliferator-activated receptor γ co-activator-1α (PGC-1α)-activated pathway to enhance mitochondrial biogenesis as an adaptation to increased oxygen consumption and ROS generation. There is also evidence that mitochondria can change their shape, localization and number by fusion and fission in response to increased metabolic demand. Potential effect of long-term oxidative stress on mitochondrial and chronic adaptation of the organelle will be discussed.

Growth factors and modulation of apoptosis induced by ROS in myogenic cells

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The loss in skeletal muscle mass and/or the alteration of muscle regeneration has been associated with a selective activation of apoptotic pathways and a general reduction in survival mechanisms. Since the range of hormones and growth factors operating upon skeletal muscle as a target organ is extensive, the analysis of diffusible effectors possibly participating in the myogenic cell maintenance, growth and differentiation will be important for the attenuation of muscle wasting.
We focused on VEGF, which expression, modulated by mechanical stimulus, is linked to muscle tissue plasticity, and on NGF, released in motor-neuron plaque and possibly related to muscle atrophy induced by denervation.
In different models of skeletal muscle cells, we demonstrated that VEGF protects myoblasts from apoptosis induced by oxidative or hypoxic stress. This protection did not correlate to the modulation of the expression of VEGF receptors, but is clearly linked to the phosphorylation of the KDR/Flk-1 receptor, the activation of NFkB transcription factor and the over-expression of the anti-apoptotic protein, αB-crystallin. Regarding NGF, we showed that, during differentiation, this growth factor determines an over-expression of myogenin, a lower susceptibility of fusing myoblasts to apoptosis, with an increase in the fusion rate into myotubes and consequent hypertrophy of multinucleated fibers. In standard conditions, NGF was not effective in protecting proliferating myoblasts, but it could sustain the survival of L6C5 myoblast growing in critical culture conditions.

Cardioprotection and longevity with wines and their cardioprotective components, resvera-trol, tyrosol and hydroxy tyrosol

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Resveratrol, a phytoalexin and polyphenolic antioxidant present in red wine increases longevity through SirT1, which is activated with NAD^+ supplied by an anti-aging enzyme PBEF. SirT1 interacts with an anti-aging transcription factor, FoxO1, which is negatively regulated by Akt. Since several recent studies have shown that red wine could have similar health benefits as resveratrol, which is present in red wine, we determined if wine and its cardioprotective components also possess anti-aging property by feeding the rats with these compounds. In addition, we examined the effects of white wine and its cardioprotective components, tyrosol and hydroxy tyrosol of the expression of longevity genes. The hearts induced the expression of SirT1, SirT3, SirT4 and caused the phosphorylation of FoxO1 and FoxO3A and stimulated PBEF in the order of white wine > resveratrol > tyrosol > hydroxy tyrosol > red wine while Akt phosphorylation and cardioprotection [lowering of infarct size and cardiomyocyte apoptosis] followed a different pattern: resveratrol> red wine> hydroxy tyrosol> white wine> tyrosol suggesting existence of different signaling pathways for the induction of longevity and survival.

Which effect of increased dietary intake of fruits and vegetables on nine markers of lipid peroxidation in type 2 diabetes patients ?

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The public recommendation is to consume at least five or more servings (400 – 600 g) of fruits and vegetables daily in order to maximize prevention against cancer, cardiovascular diseases and diabetes. Such an effect of plant foods seems to be attributed to their high content in antioxidants (vitamin C, carotenoids, polyphenols) able to decrease oxidative damages to key structures of the body including lipids, DNA and proteins. However, only few trials (with controversial results) have investigated if diets rich in fruits and vegetables will have or not beneficial effects on lipid peroxidation. In our preliminary study performed with international experts in the detection of lipid peroxidation, a group of 29 type 2 diabetes patients (age ; plasma glucose and glycosilated hemoglobine levels respectively of 1.77 +/- 0.62 g/L and 8.64 +/- 1.28 %) having a medium consumption in fruits and vegetables was divided into two groups : one following by a dietician and receiving a booklet with precise advices in order to reach an intake of 600 g of fruits and vegetables each day during two months; the other one receiving no special instructions about their diet but was asked to mention in a booklet their daily consumption in fruits and vegetables. Lipid peroxidation was evaluated by the detection of lipids peroxides (POXL), thiobarbituric acid-reactive substances (TBARS), oxidized LDL, antibodies against oxidized LDL (Abox-LDL), oxysterols (17 b – hydroxycholesterol and 7 keto cholesterol), LDL size and isoprostanes in plasma and 4 – hydroxynonenal (HNE) in urine. When compared to basal conditions, no significant increase or decrease in both plasma vitamin C and b – carotene was surprisingly observed in both groups. In the group encouraged to eat 600 g of fruits and vegetables, a decrease in the concentration of POXL, oxidized LDL, TBARS and, more particularly, 7 keto cholesterol was noted. All these parameters were, in contrast, increased in the non - controlled diet group. The concentration of 17 b – cholesterol remained unchanged in the first group while it significantly increased in the second group. In both groups, any modification was evidenced with respect to LDL size whereas the concentration of Abox-LDL and HNE was increased. Conclusions of our study highlighted two crucial points for evidencing real repercussion of increased fruits and vegetables consumption as preventive effect for our health : 1° great difficulties to control the real intake of fruits and vegetables in both groups; 2° an appropriate choice of parameters for evaluating lipid peroxidation as recognized to be involved in cardiovascular problems associated to diabetes.

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N-3 long chain polyunsaturated fatty acids : a nutritional tool for prevention of metabolic syndrome.

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Epidemiological studies in Ekimos as well as studies in rodents and in healthy humans showed that long chain n-3 PUFA prevent insulin resistance and/or hypertriglyceridemia and hypertension. *In vivo* in rodents, LC n-3 PUFA have a protective effect against high fat diet or sucrose or fructose-induced insulin resistance. Such effect is explained at molecular level by the prevention of many alterations of insulin signaling induced by the high fat diet. Indeed, the protective effect of n-3 LC-PUFA results from : a) the prevention of the decrease of phosphatidyl inositol 3' kinase (PI3 kinase) activity and of the depletion of GLUT4 protein in muscle ; b) the prevention of the decreased expression of GLUT4 in adipose tissue. In addition, LC n-3 PUFA inhibit both activity and expression of liver glucose 6 phosphatase which could explain a protective effect with respect to the excessive hepatic glucose output induced by the high fat diet. N-3 LC-PUFA also decrease muscle intramyofibrillar triglycerides and liver steatosis alleviating lipotoxicity. In healthy humans, fish oil increases insulin sensitivity, partially prevents dexamethasone-induced insulin resistance, et prevents most of overfeeding-induced metabolic and cardio-vascular alterations found in patients with metabolic syndrome. All these data in rodents and in humans demonstrate that LC n-3 PUFA have a high potency for prevention of metabolic syndrome.

Antihypertensive effects of flavonoids

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Flavanols are naturally occurring plant compounds, which biological effects could explain some of the cardiovascular benefits linked to the consumption of fruit and vegetables. Dietary intervention studies in humans and animals indicate that flavanol-rich foods, i.e. wine, tea, and chocolate may exert blood pressure (BP) lowering effects in humans and rats. Mechanistic studies have shown that such BP decrease could be dependent on NO bioavailability. Hypertension was induced in male Wistar rats by treatment with the NO-synthase inhibitor L-NAME (360 mg/l drinking water, about 10 mg/rat/d). The addition of (-)-epicatechin (EC) in the diet (0.2-4.0 mg/g diet) significantly modulated the L-NAME-dependent BP increase. EC had antihypertensive effects when EC was administered simultaneously with L-NAME, or after L-NAME-induced hypertension was established. The EC effect was dose-dependent, and disappeared with the removal of EC from the diet. The decrease in BP was associated with the presence of EC in plasma, the decrease in markers of oxidative damage, i.e. malondialdehyde and oxidized glutathione, and an increase in plasma NO. We hypothesize that the effects of EC are consistent with the modulation of oxidant production which allows a NO steady-state which finally result in the antihypertensive effect.
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OP22

Effects of diabetes and exercise on the expression of the Rcan1 protein in rat skeletal muscle. Implication of oxidative stress.

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Muscle sarcopenia is a hallmark of aging and of a number of chronic diseases including diabetes. Many findings suggest that the development of sarcopenia is a multifactorial process which might involve inflammation and oxidative stress. Fortunately sarcopenia is partly reversible with appropriate exercise interventions. Therefore understanding the mechanisms by which exercise can prevent sarcopenia has important consequences in order to develop effective prevention.

With this aim in view, we investigated the effects of diabetes and exercise on the expression of the Rcan1 protein in rat skeletal muscle. The Rcan 1 protein is an early adaptive oxidative stress protein, known to inhibit the activity of calcineurin, a key-molecule in muscle structure and function. The expression of the Rcan1 protein, as well as changes in free glucose, oxidative stress markers and antioxidant enzymes activity. were analyzed in the gastrocnemius muscle of healthy and diabetic rats, at rest and 2hours after a running exhaustive exercise performed on a treadmill. Our studies indicate that:

1) Diabetes is associated with an increase in the expression of Rcan1 in the gastrocnemius muscle, which is concomitant to high muscle free glucose and to oxidative stress, as judged by the high levels of 8-isoprostanes F2 and GPX activity.

2) Oxidative stress decreased, as well as the expression of Rcan1, in diabetic rats, 2 hours after the end of exercise, whereas no change was seen in muscle free glucose. The decrease in oxidative stress is discussed.

These findings suggest that oxidative stress could be involved in the increase of muscle Rcan1 protein expression in diabetes and that oxidative stress might play a non negligible role in diabetes sarcopenia by that way.

Effect of a fermented nutraceutical on thioredoxin level and TNF-α signalling in cirrhotic patients

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The aim of the study was to gain further insights into the possible nutraceutical effect on redox balance via thioredoxin (Trx) modulation and on the intrinsic susceptibility of monocytes to generate an inflammatory response. The study group consisted of thirty-two patients with compensated Child A-C, HCV-related cirrhosis. Patients were supplemented for 6 months with 6g/day of a GMP- and ISO-certified fermented papaya preparation (FPP). Fifteen unsupplemented, age/gender-matched healthy subjects served as controls. Patients were administered a detailed diet-life style questionnaire and blood was collected to test routine biochemistry, Trx, redox status (GSH, GSSG, GSH/GSSG ratio, 4-HNE and α-tocopherol). Moreover, isolated monocyte were tested for ex-vivo LPS-stimulated TNFα production and TNFα mRNA. As compared to control, patients with liver cirrhosis showed a significantly higher serum level of Trx. A significant correlation occurred with GSH/GSSG ratio in Child B and C patients. FPP supplementation brought about a significant reduction of Trx with levels comparable to the ones of healthy controls. Ten patients Child C (31.2%) showed borderline low levels of α-tocopherol while all cirrhotic patients, as a whole, showed a significantly abnormal redox balance. Supplementation with FPP did not modify α-tocopherol depletion but significantly improved redox balance parameters. Patients with liver cirrhosis showed a significantly upregulated TNF-α production in a time-dependent manner and this effect was more pronounced in more advanced stages of the disease and showed a significant correlation with α-tocopherol level. Supplementation with FPP significantly, although partially, downregulated TNF-α production from monocytes. Taken altogether, it would appear that the typical oxidative-inflammatory biochemical milieu of these patients is mirrored by a significant TNF-α upregulation at a monocyte level while a targeted nutraceutical might be a potential amenable intervention to be part of validated scheduled treatments.

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The energy-redox axis in aging and neurodegeneration

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Alterations in multiple molecular pathways occur during brain aging, some of which correlate with cognitive impairment. Decline in cognitive function has been suggested to be closely associated with energy changes in the brain. As such, energy hypometabolism has been considered as one of the earliest changes associated with normal brain aging, mild cognitive impairment, and Alzheimer's disease. Understanding the mechanisms that regulate bioenergetic shifts during normal aging provides insight into pathological aging, where age-related changes in mitochondrial energy production may be accelerated in an Alzheimer's disease model.

The interaction between redox and energy changes form a regulatory mechanism that controls cellular energy levels in response to redox changes (*i.e.* increased generation of H_2O_2 and $^{\cdot}NO$) through specific post-translational modification of cytosolic and mitochondrial proteins including phosphorylation (pyruvate dehydrogenase), tyrosine nitration (succinyl-CoA- transferase), and S-glutathionylation (glyceraldehyde-3-phosphate dehydrogenase). The role of these protein modifications in aging and age-related neurodegeneration is established by an age-dependent increase in the levels of $^{\cdot}NO$ –a consequence of increase expression of neuronal nitric oxide synthase and inflammation– leading to not only nitration and S-nitrosation but also S-glutathionylation of specific proteins. Acute exposure of primary cortical neurons and astrocytes to a $^{\cdot}NO$ flux, mirroring neuroinflammation, led to S-glutathionylation of proteins in a dose- dependent manner due to oxidation of the cellular redox environment. The significance of a higher redox buffering capacity was reflected in the extent of intracellular formation of S-glutathionylated proteins.

In a triple transgenic model of Alzheimer's disease, metabolic changes –decreased pyruvate dehydrogenase activity and mitochondrial respiration– and redox changes precede the occurrence of histopathology and accompany cognitive deficits and are compounded by inactivation of cytosolic glyceraldehyde-3-phosphate dehydrogenase upon S-glutathionylation. Taken together, these data clearly demonstrate that (*a*) metabolic declines are an early event in AD occurring at the onset of cognitive changes and (*b*) impaired mitochondrial utilization of energy might play a role in glucose hypometabolism. This was corroborated by dynamic microPET scanning that showed a dramatic decline in glucose uptake and metabolism occurred in 3xTg-AD mice as compared to non-Tg mice at 6 months of age. Treatment of mice with the dithiol compound, R-lipoic acid led to a partial rescue of net glucose uptake in the brain.

Zinc in brain function and dysfunction

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Zinc deficiency (ZD) associated with inadequate zinc intake and undernutrition is frequent throughout the world. A condition of ZD can also be triggered by infections or pro-inflammatory conditions. If ZD occurs during pregnancy, it can result in decreased availability of zinc to the fetus. Increasing evidence indicates that maternal ZD can lead to alterations in neonate development that result in modified infant behavior, cognitive and motor performance. Accordingly, undernourished children supplemented with zinc showed improved developmental quotients, activity patterns, and neuropsychological functions. We found that in neuronal cells, ZD affects two critical events in neurodevelopment, i.e. neuronal proliferation and apoptosis. Confirming this observation, severe dietary ZD imposed to rats throughout gestation results in multiple pregnancy/fetal abnormalities: lower litter weight, lower live fetuses/litter, lower fetal, brain and cerebellum weight, and higher percentage of malformed fetuses and resorptions, as compared to controls. In a model that more closely resemble conditions found in human populations, gestational exposure to marginal levels of dietary zinc (MDZ) do not affect general maternal condition and fetal outcome in rats. However both, ZD and MDZ lead to brain protein thiol oxidation, decreased levels of glutathione, and to alterations in redox-sensitive signaling. The exposure to MDZ diets from gestational day 0 to postnatal day 56, also causes oxidative stress in cerebral cortex, hipoccampus and cerebellum, which was associated with altered neurobehavioral patterns. In summary, besides the essentiality of zinc for the normal development of the nervous system, suboptimal zinc nutrition during early development can increase the risk for neurological disorders later in life.

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Caloric restriction and exercise as an anti-aging intervention.

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Declining physical activity and performance in the context of loss of skeletal muscle mass with age is virtually a wide-spread feature that has been documented in several species, including worms, fruit flies, rodents, non-human primates and humans. This age-related loss of muscle mass and strength (sarcopenia) represents an important risk factor for disability and mortality in older subjects and has been linked with cellular energy deficit and increased apoptosis. Several key theories on aging describing the mechanisms underlying sarcopenia have recently focused on the viability of mitochondria given their central role in controlling energy production and death processes within cells. Indeed, mitochondria have a critical role in ATP production, oxidant release and regulation of cell death (apoptosis). Primarily, unknown factors leading to a decrease in aerobic energy efficiency are linked with mitochondrial mutations which may result in apoptosis. Moreover, deregulation of autophagy (degradation of long-lived proteins and organelles, such as the mitochondria, and subsequent recycling of macromolecular constituents) in post-mitotic tissue might also be responsible for the age-associated cellular energy failure. Additionally, alterations in specific signaling pathways, such as metabolic byproducts that affect AMP-activated protein kinase (AMPK), may play a role in both the cell survival response and apoptotic response depending on energy depletion. There is supporting evidence that apoptosis in aging skeletal muscle may be due, at least in part, to the progressive decline in mitochondrial function and the resulting energy depletion within the cell. Finally, mitochondrial dysfunction is also modulated by oxidative damage to macromolecules, including mtDNA, RNA and proteins, essential components for optimal mitochondrial function. Therefore, understanding the confluence of these age-related changes in mitochondrial functioning may lead to the development of interventions to prevent functional decline. For example, life-long caloric restriction (CR) has been found to improve health and slow the aging process in numerous species. The attenuation of oxidative stress may be one key mechanism through which CR delays the onset of morbidity, increases lifespan, and reduces the risk of developing a number of pathological conditions. Therefore, the health benefits of CR may be due, at least in part, to a reduction in the amount of mitochondrial oxidant production. Interestingly, CR and, more recently, exercise training have been found to beneficially affect both mitochondrial function and apoptotic signaling in skeletal muscle of both young and old animals. In addition, data available so far suggest that chronic exercise may serve to enhance antioxidant enzyme activities, and augment certain cellular repair/removal pathways, thereby reducing the amount of oxidative tissue damage. Additionally, autophagy and protein degradative mechanisms may be critical for maintaining cellular health and tissue function. CR studies in animals have typically utilized a 30-40% restriction regimen, but we have recently shown that 8% CR provides a number of beneficial health effects and may have the advantage of being more applicable to human subjects. We have now animal data which indicate that even a small reduction in food intake can provide protective effects against sarcopenia, oxidative stress and cellular remodeling in aging skeletal muscle. Moreover, in a recent human study we compared the effects of 20% caloric restriction or 20% exercise alone over a one year period on oxidative damage to DNA and RNA, as assessed through white blood cells and urine analyses. This study provided evidence that negative energy balances induced through either CR or exercise resulted in substantial and similar improvements in markers of DNA and RNA damage in white blood cells, potentially indicative of reduced systemic oxidative stress. More recently, calorie restriction mimetics (CRM) have been developed to mimic the substantial anti-aging effects that CR has on many animal models. Examples of CRM are resveratrol or other nutritional mixtures, which presumably affect similar biochemical pathways as CR. In this presentation we will discuss the most recent evidence regarding the relevance of skeletal muscle apoptosis in sarcopenia, as well as its modulation by calorie restriction, exercise and other nutritional interventions.

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EN.REFLIST

Oxidized protein degradation and repair in ageing and oxidative stress

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Accumulation of oxidatively modified proteins is a hallmark of cellular ageing and failure of protein maintenance is a major contributor to the age-associated accumulation of oxidized proteins. Oxidized protein degradation is mainly achieved by the proteasomal system in the cytosol and the nucleus while the Lon protease has been implicated in oxidized protein degradation within the mitochondrial matrix. It is now established that proteasomal function is generally impaired with age. Concerning mitochondrial proteins, we have reported that the age-related accumulation of oxidized and glycoxidized proteins in rat liver mitochondrial matrix is affecting a restricted set of proteins and is associated with an alteration of the ATP-stimulated Lon-like protease activity. Repair is limited to few modifications, such as methionine oxidation, that can be catalytically reversed within proteins by the methionine sulfoxide reductase (Msr) enzymes, MsrA and MsrB. Msr function has been shown to be impaired with age in several rat organs and during replicative senescence of human fibroblasts. MsrB are zinc-containing enzymes and zinc has been shown to be essential for their activity. To address the effect of zinc on protein maintenance systems, oxidized protein content and proteasome peptidase and Msr activities were monitored in peripheral blood lymphocytes samples of control and zinc-supplemented subjects. Finally, to analyze the relationship between oxidative stress, protein oxidative damage, zinc and Msr, MsrA has been overexpressed in SV40 WI-38 human fibroblasts while MsrA and MsrB2 has been overexpressed in Molt-4 lymphoblastic cells. Overexpression of either MsrA or MsrB2 was found to protect the cells against oxidative stress-induced cell death and protein oxidative damage.

Neopterin - New Bioindicator of Malnutrition and Inflammation in the Elderly

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At present there are varying views on the interpretation of biochemical markers of malnutrition, and there is ongoing research on the mutual influence of nutritional indicators during a concurrent inflammatory reaction. The levels of serum proteins can also be reduced during an inflammatory reaction (negative acute phase proteins) with a concurrent rise in inflammatory markers, such as C-reactive protein (positive acute phase protein).
This phenomenon is prioritization of inflammation and malnutrition of the organism. Studies have shown a statistically significant relationship between age, plasma proteins, and positive reactants of inflammation. Revising the interpretation of malnutrition and inflammation, bioindicators reveal the necessity of not using only absolute values of plasma proteins, lipoproteins and other components, but rather the indices that integrate bioindicators of malnutrition, inflammation, sex and age.

Determination of serum proteins still ranks among principal examinations in malnutrition diagnostics, but at present the view of serum proteins as only malnutrition markers is being changed, as their values are affected also during inflammatory process.

The levels of serum proteins are, however, also decreased in the course of an inflammatory reaction (negative proteins of the acute phase) by the transfer of plasma proteins to the reactants of the acute phase, such as C-reactive protein (CRP). It is therefore suitable for the examination of the changes in serum proteins to determine simultaneously also CRP, the value of which is increased in inflammation (positive protein of the acute phase), and to compare the obtained values with each other. A decrease in serum proteins can be also due to transcapillary leak to interstitial fluid (albumin level is particularly influenced).

Since positive reactants of inflammation (CRP, lipoprotein (a), orosomucoid, fibrinogen) and biondicators of malnutrition (albumin, prealbumin, transferin etc.) are reciprocally dependent, there is non-protein and independent biomarker of inflammation essential for reliable discrimination of both processes. Neopterin produced by human macrophages after stimulation with interferon gamma is secreted by activated T-lymphocytes and is therefore a suitable indicator to monitor inflammation and immunity activation. Tab.1.

Bioindicators of malnutrition	Indices of inflammation
albumin	C-reactive protein
prealbumin	fibrinogen
transferin	orosomucoid
cholinesterase	α_1-antitrypsin
protein-binding retinol	neopterin in urine
total protein	sedimentation of erythrocytes
lipids – cholesterol, HDL, LDL and leptin	lipoprotein (a)
some hematological an immunological examinations	
– absolute number of lymphocytes, skin tests etc.	

Table 1: Bioindicators of malnutrition and indices of inflammation

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OP29

Complex relationships between smoking, physical activity and oxidant stress : Smoking negates the beneficial effects of exercise training in human.

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Amongst several lifestyle habits physical exercise and smoking have a significant influence on the risk of major vascular thrombotic events. These factors have in common to tackle the redox balance. It is wellknown that physical exercise produces reactive oxygen species (ROS) that could be exacerbated during vigorous exercise. However, regular exercise without exhaustion is associated with a reduced ROS production and with an overall decreased risk of cardiovascular diseases. In previous studies, we have shown that cigarette smoking is associated with increased platelet activation and impaired redox status in humans. Smoking has also been reported to release large amounts of ROS which were proposed to represent a potential mechanistic link between cigarette smoking and cardiovascular diseases. The increased oxidant stress observed in smokers may be due to the lower blood antioxidants usually observed in smokers. Numerous key parameters involved in pro-atherothrombotic effects of smoking such as fibrinogen, interleukin-6, platelet activation, LDL-cholesterol, oxidant stress biomarkers, etc… might be favorably modified by regular endurance exercise. Therefore, the commonly accepted idea that exercise would normalize smoking-induced alteration of biological parameters has not received experimental-based evidence. On the contrary, recent work clearly indicated that postprandial oxidant stress was reported to be exacerbated in cigarette smokers.

In the present study, we thus compared oxidant stress biomarkers in 146 healthy subjects before and after a 20-week exercise training program. Basal levels of antioxidants and oxidant stress markers were altered between non-smokers and smokers. These data were compatible with an increase in oxidized lipids and proteins in smokers. Exercise improved several parameters in non-smokers although it did not modify the LDL oxidation parameters. Significantly increased resistance was observed in the free radical–induced hemolysis in non-smokers. We found a general improvement in the antioxidant status following the exercise training session in non-smoking subjects. This effects was not observed in smokers specially in men. From our data, we can conclude that smoking negates the beneficial effects of exercise training.

Posters

A Study on the possibility to increase the antioxidant status of the plant food

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Antioxidants are substances that oppose the damaging effects of free radicals that are generated as a result of both the normal byproduct of metabolism as well as those generated through the exposure to environmental factors such as cigarette smoke, sunrays, pollution, etc. Antioxidants are vitamins, minerals and nutrients as well as enzymes created by the organism to counter the effects of oxidation and free radicals. By countering the effects of free radicals, antioxidants play a role in preventing chronic diseases and the aging process.

However, the accumulation of antioxidants by plants strongly depends on the conditions of growth as plants sensitively react to changes in conditions such as light intensity, mineral availability, temperature and water availability.

The purpose of this work was the study of the action of different factors (intensity of light and mineral nutrition) on the accumulation of some low-molecular antioxidants by barley plants.

For analyses were used 10 days after sowing barley plants (*Hordeum vulgare* L.), grown as the hydroponic culture. Plants were cultivated on Hogland's nutrient solution with and without selenium. Selenium was added in form of sodium selenate in the concentration of 1 μmol L^{-1}. The barley plants with different selenium nutrition were used by the study the effect of the light intensity (175 μmol m^{-2} s^{-1} and 500 μmol m^{-2} s^{-1}) on the accumulation of some low-molecular antioxidants (carotenoids, anthocyans, rutin, glutathione, riboflavin, ascorbic acid) by the plants.

The findings show the positive effect of selenium on the accumulation of some low-molecular antioxidants. In the barley plants by the addition of selenium to nutrient solution increased the content of glutathione by 15%, ascorbic acids by 12%, rutin by 6%. The content of carotenoids and riboflavin changed not significantly. A reduction of the content of anthocyans (to 29%) by the addition of selenium to nutrient solution can be also the proof of the positive action of selenium in the barley plants. It is known that the level of anthocyans is increased under the influence on the plants of a whole series of stress factors.

Studies of the influence of high light intensity on the accumulation of some antioxidants in the Se-deficient plants showed that the exposure of sprouts on the light with intensity of 500 μmol m^{-2} s^{-1} during 12 hours enhanced the content of all analyzed low-molecular antioxidants (carotenoids on 11%, rutin on 17%, glutathione on 17%, anthocyans on 15%, riboflavin on 24%). The increase in the content of ascorbic acid was most significant - it was more than on 50%.

Effect of high light intensity on the accumulation of some antioxidants in the Se-sufficient plants was different. The level of glutathione changed not significant in Se-sufficient plants after 12 h exposure on the high light intensity, while the content of ascorbic acid decreased by the same conditions up to 80 %.

Thus, the results of investigating the influence of the light intensity and micronutrient selenium on the accumulation of low-molecular antioxidants (carotenoids, rutin, glutathione, anthocyans, ascorbic acid, and riboflavin) by the barley plants prove the possibility of increasing the antioxidant status of plant food through the changing of conditions of plant cultivation.

The antioxidative activity of the most frequently consumed Polish mushrooms

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One of the nutritional benefits of mushrooms is the presence of bioactive secondary metabolites which are reported to reveal therapeutic effects *in vivo*. There is a lot of evidence that these metabolites display antioxidative properties. Therefore we selected the most frequently consumed species in Poland (*Boletus edulis* young – "white" and adult – "yellow", *Leccinum spp., Xerocomus bodius, Xerocomus subtomentosus, Cantharellus cibarius, Suillus spp., Suillus variegatus, Suillus bovinus, Pleurotus ostreatus, Agaricus bisporus*) and determined the content of polyphenols and flavonoids, β-carotene, lycopene in aqueous and methanolic extracts of dried bodies as well as the reducing power and ability to scavenge $ABTS^{\bullet+}$.

Aqueous extracts of *Boletus edulis* "white" and *Xerocomus subtomentosus* displayed the highest total content of phenolics, however the highest concentration of flavonoids was found for *Xerocomus bodius*. The quantity of polyphenols and flavonoids in methanolic extracts was lower than in the aqueous extracts except for the case of *Suillus bovinus*. We observed a strong correlation ($r>0.9$) between the concentration of polyphenols and reducing power and scavenging effects in both aqueous and methanolic extracts. The weakest effects were noticed for *Agaricus bisporus*, *Pleurotus ostreatus* and *Cantharellus cibarius*. The highest content of β-carotene was found in methanolic extracts of *Suillus spp., Suillus variegates* and *Suillus bovinus*, while the amount of lycopene was the highest for *Suillus bovinus*.

Mushrooms selected for our experiments were found to differ in the content of antioxidants and total antioxidative capacity.

In vivo antioxidant effect of yogurt enriched with CoQ10

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Oxidative stress and lipoprotein oxidation are known to be associated with cardiovascular disease and atherogenesis. Susceptibility of lipoproteins to oxidation relies on several factors including nutritional aspects: namely the intake of fatty acids, vitamins and antioxidants plays a pivotal role. Ubiquinol is the most reactive antioxidant in LDL and the susceptibility of these lipoproteins to peroxidation is inversely proportional to the CoQ10/PUFA ratio. Supplementation of LDL with ubiquinol has been shown to protect them from peroxidation. In the present study we evaluated the bioavailability, and antioxidant activity on lipoproteins, of CoQ10 in a commercial yogurt (Milchof Meran, Italy). 20 healthy normocolesterolemic subjects aged 24-45 volunteered in this study. Half of them were taking one dose of yogurt containing 20 mg of CoQ_{10} and the other half two doses a day, for 14 days. The analysis of CoQ10 content in LDL has shown a significant increase already with a single administration (+128%, $p>0.01$), that raised to 4X values in subject taking 40mg (+320%, $p>0.01$). The remarkable increases obtained with 40 mg/day supplementation were associated with a) enhanced antioxidant protection of LDL to peroxidation as shown by a significant prolongation of the lag-phase of dienes formation following Cu^{++} exposure (+13%, $p<0.01$); b) increased HDL paroxonase activity (+31% $p<0.05$).

Bioavailability of QH absorb, a novel formulation of reduced Coenzyme Q_{10}

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Coenzyme Q_{10} is widely used as a food supplement and, in some countries as an ethical drug, on the basis of its recognized roles in cellular bioenergetics and as a lypophilic antioxidant. Bioavailability of CoQ10 formulations is very limited, but some effects of CoQ10, such as improvement of endothelial function (1) have clearly being related to the achievement of a certain plasma level threshold. Recently the reduced form of CoQ10, Ubiquinol-10, was made available. Even though oxidised CoQ10 is promptly reduced when administered per os, animal studies have shown that the reduced form is probably better absorbed. In the present study reduced CoQ10 (QH absorb, Jarrow Formulas, LA) was given for 14 days to 11 healthy volunteers, at the dosage of 100mg t.i.d., one capsule with each of the main meals. Plasma CoQ10 levels went from 0.7 ± 0.2 µg/ml to 5.8 ± 1.6 µg/ml. These values are remarkably higher than the ones recently obtained with the same dosage of the corresponding formulations of oxidised CoQ10 (1). This finding might be of particular interest in situations where a certain threshold is required to afford vascular protection or in clinical settings such as neurodegenerative disorders, where high CoQ10 dosages were shown to be effective (2).

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Nitric oxide and ethyl nitrite in the stomach: new mechanisms underlying the physiological impact of red wine

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Nitrite may be reduced to nitric oxide (NO) in acid/reducing environments. Due to their redox properties, red wine phenolic compounds, at gastric pH, may yield NO from nitrite, inducing local relaxation (Gago et al. 2007). Also, an ethanol derivative, ethyl nitrite, can be formed in the presence of wine and nitrite and may induce relaxation of stomach *in vitro* (Gago et al. 2008). Using GC-MS we demonstrate in humans that ethyl nitrite is formed in the stomach following the consumption of lettuce (source of nitrite) and red wine. Ethyl nitrite acts as a NO donor and both the NO released from ethyl nitrite and produced from the reduction of nitrite by red wine polyphenols induces dose-dependent relaxations of rat stomach fundal strips, in a guanylyl ciclase dependent way, Moreover, real-time diffusion experiments with rat stomach show that upon ingestion of red wine and nitrite NO diffuses through the stomach wall and, therefore, more systemic effects are expected to occur. These results suggest that both major fractions of red wine, the polyphenols and the ethanol, interact with dietary-derived nitrite in the stomach leading to the production of NO and ethyl nitrite, both inducing smooth muscle relaxation in the stomach wall. These findings reveal an alternative chemical reaction pathway with the stomach acting as a bioreactor facilitating the formation of new bioactive molecules from diet-derived wine and nitrite with possible impact on gastric physiology and pathophysiology.

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Tamoxifen and raloxifen in the protection against the oxidative action of hypochlorite, peroxynitrite and AAPH.

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The antioxidative properties of selective estrogen receptor modulators (SERMs) employed in the hormonal replacement therapy and their influence on the antioxidative defence have been reported in *in vitro* as well as *in vivo* studies. The aim of thois study was to find out whether two of such compounds,, tamoxifen and raloxifen, are efficient in protection against such oxidants as hypochlorite, peroxynitrite and AAPH. The antioxidative effects were examined on the basis of ABTS cation radical reduction, prevention of fluorescein bleaching, oxidation of the thiol groups and formation of hydroperoxides in erythrocyte membranes. The ability to scavenge the ABTS cation radical was observed only for raloxifen, while both tamoxifen and raloxifen were found to demonstrate a dose-dependent protection of fluorescein against oxidation. Raloxifen was much more effective against all oxidants employed. Tamoxifen protected erythrocyte membrane thiols only against the action of hypochlorite and decreased the formation of hydroperoxides during incubation of the membranes with this oxidant, but was completly ineffective against of peroxynitrite or AAPH. The oxidative effects of peroxynitrite were not counteracted by raloxifen. In contrast to tamoxifen, raloxifen ameliorated the oxidative events triggered by AAPH and was more potent in protection against hypochlorite. Our results indicate that tamoxifen and raloxifen, which are used in clinical trials, can act as antioxidants, but show different selectivity and efficiency

P7

Evaluation of risk and benefit relative to antioxidants intakes

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Afssa's role is to assess food-related health risks. In the context of the evolving EU regulations on dietary supplements, addition of substances to foodstuffs (n°1925/2006) and claims (n°1924/2006), the aim of this work, based on an overview of Afssa opinions as regard to antioxidants molecules assessed, was to identify (i) the type of ingredients concerned, (ii) the risk evocated and (iii) whether scientific and statutory background sustaining claims is available.

Opinions published since 2000 (about 80) were first classified according to products categories : dietary supplements, ingredients, ordinary foodstuffs or intended for particular nutritional purpose. Secondly, they were classified (i) for risks assessment according to the type of antioxidants molecules (polyphenols, carotenoids and vitamins) (about 59 opinions) or (ii) for benefits assessment according to the type of claims (health claims, nutritional claims, cosmetic claims) (about 56 opinions).

This overview of opinions relative to antioxidants risks and benefits since 2000 shows the increasing number of fortified foods and foods supplements containing antioxidants for which Afssa's opinion is required. Excepted for carotenoids, there are more opinions which do not evoke risk or refute it than opinions pointing out a risk. The analysis of these opinions also highlights that the evocated risk differs according to the considered molecule. Furthermore, the lack of scientific data related to antioxidants induced risk and their biochemical specificities emphasized the difficulties of risk assessment.

Concerning benefits, the majority of claims are unfounded (93%) mainly because of the lack of clinical trials.

An in-depth review of antioxidant physiological functions, of interactions between molecules and their synergy is needed to propose recommendations relative to antioxidants intakes.

Oxidant-antioxidant balance in neonates

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Oxygen radical injury may be a pathogenic mechanism in diseases of the premature like retinopathy and bronchopulmonary dysplasia. Deficient antioxidant defense and/or increased free radical production may contribute to complications of prematurity. The measurement of the interplay between oxidant-antioxidant im/balance and inflammation, infection and unknown factors in the pathophysiology of these diseases of the premature is hampered by the small amount of blood available.
Experience and methodology in the handling of dried blood microsamples is available in neonatal screening laboratories. This knowledge was used to measure some parameters of the oxidant-antioxidant balance in small dried blood spots. An overview of the handling and methodology to measure these parameters in blood microsamples and urine will be presented.
The determination of the oxidant-antioxidant im/balance requires the measurement of different groups of parameters like the levels of antioxidant defense, antioxidant consumption and radical-induced damage. As a result it will be possible to decide whether a (non)physiological flow of radical is fighted by an (in)sufficient antioxidant defense. The presented methodology will be the basis for collaborative studies to learn more about the role of oxidative stress in "oxygen radical disease of prematurity".

Black currant seed press residue consumed in whole grain bread significantly increased serum γ-tocopherol concentrations

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During seed processing of black currant a press residue (PR) remains, which is considered as a waste product at the present though it is still rich in oils and tocopherols. The physiological effect of the consumption of black currant PR was studied in two human intervention studies conducted in women. Firstly, both studies started with a baseline period without interventions. Secondly, in study I (n=18), consecutively 10 g PR/d and 20 g PR/d were consumed in yoghurt for four weeks each. In analogical design in study II (n=36) a PR supplemented bread (20 g PR/d) was consumed in comparison with a control bread. Blood was taken after a 5-day standardised diet at the end of each period.
In study I γ-tocopherol intakes [µmol/d] were 3.50 ± 0.34 (baseline), 5.37 ± 0.61 (10 g PR/d) and 7.01 ± 0.75 (20 g PR/d), intakes in study II were 9.71 ± 1.59 (baseline), 13.5 ± 1.7 (control bread) and 17.7 ± 2.3 (PR bread). PR consumed in yoghurt caused no changes in serum γ-tocopherol concentrations. PR consumed in bread, instead, caused significantly increased serum γ-tocopherol concentrations (1.49 ± 0.58 µmol/l) compared to control bread (1.17 ± 0.46 µmol/l) and baseline (1.18 ± 0.34 µmol/l) (P<0.001). This effect is possibly due to the alkylresorcinol present in whole grain wheat and rye. It competes together with γ-tocopherol for the degradation enzyme leading to enrichment of serum γ-tocopherol.

P10

The antioxidant status of water extracts from the dried up leaves of an amaranth (*Amaranthus tricolour* L.)

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The Plant an amaranth possesses high growth rate, accumulation of a biomass, high adaptable ability to photostresses and actively functions at high intensity of illumination and raised UV – radiation. It can be promoted by that young leaves contain a considerable quantity of antioxidants. The basic pigment of the given plant is amaranthin. Maintenance research amaranthin in leaves of different age of plants of a grade of Valentine has revealed its maximum accumulation in young sheet. However it is necessary to notice that in young sheet carotinoides and a chlorophyll *a* and *b* in 5 – 6 times are less, than amaranthin that testifies to its intensive biosynthesis. Probably, at the initial stage onthogenesis amaranthin stimulates ростовые plant processes.

It is known that one of the reasons of occurrence of free radicals and active forms of oxygen are the photoinduced reactions. By some scientists it is shown that the water-soluble pigment амарантин along with SOD and other water-soluble antioxidants can participate in detoxication active forms of oxygen, that is amaranthin possesses amaranthin activity. As it is known, oxidation-reduction and antioxidants properties of molecules amaranthin, and also its ability to form complexes with ions of variable metals and to co-operate with photosyntesiert underlie mechanisms of protective function. Dynamics of the maintenance amaranthin in amaranth leaves has adaptive character to light illumination and temperature and to one of its major functions protection of components of a cage against active forms of oxygen is. Hence, considerable growthing amaranth possibility can explain to that young growing leaves differ high antioxidant activity. This activity remains and in the dried up leaves possibly at the expense of a red-violet pigment – amaranthin. Amaranthin proof enough pigment, it remains and in the dried up leaves.

Therefore the work purpose was research antioxidant status of water extracts from the dried up leaves of an amaranth. Possibility of reception of water extracts from the dried up leaves of an amaranth keeping antioxidants properties has been studied. For this purpose spent extraction at temperatures – 20ºC, 40ºC, 60ºC, 80ºC and 100ºC. For extraction used the dried up crushed leaves of two fractions. The first fraction included fragments of leaves in the size from 0,1 to 1 mm, and the second – from 5 to 10 mm. At carrying out extraction used such weights of leaves that the maintenance of leaves in water made 0,5 mg/ml or 2,5 mg/ml.

By us it is shown that the size antioxidant activity of water extracts from the dried up leaves of an amaranth depended on time extraction and temperatures. So, at temperatures 20ºC and 60ºC occurred gradual extraction antioxidants in the water solution reaching the maximum values in 24 hours. More the heat – 100˚C provided almost full extraction antioxidants already through 5 – 10 minutes. It is shown that the maintenance of antioxidants in water extracts from leaves of an amaranth after 5 minutes extraction at temperature 20˚C has slightly decreased, then the increase in an exit of antioxidants in a water solution was marked and this size has reached the maximum values after 24 hours extraction. This supervision concerned both fractions of leaves and concentration (0,5 mg/ml and 2,5 mg/ml). Time influence extraction on an exit of antioxidants from amaranth leaves in a water solution at temperature 60 ˚ With has shown that in 5 minutes extraction in a water solution antioxidants were found out. Then, within 5 hours the maintenance of antioxidants in a water solution practically did not vary, and only after 7 hour expositions increase of the maintenance of antioxidants in a water extract to the maximum sizes is noted at 24 sentry extraction. Law extraction antioxidants from the dried up leaves of an amaranth at temperatures 40ºC and 80ºC was same.

Time influence extraction on an exit of antioxidants in a water solution from the dried up leaves of an amaranth of fraction 1 and 2 has been noted at temperature 100ºC. At a heat antioxidants quickly extractise in a water solution already during the first 5 – 10 minutes and after that their maintenance in a solution did not vary almost. So, the size antioxidant activity of water extracts from the dried up leaves of an amaranth depends on time extraction and temperatures.
So, at temperatures 20˚C, 40ºC, 60ºC and 80ºC there is a gradual exit of antioxidants in the water solution reaching the maximum values in 24 hours. More the heat – 100˚C provides almost full extraction antioxidants already through 5 – 10 minutes. This conclusion concerns both fractions from the dried up leaves of an amaranth and to both concentration.

Antioxidanting properties of water extracts from the dried up leaves of an amaranth can be defined by a pigment amaranthin which has an absorption maximum at length of a wave equal 540 nanometers. The optical density (540 nanometers) the water extracts received at temperatures 20ºC, 60ºC and 100ºC has been measured. Extraction of amaranthin in a water solution from two fractions of leaves of an amaranth of concentration of 0,5 mg/ml occurred for 5 minutes irrespective of temperature (20˚C, 40ºC, 60ºC, 80ºC and 100ºC), and at increase in concentration to 2,5 mg/ml extraction occurred more slowly, but reached great values. The maximum exit amaranthin in a water solution occurred in 3 hours extraction irrespective of temperature.

Thus, considering that antioxidanting properties of water extracts from the dried up leaves of an amaranth had the maximum values only in 24 hours extraction at temperatures 20ºC and 60ºC, it is possible to conclude that not only amaranthin causes antioxidanting properties of water extracts from the dried up leaves of an amaranth.

Lipoperoxyl radical scavenging activity of the phytochemical betanin and its aglycone in aqueous/lipid membrane system. Kinetics and mechanistic aspects.

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Dietary antioxidant phytochemicals are believed to play a major role in maintaining a healthy status. Betacyanins are condensation products of betalamic acid with a *cyclo*-DOPA structure with additional glycosylation and acylation patterns at C-5 or C-6. Betanin, betanidin 5-O-b glucoside, is the major dietary betacyanin, occurring in red beet and cactus pear fruits, and its aglycone betanidin is easily formed during digestion. Both compounds contain a phenolic and a cyclic amine group that are good electron donors. Antioxidative effects of betacyanins have been demonstrated in LDL and cell cultures[1,2]. Importantly these pigments are highly bioavailable in humans[3]. Recent studies showing that betanin partitions within the core of phosphatidyl-choline vesicles[4] prompted us to evaluate the kinetics of the reaction between either betanin or betanidin and peroxyl radicals generated in an etherogeneous aqueous/soybean phosphatidyl-choline membrane model, whose oxidation was induced by AAPH. Betanin and betanidin showed a behavior consistent with the activity of their monophenolic and diphenolic moieties, respectively. Both pigments were chain-breaking antioxidants, with effectiveness of the same order as a-tocopherol. Mechanisms of the reaction of either betacyanin with lipoperoxyl radicals are proposed on the basis of the stoichiometry of reduction, and of products identified as betalamic acid and either dopachrome or dimers of cyclo-DOPA glycosides for betanidin, or betanin, respectively.

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Interaction of G3.5 polyamidoamine (PAMAM) dendrimers with human red blood cells.

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A very important factor, which also decides about a potential use of dendrimers in medicine, is their interaction with blood cells. In our investigation, the G3.5 PAMAM dendrimers were examined and the interaction of these polymers with red blood cells was investigated. The 1% haematocrit was prepared in PBS. PAMAM G3.5 dendrimer in methanol was used in concentrations: 40, 80, 120, 200µM and erythrocytes were incubated with increasing concentrations of PAMAM solution for 0.5, 1.5 and 3 hours. The measurement parameters were: percentage of haemolysis, level of reduced glutathione (GSH) (measurements with use Carl-Zeiss SPEKOL11 spectrophotometer) and generation of reactive oxygen species (ROS) (measured by flow-cytometry, Becton Dickinson LSR II). The results obtained from the experiments show that PAMAM G3.5 dendrimers possess hemolytic activity against erythrocytes depending on the concentration of polymer and incubation time. The highest hemolytic activity was achieved after 3h incubation. The GSH level decreased along with an increase in dendrimer concentration and incubation time (the highest decrease in GSH after 3h of incubation). The highest increase in ROS was observed for the 1.5h incubation with dendrimer compared to the control. The obtained results show that G3.5 PAMAM dendrimer in applied concentrations and incubation time affect erythrocytes haemolysis, GSH level and generation of ROS. The above results do not cross out using PAMAM dendrimers in medicine but further research is still needed (also with erythrocytes).

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Measurement of the antioxidant enzyme glutathione peroxidase in whole blood using an assay kit on the fully automated RX series analysers.

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Determination of the antioxidant enzyme glutathione peroxidase (GSH-Px) in total blood can be useful in monitoring antioxidant status in different pathologies and changes in life-style such as diet or exercise. This enzyme detoxifies hydrogen peroxide and also converts lipid hydroperoxides to nontoxic alcohols. Selenium in the form of selenocysteine is part of the catalytic site of the mammalian GSH-Px and correlations between selenium and enzyme activity have been reported. Automatic systems enabling in vitro quantification of GSH-Px represent an excellent tool for research, clinical, veterinary, nutritional and therapeutic applications. We report the analytical performance of an assay on the fully automated RX Series analysers for quantification of GSH-Px in whole blood samples.

The assay principle is colorimetric, the decrease in absorbance due to cofactor oxidation is measured. The assay was applied to the RX Series analysers that include dedicated software for data management.
Evaluation of the performance parameters showed an assay sensitivity of 82.88U/l with linearity up to 900U/l. Within run and total precision for three different concentration levels(n=44), expressed as %CV were typically<8. Correlation (n=49) with other commercially available system generated the following linear regression equation: Y=0.99x-2.55, r=0.99.

In conclusion, data show optimal performance of the assay for the measurement of GSH-Px in whole blood on the RX Series analysers which represents an accurate and convenient tool for applications in different settings

Stable Isotope of Magnesium-25 as Micronutrient Antioxidant

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Chemical reactions obey the law of conservation of the total angular momentum (spin): the spin state of reactants must be identical to that of products. As a result, acceleration of reactions can be achieved via magnetic fields of nuclear spins of magnetic nuclei. Among three stable isotopes of magnesium, ^{24}Mg, ^{25}Mg and ^{26}Mg with natural abundance 78.7, 10.1 and 11.2%, only ^{25}Mg has a nuclear spin magnetic moment (I = 5/2). Two other isotopes are spinless (I = 0) and, for this reason, nonmagnetic. We have for the first time shown that the cells of *E. coli*, which grows on the medium containing ^{25}Mg-sulphate, multiply by about 20 per cent faster than the cells which grows on the nonmagnetic ^{24}Mg or ^{26}Mg isotopes. Moreover, the cells grown on ^{25}Mg demonstrate essentially less activity of superoxide dismutase), by about 40 per cent, when compared to the cells grown on ^{24}Mg or ^{26}Mg. Inasmuch as activity of SOD is adjusted to the intracellular level of superoxide radicals, the lower activity of SOD can be considered as evidence for lower production of superoxide radicals as the faulty by-products of respiration. It means that the energy park's bio-nanoreactors work not only more effective but more reliable too while supplied with the magnetic isotope of magnesium by comparison with the case of the nonmagnetic isotopes of magnesium. Thus, magnesium-25, owing to its nuclear spin, produces the preventive antioxidant effect.

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Using of Cyclic Voltammetry in Assessment of Antioxidant Capacity – Clinical Application

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The main aim of this contribution is to study antioxidant activity by using of cyclic voltammetry at carbon working electrodes. Cyclic voltammetry (CV) can be used for measuring highly important antioxidants (low molecular weight antioxidant) such as glutathione, reduced nicotinamide adenine dinucleotide, carnosine. CV was also used to monitor the levels of antioxidant capacity in patients with tumours, in which the elevated concentration of reactive oxygen species can be expected due to tumour development or anticancer treatment. For measuring antioxidant capacity, we utilized carbon paste electrodes, which are easy to prepare. To assess antioxidant capacity the sum of the areas of all signals measured in the samples were used. The experimental results were compared with antioxidant capacity measured by Trolox (UV-VIS spectrometry). Changes in antioxidant capacity were observed in patients with medulloblastoma by using of both methods.
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Prediction of metabolism of a cytokinin molecule using on-line electrochemistry/liquid chromatography/mass spectrometry (EC/LC/MS)

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The analysis of reactive metabolites using conventional in vivo and/or in vitro techniques is hampered because the intermediately formed reactive species are prone to covalent binding to thiols like glutathione or to thiol group of cellular macromolecules. Therefore, the application of improved methods is required. EC/LC/MS is a new promising tool for the identification of both reactive and stable metabolites in drug development.

Cytokinins play a crucial role in regulation of proliferation and differentiation of plant cells. They also control various processes in plant growth and development, such as delay of senescence, transduction of nutritional signal, control of shoot/root balance and increased crop productivity[1]. Virtually all naturally occurring cytokinins identified to date are adenine species substituted at N6 with an isoprenoid or aromatic side chain. An additional modification of a cytokinin molecule can lead to a dramatic changes of action in a control of growth and development. Olomoucine and its derivatives roscovitine and bohemine, are a C2, N9-substituted 6-benzylaminopurine derivatives, which specifically inhibit Cdk-2 related kinases[2].

Coulometric electrochemical (EC) flow cells are widespreadly used as high performance liquid chromatography detectors (HPLC-ECD) for the study of various redox-active compounds. Primary use of EC cells has been for quantitative bioanalysis of these compounds and using the EC cells as simple means of producing redox reactions, the products of which may then be studied by mass spectrometry (MS) [3].
We present a validated HPLC/EC/MS method for quantitative analysis of above mentioned Cdk-inhibitors and compare it to existing HPLC/MS and HPLC/MS-MS methods.

The prediction of metabolism of these compounds is an important task, considering the fact that for example, R-roscovitine (also labeled as CYC202) is now in Phase II clinical trials for breast and lung cancer[4]. Besides some established in-vitro and in-vivo methods using biological systems[5], we have developed a biomimetic model using the cell as an electrochemical reactor for simulation of in situ oxidative reactions, that occur in metabolism of mentioned Cdk-inhibitors, and identification of products by MS. This utilization of electrochemical cell also opens possibilities to prepare various metabolites in a sufficient amount for further biological testing, thus bypassing the need to synthesize them.

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Oxidized nucleic acid bases as markers of oxidative stress in organisms

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Analysis of markers of oxidative damage is important to assess the extent of the risk and burden of organisms to toxic compounds present in the environment. One such molecule is 8-oxo-DG, which can be detected in easily accessible body fluids such as urine. Basic electrochemical behaviour of the studied molecules was investigated by using of square wave voltammetry. In the present study we focused on comparison amperometric and coulometric detection of 8-oxo-DG. The aim was to distinguish bases and their derivatives using liquid chromatography (adenine, guanine, 8-oxo-DG, 8-oxo-dA). For all studied molecules hydrodynamic voltammograms have been measured within the range from 200 to 1,300 mV. The oxidation signals were negligible to the potential of 500 mV and then their height rapidly increased. The calibration dependence for all studied compounds were strictly linear (R = 0.99) and detection limits were estimated down to sub pmoles level. In addition, both electrochemical detectors were used for the analysis of real urine sample. Liquid chromatography represents so called "golden standard" for analysis of complex mixtures. Its coupling with electrochemical detector brings higher sensitivity and selectivity.
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Ydroxytyrosol Supports Endurance and Alleviates Inflammation

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Hydroxytyrosol (OH-T) is the most abundant polyphenol in olives, and is considered to have the highest antioxidant potency compared to the other olive polyphenols. Oxidative stress can occur e. g. during exercise, and causes cellular stress reactions, inflammation, and tissue damage. We investigated the anti-inflammatory activities of hydoxytyrosol (OH-T) in cell-based systems and in in-vivo models. OH-T dose-dependently inhibited the production of nitric oxide (NO) and prostaglandin E2 (PGE2) in lipopolysaccharide (LPS) -stimulated macrophages. We have found by immunoblot analysis that OH-T impairs the nuclear translocation of NFκB and the associated gene activation. Accordingly, the mRNA level of distinct inflammatory genes (e.g. interleukin-1α, inducible NO synthase and PGE2 synthase) was dose-dependently modulated by OH-T. In addition, we also demonstrated that OH-T reduced the binding of monocytes to inflamed endothelial cells by impairing the expression of chemokines and intracellular adhesion molecules (ICAM). The anti-inflammatory effect of OH-T was confirmed in vivo, since it reduced the carrageenan-induced paw edema in rat. Finally we evaluated the effect of this potent antioxidant and anti-inflammatory compound on endurance exercise. Mice (C57BL/6J, 5 wks old) were treated with OH-T (50, 150 and 300 mg/kg BW) for three weeks. At the end of the study the animals had to run until exhaustion on a treadmill. Time/distance and work load and body composition were measured. OH-T significantly prolonged the running time to exhaustion in mice.

In summary, hydroxytyrosol has pleiotropic anti-inflammatory effects, which are characterized by NFκB inhibition and which are likely connected to OH-T potent antioxidant function. Moreover,
OH-T improved endurance in prolonged exercise.

Vitamin and mineral supplementation in elderly people regularly trained in endurance

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Purpose: The influence of vitamin and mineral complex supplementation on muscular activity and oxygen uptake (VO_2) kinetics was examined during a heavy cycling trial. **Methods:** Two groups of 8 elderly people regularly trained in endurance, were randomly assigned in a double-blind process to one of two treatment groups: antioxidant supplementation (As group) or placebo (Pl group) for 21 days before the end of experimentations. Each subject had to perform two 10 min sessions of cycling on cycloergometer at the same heavy power output. During each cycling bouts, VO_2 kinetics were recorded. The second session was preceded by a fatiguing mainly eccentric exercise on a leg press. MVC of knee extensors was assessed before and after fatigue. EMG activity of thigh muscles was recorded with surface electrodes. **Results:** MVC was significantly ($P < 0.05$) reduced after eccentric exercise in similar proportions for both groups (As. – 10.9%; Pl. – 11.3%), associated with a reduction in EMG frequency parameters (i.e. Fmed, MPF), with lower decrease for As group. Characteristics of VO_2 kinetics recorded during the cycling bouts were modified between conditions. The amplitude (A_2) of the VO_2 slow component (VO_2 SC), and time constants of the primary ($\tau 1$) and secondary ($\tau 2$) VO_2 components were higher after fatigue for both groups, with a lower increase for As group. VO_2 kinetics changes were associated with an increase of VL activity, lower for As group. **Conclusion:** Results of the present study report a positive effect of the vitamin and mineral complex supplementation on exercise tolerance after fatigue.

Exhaustive Exercise Induces Nrf2-driven HO-1 Expression in Human Peripheral Blood Mononuclear Cells and in Mouse Muscle

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It has been speculated that intense exercise may cause oxidative injury through generation of reactive oxygen species. However, our body tends to adapt to oxidative stress. This study was aimed to test a hypothesis that exhaustive exercise induces the expression of heme oxygenase-1 (HO-1), a key enzyme in cellular antioxidant defence. HO-1 upregulation was mediated by antioxidant response elements (ARE) which are under control of the redox sensitive transcription factor NF-E2-related factor-2 (Nrf2). Human peripheral blood mononuclear cells (PBMCs) were obtained from the venous blood of fifteen healthy volunteers before, immediately after, and 1 h after an exhaustive exercise. Isolated human PBMCs were subjected to the electrophoretic mobility gel shift assay and Western blot analysis. The Nrf2-ARE binding activity and HO-1 expression, measured immediately after exercise, were more pronounced than those achieved before exercise. After 1 h recovery, Nrf2-ARE binding activity and HO-1 expression returned fully to the basal levels. In follow-up studies with mice, HO-1 expression in muscle and colon, analyzed 1 h after exhaustive exercise, dramatically increased in comparison with that observed both before and immediately after exercise. In contrast, there was no significant increase of HO-1 expression in heart and lung. Taking all these findings into account, we conclude that exhaustive exercise transiently induces Nrf2 activation and subsequent expression of HO-1 as part of the adaptive cytoprotective response against oxidative stress.

Gestation, training and redox state: study in an animal model.

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Since antiquity, it is known that the intrauterine life environments are of major importance for health during later life. Pregnancy is a physiological state associated with a moderate enhanced oxidative stress related to high metabolic turnover and elevated tissue oxygen requirements. Moreover, oxidative stress in pregnant women has been linked to oxidative stress in fœtus. On the other hand, a too important increase in oxidative stress during pregnancy has been linked to the pathogenesis and progression of neonatal diseases. Thus it appears important to limit oxidative stress during pregnancy. In this way endurance training, known to have beneficial effects on redox balance, during pregnancy could be of potential interest on the redox state of the newborn. Therefore, we studied the effect of moderate intensity endurance training of female rats before and during gestation (TG) on the redox state and the liver and muscle mitochondrial function of newborns compared to those from untrained mothers (C). Our results show significant changes in spontaneous mitochondrial H_2O_2 release, as index of reactive oxygen species (ROS) production, depending on the substrate used to energize the electron transport chain both in liver and muscle in TG newborn rats. These changes are related to alterations in mitochondrial oxygen consumption. There are no differences in muscle or in liver glutathion peroxidase (GPX) activity or in protein damages between TG and C groups. However, in plasma, GPX activity and protein oxidation are significantly higher in rats from TG group compared to rats from C group. Such changes in plasma could be an adaptative signal transmitted from the mother to the young. This preliminary study shows that it may be possible to act on the redox state in newborns by modifying maternal physical activity before and during pregnancy.

3-months multivariate aerobic training attenuates exercise-induced lipid peroxidation in overweight adolescent girls.

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The lipid excess in obesity increases oxidative stress at rest and in response to exercise in adolescent or adult populations (Youssef et al. in press, Vincent et al. 2005). In non-obese people (NOB), aerobic training has beneficial effects by decreasing oxidative stress. We hypothesized that 3-months of multivariate aerobic training in overweight and obese (OV) adolescent girls, would reduce lipid peroxidation at rest and after maximal exhaustive exercise.

Methods: 39 adolescent girls (age: 14-19yrs, BMI: 20-35.5kg/m²) were stratified into a control group (9 NOB and 9 OV) and trained group (7 NOB and 14 OV) who accomplished 3-months multivariate aerobic training. Anthropometric parameters, VO_{2peak}, pre and post-exercise plasma lipid peroxidation markers (F_2-Isop, ROOH, ox-LDL) were measured at baseline and after 3 months.

Results: Training increased fat-free mass and decreased fat mass and hip circumference, in OV girls but did not modify aerobic capacity. At rest no significant effect of training on lipid peroxidation markers was observed in both groups (NOB and OV), whereas 3-months multivariate aerobic training attenuated the exercise-induced lipid peroxidation in NOB (ox-LDL) and OV overweight subjects (F_2-Isop, ROOH, ox-LDL).

Conclusion: 3-months multivariate aerobic training improved the tolerance to exercise-induced lipid peroxidation in OV adolescent girls.

Effect of fermented papaya preparation on indices of oxidative stress during performance sport (motor racing)

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Motor car racing is representative of a concentrative sporting activity involving mental-concentrative and psycho-emotional stress. The increased number of isometric contractions and oxygen consumption are major features. The psycho-emotional stress activities are associated with catecholamine release during this type of exercise. ROS are implicated in the molecular and tissue damage arising from increased oxidative metabolism associated with strenuous exercise. This study examined the influence of fermented papaya preparation (FPP) on urinary markers of oxidative stress, MDA and protein carbonyls in 3 elite racing drivers during the 2007 Le Mans 24hr race in France. The drivers received full details of the study and signed a consent form prior to commencement of the study. FPP was consumed 14 days (9g/day) before the race. During the race, drivers were supplemented with FPP (6g) at 8 hrs intervals (maximum consumption of 18g) during the 24hr race. Urine samples were collected at the beginning of the race, 12hr and 24hr of the race (in compliance of the conditions imposed by the logistics of race track and pit management). The levels of malondialdehyde (MDA) and protein carbonyl were measured as markers of oxidative damage. Although the MDA values were similar at the beginning of the race and at the 12hr period, there were notable differences in the values at 24hr following the race. The non-supplemented driver had the highest level of MDA and protein carbonyl with values of 1.26 mM and 0.411 mmoles/g protein respectively. The corresponding values for the FPP-supplemented drivers were 0.714mM and 0.115mmoles/g protein respectively. The implication for the elite motor racing drivers and performance sports athletes is that the higher fatigue and more muscle's oxidative attack (stiffness, cramps) due to the exhaustive exercise could be modulated by supplementation with dietary FPP.

New lychee fruit-derived polyphenol Oligonol converted into a low-molecular form improves exercise-induced fatigue.

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Oligonol (Amino Up Chem. Co., Ltd., Sapporo, Japan) is a new lychee fruit-derived polyphenol converted into a low-molecular form, which has improved absorption and strong antioxidative activity. The aim of the current study was to investigate the effects of Oligonol supplementation on perceived subjective mood states in addition to oxidative stress in young athletes during track-and-field training. The ratings of perceived exertion (RPE) responses were significantly lower following Oligonol supplementation. The results of our own questionnaire on fatigue and pain showed that Oligonol supplementation significantly improved all the fatigue scores and tended to attenuate the feeling of pains. Unexpectedly, however, there were no changes in the oxidative stress markers in urine after Oligonol supplementation. These results suggest that Oligonol supplementation has a positive effect on exercise-induced fatigue. Moreover, the animal and *in vitro* studies on Oligonol will be also discussed.

Antioxidant status, hormones and serum enzyme monitoring in trained half-marathon runners

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Physical exercise is characterized by an increase in oxygen consumption by the whole body. This leads to changes in oxidative balance and in serum enzyme levels. The objective of this study was to determine the effect of the exercise on serum enzyme activities and follow their recovery after the run, as well as assess the occurrence of oxidative stress during exhaustive exercise and the antioxidant response. Fourteen voluntary male subjects were studied. The exercise was a half-marathon (21,097 km). Blood and urine samples were taken before and immediately after the race, after 3h recovery and a week later. For the entire group, hematocrit, hemoglobin (Hb) and red blood cell number significantly decreased. Serum lactate dehydrogenase (LDH) activity significantly doubled post race and then declined but remained elevated one week later. Serum creatine kinase (CK) activity tripled from baseline after the run, and returned to baseline after 1 week. The rise of these enzymes reflects mechanically damaged muscle cells leaking contents into the interstitial fluid. Triglycerides and VLDL-cholesterol increased significantly after the race and remained high 3h later. The half-marathon induced significant increases in catalase and GR activities. Significant decreases in GPx activity were also observed. The race induced oxidative stress, as evidenced by increases in blood GSSG and in serum urate concentrations and by the pattern of change of erythrocyte antioxidant enzyme activities throughout the recovery period. The extent to which the redox alterations are associated with the recovery of muscle function should be further analysed.

Phytoestrogens enhance antioxidant enzyme defenses and reduce testosterone and estradiol circulating levels

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Our aim was to evidence the effects of diet supplementation with phytoestrogens on blood cell counts, erythrocyte antioxidant adaptive responses and sex hormone levels induced by moderate exercise. Ten female swimmers participated for 26 days in a double-blind diet intervention with either a functional beverage rich in vitamins E and C or the same beverage but also supplemented with *Lippia citriodora* extract (PLx). After the intervention all subjects participated in a swimming session. Supplementation with PLx induced lower leukocyte and erythrocyte circulating levels. Diet supplementation with PLx enhanced the activation of erythrocyte antioxidant enzymes in response to the swimming session. Thus, swimming increased the erythrocyte activity of glutathione peroxidase and glutathione reductase only in the PLx-supplemented group. PLx decreased the plasma levels of 17-b-estradiol, testosterone and free testosterone and increased the levels of the sex hormone binding protein. In summary, diet supplementation with phytoestrogens such as verbascoside decreases the number of circulating erythrocytes and leukocytes and enhances the glutathione-dependent enzyme activities in erythrocytes in response to exercise. Diet supplementation with verbascoside also decreases the plasma steroid hormone levels, pointing towards a possible agonist effect between estradiol and these phytoestrogens.

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Palmitic acid stimulates nitric oxide production in rat skeletal muscle cells by increasing nuclear factor-kappa B activation and iNOS expression

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In this work we evaluated the effect of palmitic acid on nitric oxide (NO) production in rat skeletal muscle cells as well as possible mechanisms involved, such as activation of nuclear factor-kappa B and regulation of inducible nitric oxide synthase (iNOS) expression. Primary culture of rat skeletal muscle cells was treated for one hour with 25 uM of palmitic acid. NO production by primary cultured rat skeletal muscle cells was examined by two different methods: indirectly, by nitrite measurement (Griess method) and directly, by using 4,5-diaminofluorescein diacetate (DAF-2-DA) assay. Nuclear factor-kappa B activation was evaluated by electrophoretic mobility shift assay and iNOS content by Western blotting. Palmitic acid increased nitric oxide production in skeletal muscle cells as measured by both methods: Griess method (120%; $p<0.001$) and DAF-2-DA assay (24%; $p<0.01$). This effect was abolished by using L-nitro-arginine and L-nitro-arginine methyl esther (NOS inhibitors). Nuclear factor-kappa B activation was increased 63% ($p<0.001$) by the fatty acid. Palmitic acid also raised iNOS protein content by 60% ($p<0.001$). In summary, the present study showed that palmitic acid stimulates NO production in cultured skeletal muscle cells by increasing nuclear factor-kappa B activation and iNOS protein content. Financial support: FAPESP, CNPq, and CAPES.

Novel roles of Rottlerin in redox signaling

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Rottlerin, a polyphenol isolated from Mallotus Philippinensis, has been used in recent years as a selective inhibitor of PKCd, although it can inhibit many kinases and has a number of biological effects. Among them, our group recently found that Rottlerin inhibits the transcription factor NFκB activated by phorbol esters in MCF-7 breast cancer cells and by hydrogen peroxide in HaCaT keratinocytes and in both cases, a drop in cyclin D1 was observed, along with a marked growth arrest. Because of the redox sensitivity of NFκB and on the basis of the Rottlerin structure (presence of five phenolic hydroxyl groups that might act as hydrogen donors), we hypothesized that Rottlerin could prevent NFκB activation acting as a free radicals scavenger, in analogy with other natural polyphenols with known antioxidant properties. The results of the current study document the antioxidant activity of Rottlerin against the artificial radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in vitro and against hydrogen peroxide added to a culture cell model. We also demonstrate that Rottlerin prevents TNF a-dependent NFkB activation in HT-29 cells transfected with the NFkB-driven plasmid pBIIX-LUC, indicating that Rottlerin is a general inhibitor of NFkB activation and transcriptional activity regardless the cell type and the activation pathway. We believe that this old/new compound has the potential to be developed as an antioxidant/antiproliferative/anti-inflammatory drug.

New *in vitro* screening tools to study the Antioxidant activities of natural substances on stimulated neutrophils: application to red wines.

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Nowadays, there is a growing interest in the use of natural substances for their anti-cancer, anti-atherosclerosis and antioxidant properties. Antioxidant molecules act either by trapping reactive oxygen species (ROS) (stœchiometric activity) or by inhibiting the oxidant enzymes responsible for the ROS production (catalytic activity, by interaction with the active site of the enzyme). New insight is that a catalytic activity is more interesting and efficient than a stœchiometric one, because low concentrations of antioxidants are sufficient to reduce an acute production of ROS. This study describes an original method able to distinguish between stœchiometric and catalytic activities of natural compounds on stimulated equine neutrophils or on the purified oxidant enzyme, equine myeloperoxidase (MPO). The method was applied to 45 "Bourgogne" red wines with a 13% ethanol solution (as control sample). Isolated neutrophils were stimulated with phorbol myristate acetate (PMA). The stœchiometric antioxidant effect of the wines on ROS production was quantified by measurement of the lucigenin-dependent chemiluminescence (CL), and the ethylene produced from the oxidation of a-keto-g-methylthiobutyric acid (KMB) upon the ROS action was quantified by gas chromatography. The catalytic effect of the wines on the activity of purified MPO was assessed by SIEFED ("Specific Immunological Extraction Followed By Enzymatic Detection"), an original method that allows the study of drug interaction with the enzyme without interference with the reaction medium. About half of the tested red wines showed a stœchiometric inhibition ≥ 60 % by CL, and up to 80 % inhibition of the ethylene production. But, more than 75 % of the tested red wines had a catalytic inhibitory effect ≥ 80 % on the activity of MPO as measured by SIEFED. These results confirm the well-recognized antioxidant capacity of red wines on the ROS production by neutrophils, but highlight their important catalytic action on the MPO activity. In a further step, an index of catalytic antioxidant activity will be established for well-recognized stœchiometric antioxidant molecules or a mixture of them, allowing so a scaling of the catalytic antioxidant properties of red wines that could be correlated with their composition in anthocyanins, polyphenols and flavonoids.

Effect of acepromazine on the release of reactive oxygen species and myeloperoxidase by activated equine neutrophils

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Acepromazine (ACP) is a phenothiazine tranquilizer widely used for pre-medication in equine anaesthesia. Interesting clinical effects have been reported regarding the ACP utilization for laminitis treatment and are attributed to its vasodilatory properties [1]. We have demonstrated, based on *in vitro* [2] and *ex-vivo* [3] animal models that ACP had potential antioxidant and anti-inflammatory effects on neutrophil stimulation. Herein, we investigated the effect of ACP on the production of reactive oxygen species (ROS) and the release of myeloperoxidase (MPO) by phorbol myristate acetate (PMA)-activated equine neutrophils. A special emphasis was put to understand better the mechanism of action of ACP by electron paramagnetic resonance spectroscopy (EPR) study. Isolated equine neutrophils were incubated for 10 min with ACP, at final concentrations ranging from 10^{-4} M to 10^{-6} M, before their activation with 10^{-7} M PMA. The whole ROS production was measured by lucigenin-dependent chemiluminescence (CL), and ethylene produced by the ROS dependent-oxidation of a-keto-g-methylthiobutyric acid (KMB) was quantified by gas chromatography (GC). The specific effect of ACP towards free radicals was investigated by means of EPR spectroscopy in the presence or absence of 100 mM DMPO, a spin-trapping agent. In parallel, the amount of MPO released by neutrophils was quantified by a specific ELISA raised against equine MPO.

ACP showed a dose-dependent inhibitory effect on the production of ROS measured by CL (95 ± 4 % at 10^{-4} M and 55 ± 12 % at 10^{-5}M, $p<0.05$) and GC (85 ± 4 % at 10^{-4} M, 52 ± 23 % at 10^{-5}M; $p< 0.05$), while the MPO release was significantly reduced only at 10^{-4} M (57 ± 12 %, $p<0.05$). EPR-spin trapping results showed that a 10 min pre-incubation of neutrophils with 10^{-6} to 10^{-4} M ACP caused a decrease of the EPR spectrum intensity of DMPO-adducts, while direct EPR indicated the appearance of a cation-radical species that is attributed to the formation of an acepromazyl radical. Our study confirms that ACP decreases the oxidant activity of activated neutrophils, and also highlights the radical property of this molecule that may have interesting therapeutic perspectives in inflammatory diseases in horses involving neutrophil activation.

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Cardiovascular complications and oxidative stress induced by long-term high energy fructose diet in the normal and aged rat are attenuated by different types of algae and marine extracts : an hemodynamic, ^{31}P-NMR and ESR study

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Previous studies have shown that rats consuming a high energy fructose (HEF) diet develop some of the metabolic modifications similar to that observed in the human polymetabolic syndrome such as insulin resistance, hypertriglyceridemia, decreased HDL-cholesterol, and vascular complications such as hypertension and cardiac hypertrophy. A 6-weeks high carbohydrate consumption is also associated with oxidative damage involving formation of reactive oxygen species (ROS) including superoxide and hydroxyl radicals. Although these effects of short-term HEF diet in rats have been linked to human type 2 diabetes profile HEF diet is paradoxically protective[1] to the ischemic myocardium while an increase in the myocardial sensitivity and a decrease of post-infarction survival occurs in diabetic patients. Thus, to clarify this issue further,we have compared the metabolic changes and blood antioxidant status in rats fed for up to 12 months with either HEF (60% w/w) or standard diets (n = 60/group). After sacrifice, differences among groups in fasting insulin, cardiovascular indices (functional postischemic recovery of isolated hearts, free radical production of cultured aortic rings), markers of oxidative stress (plasmatic vitamins C and E, erythrocyte glutathione peroxidase, glutathione reductase and superoxide dismutase; left ventricular myeloperoxidase activity), energetic metabolism (^{31}P-NMR ATP determination) and direct detection of ROS in liver and kidney using the spin-trap DEPMPO[2] were assessed using hemodynamic, biochemical and electron spin resonance techniques. The data evidenced that (i) HEF diet was overall protective to hearts when it did not exceed 2 months while it became highly detrimental after 6 months duration, (ii) the development of impaired insulin activity was not related to hyperglycemia but to the antioxidant activity (i.e., vitamin E elevation and enzyme activity, trace element and glutathione depletion) and high lipid levels, and (iii) supplementation with algae extracts rich in phycocyanins efficiently improved all studied parameters in a 12-months HEF diet. These results could be of interest in the field of human nutrition because of the increasing consumption of fructose-enriched foods and beverages in many industrialized countries.

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Supplementation with low doses of antioxidants is effective to induce protection against myocardial ischemia-reperfusion injury in normal and hypertrophied rat myocardium.

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It is believed that antioxidant supplementation induces myocardial protection against myocardial ischemia-reperfusion injury in rats. Nevertheless, most of the studies were conducted with high doses of antioxidants. Therefore, the aim of this study was to investigate whether supplementation with antioxidants at low doses can improve the ability of normal and hypertrophied cardiac muscle (SHR or trained rats following a 3 months swimming program) to recover its function after an ischemic period in Sprague-Dawley rats. Seventy-two animals (body mass 300-350 g) were randomly allocated to control (placebo groups) and experimental groups (n = 12/group). In this latter, supplementation with a nutritional supplement (Oxytonic®) which contained an association of vitamins C, E, B_2, selenium, copper, manganese, zinc, beta-carotene and an extract of brown algae at low doses (respectively ± 1800, 179, 90, 0.9, 14.2, 25, 300, 85.7, 22.3 µg/day) was realized during three months. At the end of the supplementation period, the hearts from control and experimental groups were removed and perfused at constant pressure (90 cm H_2O) according to the Langendorff technique. After 30 min stabilization, the hearts were subjected to 10 min low-flow normothermic global ischemia followed by 30 min of total normothermic global ischemia and 60-min reperfusion. Left Ventricular Developed Pressure (LVDP), Rate × Pressure Product (RPP) and Coronary Flow (CF) were determined. An index of lipid peroxidation by measuring thiobarbituric acid reactive substances in coronary effluents and frozen hearts was also evaluated. Hemodynamic parameters measured at the end of stabilization period were unaffected by supplementation. During reperfusion, post-ischemic recovery of LVDP, RPP and CF were significantly improved in the experimental groups, as compared to the control groups ($p < 0.0001$). In addition, index of lipid peroxidation measured in heart tissue, as well as in coronary effluents, was significantly decreased in experimental group *versus* control groups ($p < 0.05$). In conclusion, supplementation with antioxidants mixture at low doses confers a protection against ischemia- and reperfusion-induced injury in isolated normal and hypertrophied hearts

The modality of supplementation with vitamin E and C on plasma oxidative stress parameters, hsCRP, and ischemia modified albumin (IMA) in patients with PAD

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Introduction: Plasma hsCRP and ischemia modified albumin (IMA) are recently introduced as the cardiovascular ischemia risk markers. Antoxidants: Vit E and C, exert an antiatherogenic effects.
The aim of study was to assess the relation between the modality of vitamin intake with plasma markers of oxidant stress and the effects of short-term dietary supplementation with vit E and C on the relation between hsCRP, IMA and plasma oxidative stress/antioxidant capacity parameters.
Patients and methods. Men (n=108): the control, healthy group (n=20), cardiovascular event-free obese subjects (n=38) and peripheral vascular disease (PVD) patients after surgical revascularization (n=44) received Vit E with vit C (2 x 100mg and 2 x 200mg daily, respectively) on an empty stomach or during meal, for 14 days in the cross-over study.. Plasma hsCRP, IMA, parameters of oxidative stress: TBARS, LOOH, LDL oxidative (Cu^{2+}) susceptibility, and antioxidant potency: FRAP, thiol/albumin ratio, vitamin E, C, redox compensation index were assessed.
Results: Food intake increased absorption of vitamins E and C, followed by decrease of plasma oxidative stress parameters. Vascular surgery of PVD patients did not reduce the oxidative stress, which markers were markedly increased even in comparison with obese patients. Supplementation of vitamins significantly reduced plasma oxidative stress parameters and improved plasma antioxidant potential more efficiently in subjects in the highest quartile of hs-CRP and IMA levels.
Conclusion: Food intake increases the bioavailability of antioxidant vitamins and improves their free-radical-scavenging activity. Plasma hsCRP and IMA may serve as marker of oxidative stress and may be useful for selecting patients which will benefit with such therapy.

Effect of vitamin C-rich diet on oxidative stress induced *in vivo* by hyperbaric oxygen therapy

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By using hyperbaric oxygen therapy (HBO) as an *in vivo* oxidation model we investigated the effect of a diet rich in ascorbic acid (AA) on the HBO-induced oxidative stress. 22 subjects were enrolled at the HBO centre and included in the AA-rich diet group or in the control group. Blood sample was collected at the basal time (T1), after 1-week of diet before (T2) and after HBO treatment (T3). The AA plasma level, antioxidant capacity, plasma hydroperoxides, lymphocytes DNA oxidation, and DNA repair capacity were assessed. The antioxidant status of plasma was increased by consumption of AA- rich foods. HBO exposure slightly reduce the AA levels in both groups without affecting the plasma antioxidant capacity. The plasma hydroperoxide levels observed in control group after HBO exposure was reduced in the AA-diet group. Levels of endogenous DNA oxidation were similar between groups and no differences were observed at T1 and T2 time points. However, the lymphocytes isolated from dietary supplemented subjects were resistant to *ex-vivo* DNA oxidation showing an increased DNA repair capacity, compared to non supple- mented lymphocytes. Dietary intake of AA-rich foods provides dual protection against oxidative stress, enhancing antioxidant plasma levels and stimulating DNA repair.

Farnesol-mediated chemoprevention of DMBA/TPA-induced skin tumorigenesis: Involvement of inflammation, Ras-ERK pathway and apoptosis

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Naturally-derived farnesol has been reported for its chemopreventive and chemotherapeutic efficacy in variety of cancers. However, the mechanism of action of farnesol is still in question. In the present study, chemopreventive potential of farnesol on 9,10- dimethylbenz(a)anthracene (DMBA)-initiated and 12-O-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumorigenesis in Swiss albino mice has been investigated. Topical application of farnesol at the dose of 25 and 50 mg/kg body weight, significantly reduced TPA-induced skin edema, hyperplasia, expression of cyclooxygenase-2 (COX-2), oxidative stress response whereas at higher dose (100 mg/kg body weight) did not. Pretreatment of farnesol significantly decreased TPA-induced ornithine decarboxylase (ODC) activity and [^{3}H] thymidine incorporation in dose dependent manner. During promotion phase, farnesol with higher dose significantly regressed tumor incidence and tumor burden with an extension of latency period 4-8 weeks. More importantly, low doses of farnesol significantly inhibited Ras/Raf/ERK1/2 signaling pathway in mouse skin tumors whereas higher dose of farnesol induced the pathway. Moreover, farnesol at all doses altered Bax/Bcl-2 ratio which leads to induction of apoptosis confirmed by DNA fragmentation. Taken altogether, these results imply a crucial role of oxidative stress, inflammation, Ras/Raf/ERK1/2 pathway and apoptosis in the chemopreventive activity of farnesol.

Key words: Farnesol, Oxidative stress, Inflammation, Ras pathway, Apoptosis, Skin tumorigenesis

Xanthohumol, a chemopreventive chalcone from hops (*Humulus lupulus* L.), generates superoxide anion radicals *via* a mitochondria-mediated mechanism

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Xanthohumol (XN) exerts a broad spectrum of potential cancer chemopreventive activities, including the induction of apoptosis (Gerhauser *et al*., Mol. Cancer Ther. 2002).

The aim of the present study was to investigate whether XN may exert pro-oxidant effects resulting in apoptosis induction. We used 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA) to monitor formation of reactive oxygen species (ROS) in benign prostate cancer cells (BPH-1). We detected an immediate XN-mediated dose- and time-dependent increase in fluorescence, indicative of ROS production, with a maximum at 12.5 µM concentration. ROS detection was not due to artificial H_2O_2 generation, as described previously for selected polyphenols. Rather, after treatment with XN up to 25 µM, we monitored a dose-dependent intracellular increase of superoxide anion radicals (O_2^{-}*) by dihydroethidium oxidation. Fluorescence microscopy images of BPH-1 cells stained with MitoSOX Red specific for mitochondrial O_2^{-}* suggested a mitochodrial origin of O_2^{-}* formation. This was confirmed by XN-mediated induction of O_2^{-}* in isolated mitochondria. Furthermore, in rho-zero BPH-1 cells harboring non-functional mitochondria, XN treatment did not induce O_2^{-}* formation. Detection of O_2^{-}* in BPH-1 cells was significantly reduced by co-treatment with ascorbic acid, N-acetyl cysteine and the superoxide dismutase mimetic MnTMPyP. Also, co-treatment of BPH-1 cells with MnTMPyP significantly prevented XN-mediated anti-proliferative activity and induction of apoptosis, monitored by flow cytometry and poly(ADP-ribose)polymerase cleavage. These data suggest that apoptosis induction by XN is a functional consequence of O_2^{-}* production.

Anticancer activity of dendrimers - *in vitro* studies

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Dendrimers are highly ordered, regularly branched, globular macromolecules that possess unique properties. These synthetic polymer-based nanoparticles are widely investigated as promising therapeutics. However, there is still a gap in understanding of mechanisms of dendrimer action *in vitro*. Therefore, it is necessary to undertake studies to determine biological properties of these compounds. The aim of the present study was to compare the effects of poly(amidoamine) (PAMAM) carboxy-terminated dendrimer and two types of poly(propyleneimine) (PPI) dendrimers (amino-terminated PPI and PPI with maltotriose units attached to the surface) on cultured human ovarian cancer SK-OV-3 cells. The cells were exposed to six different concentrations of dendrimers (1, 10, 40, 80, 150 and 300 μM) for 24 h. The cytotoxicity of dendrimers was studied by MTT assay immediately after the incubation with dendrimers or 24 hours after removing the dendrimer from the medium. The morphological features of apoptosis were examined by a confocal laser scanning microscope (CLSM). Orange acridine (OA)/ ethidium bromide (EB) staining technique was employed to determine whether the induction of apoptotic and necrotic cell death took place upon dendrimers. The level of reactive oxygen species (ROS) was estimated by DCFH-DA assay. Mitochondrial membrane potential changes were monitored using a fluorescent probe JC-1. In summary, the present data provide the evidence that amino-terminated PPI demonstrate higher anticarcinogenic and cytotoxic activity when compared to PPI dendrimers modified by attaching maltotriose to the surface and PAMAM dendrimers. These findings will lead to better understanding of biological properties of dendrimer and will open the possibility to use them in medical applications.

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Flavonoids quercetin and epigallocatechin modify fluidity of plasma membrane of breast cancer cells treated with paclitaxel

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Quercetin (Q) and epigallocatechin (Ep) are naturally occurring flavonoids with well documented antioxidant properties. In certain conditions and cell types they have been shown to display also prooxidant and anticancer properties and thus could be potentially useful as low toxic antioxidants in adjunctive therapy with anticancer drugs. Using fluorescence spectroscopy technique we analyzed the effect of these flavonoids on fluidity of the plasma membrane of MCF-7 breast cancer cells treated with anticancer drug paclitaxel (PTX). TMA-DPH fluorescent probe was selected to monitor fluidity in the polar head-group region of the lipid bilayer.

We demonstrated that low concentrations of PTX (0.05-0.1 μM) increased membrane fluidity, while higher drug concentrations (0.3-20 μM) caused its progressive decrease. Quercetin showed dual action: increased membrane fluidity at lower concentration (10 μM) but caused its significant decrease at higher concentrations (50-1000 μM). Flavonoid disturbed the structure of the plasma membrane to a considerably higher degree than PTX. Low concentrations of quercetin (bellow100 μM) in combination with PTX caused significant membrane fluidization while flavonoids concentrations higher than 100 μM led to membrane rigidifications. Epigallocatechin used alone showed opposite effect to this of quercetin and significantly decreased membrane fluidity. Employed in combination with paclitaxel epigallocatechin showed profound effect on the plasma membrane structure and reduced its fluidity by 30-50% .

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Pyrroline and pyrrolidine nitroxides increased oxidative stress induced by paclitaxel in breast cancer cells

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Nitroxides Pirolin (2,2,5,5-tetramethyl-3-carbamoyl pyrroline-1-oxyl, PL) and Pirolid (2,2,5,5-tetramethyl-3-carbamoylpyrrolidine-1-oxyl, PD) are cell-permeable, stable radical antioxidants with broad spectrum of activity. In light of our previous SAR (*structure activity relationship*) studies on nitroxides acting as less toxic anticancer agents and antioxidants, we aimed at investigating their effect on anticancer activity of paclitaxel (PTX) with an emphasis on ROS generation. Paclitaxel is a clinically effective antineoplastic agent against a variety of tumors, especially drug-refractory ovarian and metastatic breast cancers. The exact mechanism of PTX cytotoxicity against tumor cells is still under extensive study. It is commonly accepted that drug interacts with microtubules and induces apoptosis in various tumor cells but ROS involvement in PTX-induced cell death has been shown as well.

Using 2′, 7′- dichlorofluorescin diacetate (DCFH-DA) as a probe for intracellular peroxide formation we have found significant enhancement of H_2O_2 production by investigated nitroxides independently of their single or adjunctive employ with PTX in MCF-7 breast cancer cells. Cells were pretreated with 50 μM PL or PD for 1 h before their incubation with IC_{50} concentration of PTX (0.4 μM) for 2h in presence of nitroxides. Intracellular level of ROS (H_2O_2) was monitored directly in cell monolayer over 0-180 min time period. We observed about 3-fold (PL) and 4-fold (PD) higher amount of ROS generated in cells by nitroxides than by PTX. Moreover, nitroxides caused considerable enhancement of oxidative stress induced by PTX in cancer cells.

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Two-step mechanism of induction of the gene expression of a prototypic cancer-protective enzyme by diphenols

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Cancer-preventive activity by exogenous molecules can be mediated by enhancing the expression of cytoprotective enzymes [e.g, glutathione-*S*-transferase (GST) or 1 (NAD(P)H-quinone oxidoreductase NQO1)] *via* antioxidant-response elements (AREs) present in the promoter regions of their genes. Previously, potency of induction of NQO1 has been linearly correlated with the ability to release an electron from different classes of inducers, including diphenols, phenylpropenoids and flavonoids.

In the present work, we focus on the induction of NQO1 by diphenols, which we consider as a model underlying the mechanisms of action of other phenolic inducers such as phenylpropenoids and flavonoids. A two-step mechanism of NQO1 activation is proposed involving (i) oxidation of diphenol inducers to their quinone derivatives and (ii) oxidation of two highly reactive thiol groups, by these quinones, of a protein involved in NQO1 induction. These two putative routes are supported by linear correlations between the inducer potencies and the redox properties of diphenols and of their corresponding quinones. The linear correlations demonstrate the possibility to predict the enhanced gene expression of enzymatic defenses by diphenols, from quantum mechanical calculations (i) of the ability of diphenols to release electrons, and (ii) of the electron affinity of their corresponding quinones.

Changes of blood platelets apoptotic markers induced by hydrogen peroxide

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Reactive oxygen species (ROS) are formed in all aerobic organisms by enzymatic and nonenzymatic reactions and are responsible for changes of various types of biological molecules. In blood platelets ROS may modulate the signal transduction and affect platelet different metabolic pathways. Blood platelets, like other circulating cells are able to produce ROS, which induce changes in intraplatelet Ca^{2+} concentration and may behave as a second messengers in thrombin- or collagen-activated platelets. ROS can also promote programmed cell death in various types of nucleated cells. Platelets are anucleated cells, that upon activation by agonists or during storage may develop apoptotic events. However, the role of ROS in platelet apoptosis remain unclear. The aim of present study was to determine the effects of hydrogen peroxide (H_2O_2) on apoptotic processes in human platelets *in vitro*. Apoptotic events in gel-filtrated platelets after treatment with H_2O_2 at the concentrations of 0.01, 0.1, 0.2 and 1.0 mM were studied. Apoptotic markers such as exposure of phosphatidylserine (PS), platelet mitochondrial membrane potential, activation of caspase-3 and platelet microparticle(PMP) formation in gel-filtrated platelets (3 x 10^7 plt/ml) were measured by flow cytometry. In platelets treated with different concentrations of H_2O_2 (0.01, 0.1, 0.2, 1.0 mM) a distinct increase of PS exposure and PMP formation were observed. Moreover, platelet incubation with H_2O_2 affected mitochondrial membrane potential and induced activation of caspase-3. The obtained results indicate that H_2O_2 responsible for oxidative stress may promote *in vitro* apoptotic events in human platelets.

Effect of epicatechin on blood platelets apoptotic markers induced by peroxynitrite

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Peroxynitrite ($ONOO^-$) is a potent and a relatively long-lived cytotoxic oxidant formed *in vivo* in the reaction between superoxide anion and nitric oxide. In blood platelets, due to their ability to production of different reactive oxygen species, $ONOO^-$ may also be formed. Our previous studies indicated, that $ONOO^-$ is not only responsible for changes of platelets function, but also induce apoptotic events in anucleated platelets. The role of antioxidants in the defense against $ONOO^-$ action on platelets is still unknown. The aim of our study was to assess if epicatechin may protect platelet proteins against nitrative/oxidative damage induced by $ONOO^-$ and establish its effects on the level of apoptotic markers *in vitro*. The monitoring of platelets apoptotic events as well in control platelets as in platelets preincubated with epicatechin, was carried out in suspension of gel-filtrated platelets (3×10^7 plt/ml). Exposure of phosphatidylserine (PS), platelet mitochondrial membrane potential ($\Delta\Psi m$), activation of caspase-3 and platelet microparticle (PMP) formation in platelets were measured by flow cytometry. Detection of nitrotyrosine-containing proteins was performed by a competition ELISA method. Preincubation of platelets with 5 and 25 mM epicatechin resulted in a distinct decrease of PS exposure, reduction of PMP formation, caspase-3 activation and $\Delta\Psi m$ depolarization after treatment with $ONOO^-$ (0.01 – 1.0 mM). Furthermore, platelets preincubation with epicatechin significantly reduced formation of nitrotyrosine in platelets proteins induced by $ONOO^-$. The obtained results indicate that epicatechin may protect platelets proteins against nitration caused by $ONOO^-$ and its derivatives and decreases apoptotic events in blood

Apoptotic effects of genistein and genistein-8-C-glucoside from *lupinus luteus* on human ovarian cancer cells

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The flavonoids comprise a class of natural phytochemicals that possess many biological properties offering possible new strategies for cancer chemotherapy. The isoflavones are a flavonoids subclass that includes genistein, which is the predominant isoflavone of legumes such as soybeans and is commonly found in a variety of human foods. Like most flavonoids, most genistein exists in nature in its glycoside form, rather than in its aglycone form. In the present study, we investigated anticancer activity of genistein and its natural glucoside, genistein-8-C-glucoside (G8CG) isolated from flowers of *Lupinus luteus*. We examined the effect of two isoflavones alone or in combination on cultured human SK-OV-3 ovarian carcinoma cells. The cells were exposed to genistein and G8CG at various concentrations (ranging from 1 to 90 µM) for 24h and 48h. The cytotoxicity was determined by measuring intracellular esterase activity using Calcein-AM assay. The apoptosis was detected by assessing caspase-3 and -9 activity. The morphological features of SK-OV-3 cells were examined by a confocal laser scanning microscope (CLSM). In summary, the present data provide the first evidence *in vitro* that genistein-8-C-glucoside and combination genistein-G8CG could be a potential candidate for ovarian cancer therapy.
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Button battery induced cell damage: A pathophysiological study

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Button batteries (BB) are frequently swallowed and must be removed immediately as, if they remain in the esophagus, they can cause severe damage and sometimes fatal prognosis. To better understand the molecular mechanism of esophagus damage, we performed a study aimed to analyze heat production, pH variation, electrical work and metal release from BB incubated in cell culture media. Cytotoxicity and apoptosis induced in a human monocytes cell line (U937) was also investigated. Calculated transferred heat was around 244 J with a maximal increase of temperature of 7.26 K/g medium whose pH, reflecting medium electrolysis, was 12.2 after about 2 h incubation. Release of metals (Cr, Fe, Ni, Mn and Li) also occurred. Only 49.7 $\pm$ 1.9% of U937 monocytes remained viable after 90 min of incubation in DMEM containing BB. No apoptosis was evidenced. Those results suggest that BB discharge induce cytotoxicity and tissue injury probably due to multiple combinations of, at least, thermal and caustic phenomena as well as metal toxicity. As maximal effects occurred in less than 2 h, our results prompt for fast removal of battery from esophagus.

Chevin JC, Attik G, Dika H, Duval RE, Bottin MC, Raspiller MF, Lanhers MC, Saka-Amini MA, Marcocci L, Rihn BH. Button battery induced cell damage: A pathophysiological study. Electrochemistry communications. 2008;10(11):1756-60. DOI: 10.1016/j.elecom.2008.09.002

Cytotoxicity and apoptosis in rat alveolar macrophages exposed to various colloidal nanoparticles

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Over the last decade, there has been huge interest in the use of polymeric nanoparticles as oral drug delivery system for low molecular weight heparin. In this work, we analyzed both cytotoxicity and apoptosis in NR8383 cells after exposure of various heparin containing nanoparticles prepared with pharmaceutical polymers either by nanoprecipitation or double emulsion.

Cell viability ranged from 94 % to 6 % depending on dose, size and zeta potential of tested particles. Indeed, when endpoint was viability measured by trypan blue exclusion, particles diameter was a good marker of cytotoxicity, as the particles with shorter diameter were the most toxic. When endpoint was mitochondrial metabolism assayed with MTT, *zeta* potential seemed to be a valuable marker of cytotoxicity, as the higher was *zeta* potential, the lower was cell viability.

Apoptosis was assessed by detection of nuclear DNA fragmentation after 6, 24 and 48 h of incubation with nanoparticles. No nuclear fragmentation was evidenced with 400 µg/mL after 6, 24 and 48 h, whatever the tested nanoparticles. However *in situ* labelling in NR8383 cells with Valine-Alanin-Aspartate-FluoroMethylKetone caspase inhibitor and DNA-binding dye, that assayed respectively active caspase and nucleus integrity, evidenced activated caspase within the cytoplasm following a 24-h incubation period with 400 µg/mL nanoparticles. Whether apoptosis was due to independent caspase activation or mitochondrial dysfunction should be further assayed.

Our study showed that interaction of nanoparticles and macrophages should be assayed using various endpoints of, at least, cytotoxicity and apoptosis, and exact balance and role of both of them should be checked when nanoparticles are tested in vitro.

Anti-inflammatory and antioxidant PUFA-rich marine oils : Beneficial effects on the cornea

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Purpose: The cornea is susceptible to attack by reactive oxygen species (ROS), which may be generated through direct attack by UV light or indirectly through formation of toxic compounds or inflammation. The aim of this work was to study the modulation of oxidative stress and inflammation in cornea using polyunsaturated fatty acids (PUFA)-rich marine oils. **Methods:** First, rats were fed with different PUFA-rich oils (prepared by Yslab, Quimper, France) by gavage during 28 days: microalgae DHA, fish oil rich in EPA and DHA (10% and 50% of the fatty acid profile) and fish oil rich in EPA (60% of the fatty acid profile). Fatty acids composition of corneal cell membranes was analysed by chromatography. Second, human corneal epithelial cells (HCE-T cell line) were exposed to different PUFA-rich marine oils (EPA and DHA) for 15 minutes, followed by a 24-hour recovery period. Oxidative stress or inflammation was chemically induced with tert-butylhydroperoxide (tBHP), sodium lauryl sulfate or IL-1β. Cell viability and reactive oxygen species were quantified with neutral red and DCFH-DA using cytofluorometry. Inflammation was evaluated through IL-6 and IL-8 production using ELISA kits. **Results:** Dietary oils changed the fatty acids composition of corneal cell membranes, indicating that *per os* administered fatty acids are able to reach the cornea. *In vitro* incubation of corneal cells with marine oils rich in EPA and DHA resulted in a decrease in tBHP-induced ROS overproduction and inflammation whereas fatty acids alone did not show any antioxidant effects. **Conclusions:** The marine oils (prepared by Yslab, Quimper, France) we tested contain omega-3 fatty acids in appropriate proportions to exert beneficial properties on corneal cells. Nutritional supplements rich in marine DHA and EPA could be prescribed as ocular treatment: they can target the cornea and prevent ocular surface pathologies implicating oxidative stress and inflammation, such as ocular dryness.

High molecular weight hyaluronan decreases UVB-induced apoptosis and inflammation

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Purpose: The aim of this study was to investigate high molecular weight hyaluronan (HMW-HA) protection on human corneal epithelial (HCE) cells against ultraviolet B (UVB) radiations-induced toxic effects.

Methods: Human corneal epithelial (HCE) cell line was incubated with HMW-HA or phosphate-buffered salt solution (PBS), rinsed, and exposed to UVB radiations. Cell viability, reactive oxygen species (ROS) and glutathione (GSH) levels, 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) release, p53 phosphorylation, caspase-3, -8, -9 activation, IL-6 and IL-8 production were assessed to evaluate and to compare UVB-induced toxicity between cells treated with HMW-HA and cells treated with PBS.

Results: Data indicate that HMW-HA had significant protective effects against UVB radiations. HMW-HA increased HCE cell viability, decreased IL-6 and IL-8 production, decreased caspase-3 and -8 activation. However, HMW-HA had no significant effect on ROS and GSH levels, 8-oxo-dG release and p53 phosphorylation.

Conclusions: To our knowledge, we report for the first time the ability of HMW-HA to protect cells against UV-irradiation. According to our results, HMW-HA provides anti-inflammatory and anti-apoptotic signals to cells exposed to UVB.

Tropical Calophyllum nuts oil induces Melanoma cell proliferation modulation With oxidative stress, apoptosis, and P2X7 cell death receptor activation

Kamélia Ghazi, Melody Dutot, Toihiri Said, Jean-Michel Warnet, Patrice Rat

INTRODUCTION: Calophyllum Inophyllum oil is a vegetable oil used in tropical medicine. It is composed of 71% unsaturated fatty acids (especially oleic and linoleic acids) and is also rich in phenylcoumarins, which are known to be anti-tumor substances. Cancer is the second leading cause of death after cardiovascular diseases. Melanoma cancer that is generally resistant to treatment (radio and / or chemotherapy) is increasing.
The objective of this study is to investigate the cellular proliferation modulation of melanoma cells incubated with Calophyllum oil and to better understand the cell death mechanisms induced by this oil on melanoma cells (B16-F0).

METHODS: The B16-F0 cells (mouse melanoma cell line) were incubated for 15 minutes with Calophyllum inophyllum oil. We evaluated the ability of the oil to induce intracellular redox potential alteration (Alamar blue test), mitochondrial injury (NonylAcridine Orange and JC-1 tests), reactive oxygen species overproduction (DCFH DA test), caspase-dependent apoptosis (Rhodamine 110 - IETD test) and P2X7 cell death receptor activation (YO-PRO-1 test). Tests were performed using cytofluorometry adapted to microplates and fluorescence microscopy. Benzalkonium chloride 0.05% was used as a positive control of apoptosis (P2X7 cell death receptor inducer).

RESULTS: Calophyllum inophyllum oil from 5 to 100% induced a sharp decrease in intracellular redox potential with induction of oxidative stress and disruption of mitochondrial activity. At this concentration, we observed caspase 8 and P2X7 cell death receptors activation, occurring during apoptosis.

DISCUSSION: Our study showed that Calophyllum inophyllum oil induced cytotoxic effects with oxidative stress and apoptosis stimulation. The activation of P2X7 receptors could be a new therapeutic strategy to modulate the proliferation of melanoma cells. Calophyllum oil could be proposed as a topical complementary therapeutic for skin cancer treatment.

Importance of the spectrofluorimetric technique in the confirmation of the diagnosis of glycogen storage disease type III

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Type III glycogen storage disease (GSD) is the phenotypic expression of a rare hereditary disease due to the specific enzyme deficiency of glycogen metabolism. This anomaly manifests with few symptoms other than hepatosplenomegaly but this is not sufficient to specify the type of the GSD. The purpose of this study was the determination of amylo-1,6-glucosidase activity on leucocytes by the spectrofluorimetric technique in order to confirm the diagnosis of the type III GSD and to distinguish this pathology from all other types, the healthy and the heterozygote.

Eighteen patients are described with hepatic glycogenosis (12 with GSD III, 4 with GSD I and 2 with GSD VI). The glycogen content and the activity of amylo-1,6-glucosidase (measured by the spectrofluorimetric technique) were determined in the leucocytes of all patients.
The glycogen content was elevated in the leucocytes of all patients with type III GSD (430 – 1839 µg/g d'Hb). As for the amylo-1,6-glucosidase, was nearly inactive (healthy volunteer 0.81 ± 0.41 nmol Glc/min/mg P). On the other hand, this activity was normal for type I GSD (1.99 ± 0.17 nmol Glc/min/ mg P) and for type VI GSD (2.43 ± 0.02 nmol Glc/min/ mg P). The mean enzymic activity of the parents was intermediary between the normal value (0.4 – 1.22 nmol Glc/min/mg P) and that of their affected children.

The determination of the enzymatic activity in leucocytes of the amylo-1,6-glucosidase has been proposed like being the only diagnosis of certainty that can be achieved in the majority of the clinical biology laboratories. The obtained results – via a blood sampling on leucocytes – confirm type III GSD. Hence, we needn't any hepatic biopsy nor an enzymatic dosage in the liver or in the muscles, which are too painful to the child and refused by the parents.
During this study, the spectrofluorimetric technique represents an considerable discriminatory capacity for the detection of the disease : it distinguishes the type III GSD from all other types, the healthy and the heterozygote.

Lifestyle factors and ageing generate oxidative damage to DNA

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Oxidative stress is a cellular or physiological status of elevated concentrations of reactive oxygen species (ROS) that cause molecular damage to vital structures and functions of DNA. A growing number of evidence points toward ROS as one of the primary determinants of ageing. Numerous lifestyle factors may influence the susceptibility to oxidative stress by modifying the antioxidant status and the free oxygen radical generation. The amount of 8-oxo-2'-deoxyguanosine (8-oxodG) excreted in urine can be used as a biomarker of oxidative DNA damage.
The purpose of the study was to evaluate the oxidative stress including ageing and lifestyle factors that may influence oxidative DNA damage, such as smoking and abnormal body mass index (BMI). The urinary concentration of 8-oxodG from 242 subjects aged 18-75 were determined with HPLC and electrochemical detection.

The mean level of 8-oxodG was 5.9 µg/g creatinine. The 8-oxodG levels were significantly higher in smokers compared to non-smokers. Further, a low BMI (<20) and a high BMI (>30) were associated with an increased 8-oxodG excretion. Especially within the group of young lean smoker females, the 8-oxodG excretion was significantly higher compared to the other groups. The level of urinary 8-oxodG correlated significantly with the age of the examined subjects. Interestingly, in all age groups, the subgroup with rheumatoid arthritis showed higher concentrations of 8-oxodG compared to healthy subjects.

On the basis of the presented results we suggest that lifestyle factors as smoking and abnormal BMI values influence the formation of oxidatively generated DNA damage. Furthermore, ageing is obviously an important factor for oxidative DNA damage.

Antioxidants, trace elements and metabolic syndrome in elderly subjects in Croatia

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Metabolic syndrome (MetS) parameters were evaluated in relation to the antioxidant and trace element status in mobile elderly population, predominantly from rural areas in Croatia. The study group consisted of 100 subjects, aged 71-88 years: 20 women and 17 men from coastal and 38 women and 25 men from continental region of Croatia. Anthropometric measurements and biochemical analyses of fasting blood samples were performed by standardised methods. MetS was diagnosed according to the International Diabetic Federation (IDF) criteria. The following parameters were determined in all subjects: concentration of glucose, triglycerides, lipoproteins and C-reactive protein (CRP) in plasma, selenium (SSe), zinc (SZn) and copper (SCu) in serum, activity of glutathione peroxidase (GPx) in blood, and superoxide dismutase (SOD) and catalase (CAT) in erythrocytes. Significantly higher GPx was found in 64 subjects with MetS compared to 36 subjects without MetS. Although there was no significant difference in SOD, CAT, SSe, SZn and SCu between the groups regarding the MetS, the lowest SSe and SZn, and the highest CAT and SOD were found in obese subjects with MetS. The results of Spearman correlation between each of the variables measured in the all 100 subjects showed significant positive association between SCu and CRP, SCu and total- and LDL-cholesterol, and between GPx and triglycerides. These findings contribute to a better understanding of the relationships between MetS, oxidative stress and the inflammatory process.

The effect of n-3/n-6 PUFA ratio on the postprandial transformation of LDL phenotype and Ischemia Modified Albumin (IMA) in patients with metabolic syndrome. The LIPGENE study.

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Background and aims: Atherogenic sd-LDL phenotype B is connected with obesity, insulin resistance, and oxidative stress (OS). Postprandial lipemia and associating OS is the risk of cardiovascular disease. The aim of the study was to evaluate the long term and short term post-dietary effect of the plasma n-3/n-6 PUFA ratio on the LDL phenotype transformation, and plasma Ischemia Modified Albumin (IMA) in patients with metabolic syndrome (MetS). Research Design and Methods. Work was done within the EU F6 LIPGENE study. Ninety nine MetS patients were randomized to the one of the following dietary regimes: Diet A(n=24) High-fat (38% energy) SFA-rich diet (16% SFA, 12 % MUFA 6% PUFA), Diet B (n=25) High-fat (38% energy), MUFA-rich diet (8% SFA, 20% MUFA, 6% PUFA), Diet C (n =26) Isocaloric low-fat (28% energy), high-complex carbohydrate diet (8% SFA, 11%; MUFA; 6% PUFA), with 1.24g/d high oleic sunflower oil supplement., Diet D (n =24) Isocaloric low-fat (28% energy), high-complex carbohydrate diet (8% SFA, 11% MUFA; 6% PUFA), with 1.24 g/d LC n-3 PUFA supplement (EPA, DHA). Pre- and post- 12 weeks dietary intervention, patients completed an oral lipid tolerance test (OLTT) with the same fat composition as consumed on the assigned dietary period.

Results: Post-dietary change of LDL density was the dominant response in all groups. Long term administration of the diet B and D diminished plasma triglyceride and cholesterol level and the density of the main LDL subfraction. Fat modification caused by the LF n-3 PUFA (D) as well as MUFA-rich diet (B), resulted in favorable transformation of LDL phenotype from B to A. All type of functional oral tests (OLTT) primarily transiently increased plasma triglyceride, what was followed by the increase in LDL density, IMA and TBARS level, concomitantly associated with the transient decrease in plasma total cholesterol, LDL and HDL cholesterol. The long term effect of diet on postprandial response documented that diet C increased postprandial plasma total cholesterol, TG and TBARS response (ΔiAUC), as well as delayed TG clearance, reflected by increased value Δ0h8h of TG, TC and LDL-C. No such adverse effect of diet D were observed. Diet D decreased significantly ΔiAUC LDL-cholesterol.

Conclusion. The study demonstrate the efficacy of dietary n-3 PUFA in changing the pro-atherogenic LDL phenotype to less atherogenic in patients with metabolic syndrome. EPA and DHA supplementation even in combination with the high carbohydrate diet normalize plasma lipid level, lipoprotein subclass pattern and moderate the oxidative stress accompanying postprandial state.

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Saturated fatty acid-induced insulin resistance in skeletal muscle cells is associated with mitochondrial dysfunction and increased ROS production

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Increased plasma levels of free fatty acids (FFA) occur in states of insulin resistance such as obesity and type 2 *diabetes mellitus*. These high levels of plasma FFA are proposed to play an important role for the development of insulin resistance but the mechanisms involved are still unclear. This study investigated the effects of saturated and unsaturated FFA on insulin sensitivity in parallel with mitochondrial function and reactive oxygen specie (ROS) production. C2C12 myotubes were treated for 24 h with 0.1 mM of saturated (palmitic and stearic) or unsaturated (oleic, linoleic, eicosapentaenoic, and docosahexaenoic) FFA. After this period, basal and insulin-stimulated glucose metabolism, mitochondrial function and ROS production were evaluated. Saturated palmitic and stearic acids decreased insulin-induced glycogen synthesis (22% and 18%, respectively; $p<0.01$), glucose oxidation (36% and 34%, respectively; $p<0.001$), and lactate production (32% and 25%, respectively, $p<0.001$). Basal glucose oxidation was also reduced (44% and 30%, respectively; $p<0.01$). Palmitic and stearic acids impaired mitochondrial function as demonstrated by decrease of both mitochondrial hyperpolarization (62% and 48%, respectively; $p<0.001$) and ATP generation (42% and 22%, respectively; $p<0.01$). These fatty acids also increased ROS production. As opposed to saturated FFA, unsaturated FFA did not impair glucose metabolism and mitochondrial function. Primary cultures of rat skeletal muscle cells exhibited similar responses to saturated FFA as compared to C2C12 cells. These results suggest that the impaired mitochondrial function and increased ROS production induced by saturated palmitic and stearic acids could be an early event in the development of insulin resistance in skeletal muscle cells. Financial support: FAPESP, CNPq, and CAPES.

Neuroprotective effects of antioxidants on rat diabetic retina

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Background/aims: Diabetic retinopathy is the major cause of adult blindness in developed countries. However, it is still not known the pathogenic link between hyperglycaemia and the establishment of diabetic complications. It has been repeatedly suggested that oxidative stress may play a role in the process and in this sense, antioxidants can be useful as a supportive therapy in diabetes. We have studied lipid peroxidation on diabetic rat retina and the neuroprotective effects of the antioxidant lutein and docosahexaenoic acid (DHA).

Methods: Male Wistar rats were made diabetic with streptozotocin. They were studied over three months after onset of diabetes. Immediately before death electroretinograms were recorded. One retina was fixed, cryoprotected and cryosectioned for detection of apoptotic cells by TUNEL analysis and activated caspase-3 and the other retina was homogenized in prechilled 0.2 M potassium phosphate buffer. This homogenate was used to assay MDA, GSH content and GPx activity.

Results: After 3 months of diabetes, antioxidant administration prevented not only the alterations of oxidative stress markers (tissue GSH and malondialdehyde (MDA) concentrations, and GPx activity) but also the impairment of retinal function (as assessed by the modifications in electroretinogram latency time and b wave amplitude). There was a decrease in the thickness of the whole retina and the inner and outer nuclear layers in the rats that have been diabetic for 3 months. These thickness modifications were not observed in diabetic animals treated with lutein or DHA. The number of TUNEL-positive nuclei and caspase-3 positive cells increased significantly in retina after 3 months of diabetes and both antioxidants normalized these numbers.

Conclusions: The data suggest that lutein and DHA are useful in diabetic protecting retina though further studies are needed to understand the exact mechanism of this beneficial action.

Chronic treatment with tocotrienol, an isoform of vitamin E, prevents intracerebroventricular streptozotocin-induced cognitive impairment and oxidative-nitrosative stress in rats

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Introduction

Oxidative stress has been implicated in neurodegenerative disorders including the Alzheimer's disease (AD). Intracerebroventricular (ICV) streptozotocin (STZ) has been shown to cause cognitive impairment, which is associated with increased oxidative stress in the brain of rats. Vitamin E is a potent free radical scavenger and antioxidant. In the present study, we investigated the effect of both the isoforms of vitamin E, α-tocopherol (100mg/kg p.o) and tocotrienol (50 and 100 mg/kg p.o) against ICV STZ-induced cognitive impairment and oxidative-nitrosative stress in rats.

Materials and methods

Adult male Wistar rats were injected with ICV STZ (3 mg/kg) bilaterally on days 1 and 3. α-Tocopherol and tocotrienol were administered chronically to rats for 21 days starting from day 1 of STZ injection. The learning and memory behavior was assessed using morris water maze and elevated plus maze on days 15, 16, 17, 18, 19 and 20. The rats were sacrificed on day 21 and whole brain homogenate was used for estimation of parameters of oxidative stress i.e. malondialdehyde, reduced glutathione, superoxide dismutase and catalase, nitrite levels and acetylcholinesterase (AChE) activity.

Results

α- Tocopherol as well as tocotrienol showed significantly less cognitive impairment in both the behavioral paradigms but the effect was more potent with tocotrienol. Both isoforms of vitamin E effectively attenuated the reduction in glutathione and catalase and reduced the malonaldehyde, nitrite as well as cholinesterase activity in the brains of i.c.v STZ rats in a dose dependent manner.

Conclusion

The study demonstrates the effectiveness of vitamin E isoforms, of which tocotrienol being more potent in preventing the cognitive deficits as well as the oxidative and nitrosative stress caused by ICV STZ in rats and suggests its potential in the treatment of neurodegenerative diseases such as Alzheimer's disease.

In Vivo Hypoglycemic Activity of *Pinus brutia* Extracts

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One of the most important factors in the diabetes mellitus is the oxidative stress which is significantly increased in both type I and II. High glucose levels induce glucosyliosis of cellular components such as proteins and DNA which can be further oxidized by different reactive oxygen species. Oxidative stress molecules as isoprostanes, lipid peroxides and 8-hydroxydeoxyguanosine are reported to be increased while respectively cell antioxidants such as vitamin C and glutathione decrease. In the literature few reports relate diabetes and oxidative stress - antioxidants.
Pinus brutia needles and bark extracts, which are rich in phenolic antioxidants were studied for their possible hypoglycemic activity. The study was realized in normal and diabetic male and female hairless mice, type SKH1. Diabetes type I, was experimentally induced after intravenous injection of streptozotocin (200 mg/kg). The extracts were administrated intraperitoneally (mean of 0,2mg per mouse). The glucose levels were measured with electrochemical element.
It is observed a remarkable and significant decrease of glucose levels in both normal and diabetic mice. The study needs further investigation especially concerning the mechanism of action of the extracts.

Tobacco smoke and UV light damage on murine skin: Protection by Pycnogenol

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UV light has been found to interact synergistically with cigarette smoke (CS) in inducing molecular and cytogenetical alterations in the respiratory tract of mice, while significant alterations in the skin have been observed after of short-term exposure. Data described in literature clearly shows that not only UV light but also CS affects the condition of the skin. UV light and CS are potent oxidative stressors. Pycnogenol is a polyphenol-rich French maritime pine bark extract, with important antioxidant capacity. The long-term skin exposure to CS on the skin of hairless mice, in combination or not to UV light and its possible protection by Pycnogenol, was investigated.

In vivo, male and female hairless SKH-2 mice were exposed for ten months to tobacco smoke and/or UV-light, protected or not by pycnogenol extract, which was administrated as an oral supplementation (0,2gr/lit) instead of tap water. Total oxidative stress on skin biopsies was estimated by fluorescence, after six months of exposure. A biophysical parameter, the development of transepidermal water loss, was being measured during 12 months, while for the same period the mortality and carcinogenicity were also evaluated.

The results showed that UV and CS may be acting synergistically, as estimated by the enhanced oxidative stress, transepidermal water loss, increased mortality, Squamous Cell Carcinomas (SCC) observed. The oral consumption of pycnogenol showed in vitro that the pine bark extract is a significant scavenger of reactive oxygen species, leading in vivo to the reduction of the number of carcinomas and the prolongation of the period in which carcinomas appear.

Oxidative Stress and Gestational Diabetes

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The main objective of this research work was to evaluate if there was oxidative damage and the level of that damage in gestational diabetes.

In the present study thirty-seven healthy women and thirty-seven women with gestational diabetes were analyzed in the three trimesters of pregnancy regarding their levels of oxidative stress. These women were diagnosed with diabetes in the second semester of pregnancy

None of the women had an acute disease or clinical complication during the pregnancy. All of them received the same polivitaminic treatment and the same diet. There were no significant differences in maternal age, parity and sex and body weight of the newborns.

The glucemic control which was measured by glycosylated hemoglobin was similar for both groups of women in the first trimester, going up in the gestational diabetic group in the second and third trimester with significant differences among them
($p < 0.001$).

Regarding the mean values of MDA the group of women with gestational diabetes had all the values with higher levels and with significant differences in the first and in the second trimester ($p < 0.019$ and $p < 0.028$ respectively). There were no significant differences within each of the groups during the gestational period.

The mean values of GPx for the diabetic women were always lower with significant differences in the first trimester ($p< 0.001$). In the group of gestational diabetes there was significant differences between the first and the other two following trimesters.

The correlation study through Pearson's chi-square test shows that in the group of women with gestational diabetes there is an inverse linear correlation between MDA and GPx in the second and third ($p<0,019$, $r = -0,494$, and $p<0,011$, $r = -0,600$ respectively).

In this observational and longitudinal study in pregnant women, the damage due to oxidative stress was present before the biochemical detection of the gestational diabetes, and its treatment (diet and exercise, without medication) lowered it. The GPx activity in women with gestational diabetes increases during the gestational period, possibly as an attempt of compensation at the high levels of oxidative stress.

Oxidative stress in pulmonology

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Introduction: EPR-imaging of metalloproteins ceruloplasmin and transferring with antioxidant system ceruloplasmin-transferrin characterize antioxidant answer of the organism to free radical oxidation initiation.

Methods: we defined EPR parameters dynamic of ceruloplasmin and transferring at patients with bronchial asthma, chronic obstructive lung disease(COLD) and pneumonia. 45 ambulance patients with pneumonia, 81 patients with bronchial asthma, 20 patients with COLD were examined. All patients were divided into groups according international standards severity of the disease. Blood serum of 120 healthy donors was used as control. EPR study included cerruloplasmin, transferring level detection and antioxidant system ceruloplasmin-transferrin.

Results: It was shown that at bronchial asthma and COLD patients with light and mild severity in case of actuation there was no significant changing of ceruloplasmin EPR-tests before and after standard treatment. Severe flowing of bronchial asthma and COLD is accompanied with significant ceruloplasmin signal decreasing and decreases even more during the process of treatment. Transferrin level was decreased in patients of all groups (on about 22-28% comparatively to norma) but especially at patients with severe flowing of the disease where after treatment level decreased on 28% out of initial. According to this situation antioxidant system ceruloplasmin-transferrin had the highest definition at patients with severe flowing. At patients with pneumonia it was shown increasing ceruloplasmin level, decreasing of transferring level and increasing of antioxidant system ceruloplasmin-transferrin data as 150% of norma before as 159% of norma after treatment these parameters did not change significantly.

Conclusions: Investigated data showed the possibility of predicting of disease treatment, differentiation of disease severity, necessarity of ceruloplasmin including in treatment of pulmonological patients in case of its initial low level or negative ceruloplasmin level dynamic treatment process. EPR-imaging is a very useful method in control on disease severity, treatment and prognosis in pulmonological clinical practice.

Fifty-one weeks' consumption of purified city-water decreases oxidative stress in senescence accelerated mice

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Redox potential, conductivity, pH, soluble oxygen, hydrogen and chlorine in Sakata city-water were 665 mV, 73.3 micro s/cm, 6.97, 12.87 mg/L, 0 mg/L and 0.04 mg/L, respectively. Those values in purified city-water were 250 mV, 16.8 micro s/cm, 6.72, 8.77 mg/L, 0.012 ppm, and 0 mg/L. The purified water has mild scavenging activities against hydroxyl and 1,1- diphenyl-2-picrylhydrazyl radicals compared with the city-water. For 51 weeks we orally administered as drinking water either purified or untreated city-water to either male senescence accelerated mice (SAMP10, a strain which has brain atrophy and deficits in learning and memory), or SAMR1 mice (which have a longer life span) as controls. The 8-hydroxy- 2'-deoxyguanosine (8-OHdG) concentration in blood plasma of SAMP10 mice was dramatically elevated compared to that of SAMR1. The level of carbonyl protein in brain of SAMP10 mice was higher than that of SAM 1. However, consumption of purified water lowered the increasd levels of 8-OHdG in blood plasma, carbonyl protein in brain and carbonyl protein in liver of SAMP10 mice. These effects were not observed in SAMR1. In addition, the purified water treatment lowered the ratio of methylguanidine to creatinine in the urine of SAMP10. Methyl guanidine is a marker of oxidative stress and kidney diseases. No effect of water treatment was found in LDL- or HDL-cholesterol, blood sugar or triglycerides in blood plasma of either strain of mice. These data give warning, especially in the case of aged people, concerning the direct consumption of city-water in daily life.

Oxidative stress markers in senescence accelerated and control mice (SAM); effect of purified or city water intake

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Senescence accelerated mice (SAMP10), which have brain atrophy and deficits in learning and memory, and SAMR1 having a longer life span was used as a control, compared to SAMP10. We orally administered to male SAM either purified water or Sakata City drinking water for 51 weeks. The values of redox potential, conductivity, soluble oxygen and released chlorine in the Sakata city water were much higher than those in purified city water. A small amount of soluble hydrogen , but no chlorine was found in the purified water. 8-Hydroxy-2'-deoxyguanosine(8-OHdG) concentration in blood plasma of SAMP10 dramatically elevated compared to that of SAMR1. The level of protein carbonyls in the brain of SAMP10 was higher than that of SAMR1. However, water purification treatment inhibited the increases in levels of 8-OHdG and protein carbonyls in SAMP10, and decreased their level in the liver of SAMP10. None of these effects were found in SAMR1. Methylguanidine is a marker of oxidative stress and kidney diseases. Purified water treatment lowered the ratio of methylguanidine to creatinine in the urine of SAMP10. No differences in effects were found in LDL or HDL cholesterol, blood sugar, or triglycerides in blood plasma of either mice. In conclusion, fifty-one weeks intake of city-water obtained by a purification system did not enhance production of oxidative stress markers in senescence accelerated mice as compared with Sakata water intake. The data suggest that direct intake of city-water in daily life may have adverse effects in the aging population.

Degradation of phospholipids by oxidative stress : exceptional significance of cardiolipin

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This study aims to elucidate the effect of oxidative stress on the phospholipids phosphatidylethanolamine (PE), and phosphatidylcholine (PC) as essential structural constituents of biological biomembranes and on cardiolipin (CL), mainly located in the mitochondrial inner membrane and required for mitochondrial membrane integrity and functionality. Abnormalities in cardiolipin content and composition are associated with mitochondrial dysfunction. We used three model systems to study the effect of oxidative stress on phospholipids and in particular, on CL. Oxidative stress was induced in isolated, functionally intact rat brain and heart mitochondria either by iron/ascorbate or by hypoxia/reoxygenation in combination with elevated calcium ion concentrations. Additionally, commercial CL was exposed to hydrogen peroxide or to superoxide anion radicals *in vitro* and the role of peroxidases in the process of CL oxidation and degradation was elucidated. The content of PE, PC and CL was determined by using HPLC analysis. In the *in vitro* experiments with commercial CL, the concentrations of CL species and oxidized CL were determined by ESI-MS/MS.

In comparison with PE and PC, CL was significantly more degraded during iron/ascorbate-induced peroxidation of rat brain mitochondria. Hypoxia/reoxygenation in the presence of increasing calcium ion concentrations disturbed the respiration of functionally intact rat heart mitochondria and drastically diminished the content of CL (decrease of active respiration by about 50 %, decrease of CL content by about 60 %). Exposure of bovine heart CL to hydrogen peroxide and to superoxide anion radicals generated by the xanthine/xanthine oxidase system did not cause oxidation or loss of CL species. Moreover, hydrogen peroxide caused only small losses of CL species in the presence of horse raddish peroxidase and glutathione peroxidase (with and without GSSG). However, after incubation of CL with hydrogen peroxide together with cytochrome c, the CL species were highly degraded (by about 80 %) and the generation of CL monohydroperoxide was remarkably enhanced.

Conclusion: Cytochrome c specifically mediates the oxidation and degradation of CL by hydrogen peroxide. This may be of impact in situations of oxidative stress that are accompanied by functional impairment and loss of CL such as hypoxia/reoxygenation in the presence of elevated calcium ion concentrations.

Plasma proteins are specifically oxidized in Alzheimer's disease

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Oxidative stress has been widely implicated in the progression of Alzheimer's disease (AD) with several studies reporting increased markers of oxidative stress in brain and peripheral tissue (Markesbery, 1997; Rinaldi et al., 2003). To our knowledge there are a limited number of proteomic studies which report the specific oxidation of plasma proteins in AD (Choi et al., 2002; Yu et al., 2003), albeit several studies do report specific oxidation to proteins in brain tissue (Butterfield et al., 2006). Proteomic studies identifying specifically oxidized proteins in plasma, in addition to other markers of oxidative stress have the potential for use as a biomarker to enable an earlier and more directed treatment of this disease. In this study total antioxidant capacity (TAC) was measured using the ferric reducing ability of plasma assay (FRAP) in 103 plasma samples (47 AD and 56 age matched controls). TAC was reduced in AD compared to control subjects (1004.838 ± 205.197µM and 952.191 ± 210.928µM respectively; repeated measures ANOVA, $p > 0.05$) although this was not statistically significant. In addition, oxidation to specific plasma proteins was examined using a subset of samples (n = 50; 25 AD, 25 age matched controls) by 2D gel electrophoresis with immunological detection. One protein was found to be significantly oxidized in AD ($p<0.05$ student t-test) and a number of oxidized proteins were present in AD but absent in controls. These data suggest that specific proteins are oxidized in parallel with a tentative depletion of antioxidants in AD plasma. These specific oxidative alterations to plasma proteins may have a functional relevance to the underlying pathology of AD.

Regulation of the p53 protein levels by aerobic training. Protective effect of exercise on paraquat-induced toxicity.

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Background: The beneficial effects of regular physical exercise and the effectiveness in the prevention of several chronic diseases and premature death have been studied. However the molecular mechanisms associated to this prevention have not been completely determined.
Aim: The aim of our study was (i) to determine the effect of 8 wks of aerobic exercise training on the survival of a group of rats treated with a lethal dose of paraquat (ii) to study the effect of 8 wks of aerobic exercise training on the expression of p53, p21 and p19 in liver, skeletal muscle, lungs and heart.
Methods: For the paraquat experiments 25 four-months-old male Wistar rats were randomly divided into 2 groups, exercise group (n=9) and a sedentary group (n=16). Endurance-trained animals were exercised 5 d/wk during 8 wks on an animal treadmill at an intensity of 75%VO_{2max}. After this period, all animals received an intraperitoneal injection of paraquat (35 $mg.kg^{-1}$). $Sa0_2$ was measured every four hours and a survival curve was performed.
For the p53, p19 and p21 expression experiments 10 four-months-old male Wistar rats were randomly divided into 2 groups, exercise group (n=5) and a sedentary group (n=5).
Results: Our data indicate that moderate aerobic exercise significantly protects against paraquat induced toxicity. We also found a training-induced over-expression of p53 in lung, skeletal muscle and liver, but not in heart. No changes in any of the tissues studied were found in the protein levels of p21 and p19.
Conclusions: Our results show that aerobic exercise training protects against paraquat-induced mortality. Exercise induced overexpression of p53 could, at least in part, explain the beneficial effect of this type of exercise in our experimental model.

In vitro Anti-inflammatory Activity of 3-O-methyl-flavones Isolated from *Siegesbeckia glabrescens*,

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The overproduction of nitric oxide (NO) and prostaglandin E_2 (PGE_2) by microglia may cause neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. The toxic peroxynitrite, the reaction product of NO and superoxide anion ($\cdot O^{2-}$) further contributes to oxidative neurotoxicity. From the activity-guided purification of EtOAc soluble fraction of *Siegesbeckia glabrescens*, four flavones were isolated as the inhibitors of NO and PGE_2 production in LPS-activated microglia BV-2 cells. The structures were determined as 3,4'-O-dimethylquercetin **(1)**, 3,7-O-dimethylquercetin **(2)**, 3-O-methylquercetin **(3)** and 3,7,4'-O-trimethylquercetin **(4)** by spectral data analysis, and their IC_{50} values of NO inhibition were 11.1, 4.2, 3.8 and 25.1 μM, respectively. They suppressed the expression of protein and mRNA of inducible nitric oxide synthase and inducible cyclooxygenase through the inhibition of I-kB-a degradation and NF-kB activation. Furthermore, compounds **2** and **3** showed scavenging activity of peroxynitrite with the respective SC_{50} values of 1.75 and 0.77 μM. Therefore the active compounds from *Siegesbeckia glabrescens* may have therapeutic potential in the treatment of neuro-inflammatory diseases through the inhibition of overproduction of NO, PGE_2 and scavenging of peroxynitrite.

Effects of endurance training on both β3-adrenoceptor and neuronal nitric oxide synthase (NOS 1) expression in streptozotocin-induced diabetic rat hearts.

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Aims **–** In diabetic cardiomyopathy, β3-adrenoceptor overexpression plays an important role in both altered β-adrenergic responses and nitrosative stress. These effects are mediated in part by NOS 1 - derived nitric oxide in diabetic heart. Endurance training induced beneficial effects on diabetic cardiac function that may result from changes in the expression of β3-adrenoceptor and NOS 1. The present study was undertaken to assess the effects of endurance training on β3-adrenoceptor and NOS 1 protein expression in diabetic rat hearts.

Methods **–** Type 1 diabetes was induced in male Wistar rats using streptozotocin (STZ, 45 mg/kg, iv). One week after induction, rats were randomly placed into an 8-wk endurance training (TD; 25 m/min, 60 min/d, 5 d/wk on a rodent treadmill) or sedentary (SD) groups. Age-matched rats were sedentary controls (SC). At the end of the protocol, left ventricular (LV) β3-adrenoceptor and NOS 1 protein abundance was assessed using Western blots.

Results **–** Diabetes elicited a significant increase of both LV β3-adrenoceptor and NOS 1 protein expression in SD compared with SC (57% and 34%, respectively; $p<0.05$). After endurance training, both β3-adrenoceptor and NOS 1 protein expression was significantly decreased in TD compared to their sedentary counterparts ($p<0.01$ and $p<0.001$, respectively). Expression of β3-adrenoceptor in TD was similar to those observed in SC animals while NOS 1 protein expression was lower in TD than in SC ($p<0.05$).

Conclusions **–** Endurance training prevented diabetes-induced increase of both β3-adrenoceptor and NOS 1 protein expression. These data are the first to demonstrate that endurance training during diabetes normalizes β3-adrenoceptor and decreases NOS 1 protein expression. These changes support an important role of physical activity in the management of diabetic cardiomyopathy.

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