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Abbreviations

PL	plenary lecture
L	lecture
WL	workshop lecture
YIL	young investigator lecture
P	poster

Content

Introductory Remarks

The 2008 meeting of the Society for Free Radical Research – Europe (SFRR-E) is held from July 5th–July 9th, 2008 in Berlin (Germany). As in previous years this meeting is thought as a forum of scientists in the field of free radicals and oxidants. Scientists from various fields of life sciences, medicine and chemistry will present their latest results and debate the consequences resulting from these facts.

For the first time such a meeting is not only sponsored by numerous companies working in this field but also by a number of other organizations. Most of these organizations sponsored workshops: the co-sponsored workshop on 'Redox Biochemistry and Micronutrients' by the Oxygen Club of California, the Linus Pauling Institute and the Collaborative Research Center 728 of the German Research Council (SFB728).

Furthermore several EU-funded pan-European projects are presenting their research results during this meeting. This includes the COST B35 initiative on lipid peroxidation and diseases, the integrative or collaborative projects PROTEOMAGE, MIMAGE and MARK-AGE. Especially the latter projects are focused on the promotion of aging research in Europe. Hopefully a new level of interaction between the different branches of science will arise through the impact of these workshops within a meeting of the Society of Free Radical Research – Europe.

The fact that oxygen radicals and other oxidizing species are important in normal and pathological conditions is now widely accepted. This is surely to be considered an achievement of the Society of Free Radical Research activities.

In the name of the organizing committee I cordially welcome all participants to the Society of Free Radical Research – Europe meeting 2008 and all the readers of this book of abstracts to share the presentations in order to foster future results.

Tilman Grune

Plenary Sessions

Plenary Session: July 5th 2008, 16:30–18:30
Chairs: T. Grune, M. Jackson, H.E. Poulsen

PL-1
H. Daniel — Surprising links: membrane transporters and ROS-resistance
Informa-Lecture

PL-2
J. Lykkesfeldt — Does vitamin C matter? A quest for in vivo effects of vitamin C deficiency
Cathérine-Pasquier-Awardee-Lecture

Plenary Session: July 6th 2008, 9:00–11:00
Chairs: T. Grune, J. Laranjinha

PL-3
M. Jackson — A transgenic approach to understanding the role of ROS in muscle ageing

PL-4
J. Vina — Identification of new longevity-associated genes and their redox modulation

PL-5
N. Hunt — Tryptophan in physiology and pathophysiology

Plenary Session: July 7th 2008, 9:00–11:00
Chairs: H.K. Biesalski, H.E. Poulsen

PL-6
J. Laranjinha — Nitrite in nitric oxide biology in the stomach: Impact of red wine

PL-7
G. Rimbach — Impact of apoE genotype on oxidative stress, inflammation and disease risk

PL-8
H.E. Poulsen — Prediction of carcinogenicity by oxidative DNA modifications

Plenary Session: July 8th 2008, 9:00–11:00
Chairs: G. Bartosz, M. Jackson

PL-9
H. Sies — Life with oxidative stress
SFRR-Europe-Lecture

PL-10
A. Taylor — Relationships between dietary carbohydrate, oxidation, proteostasis and increased risk for AMD and cataract

PL-11
R. Brigelius-Flohe — Selenium, glutathione peroxidases and cancer

Sessions

Session 1 **Redox Biochemistry and Micronutrients**
(OCC/LPI/SFB728/Mars sponsored)

July 6th 2008, 14:30–16:00 and 16:30–18:00
Chairs: L.O. Klotz, W. Stahl

S1-1
E. Cadenas Cell function and mitochondrial protein post-translational modifications

S1-2
D. Jones Redox systems biology: Is there a redox code?

S1-3
J. Erdman Are health attributes of carotenoids related to their antioxidant functions?

S1-4
V. Ullrich Redox signaling by nitrations and nitrosations

S1-5
T. Schewe Biochemical flavonoid research – recent progress and perspectives

S1-6
M. Kelm Novel aspects of nitrogen monoxide action in the vasculature: modulation by nutritional flavonoids

Session 2 **Mechanisms of antioxidative defences**

July 7th 2008, 11:30–13:00 and 14:30–16:00
Chairs: G. Poli, G. Mann

S2-1
M.B. Kadiiska Products of oxidation as measurable indicators of oxidative stress in experimental animal studies

S2-2
G. Martinez Clinical diagnostic of redox balance: an up-date

S2-3
B. Catalgol Protein oxidation and proteasome inhibition during UVA irradiation

S2-4
C. Fraga Flavonoids antioxidant actions: starting at the membrane?

S2-5
R. van den Berg Potential role of antioxidants in protection against photodamage of skin

S2-6
M. Ristow A transient increase in oxidative stress promotes health and extends life span: The concept of mitohormesis

Session 3 **Selenium and Sepsis**

July 8th 2008, 14:30–16:00
Chairs: N. Kartal-Özer, C. Fraga

S3-1
J. Köhrle Selenoproteins, the thyroid hormone axis and redox regulation

S3-2
K. Reinhart The role of selenium in the critically ill patient

S3-3
M. Maiorino — Why selenium rather than sulphur catalysis in peroxidases? More we learn, less we understand it.

Session 4 — **Oxidants and Signalling**

July 8th 2008, 14:30–16:00
Chairs: E. Cadenas, N. Hunt

S4-1
A. Azzi — Signaling by alpha-tocopheryl phosphate

S4-2
L.O. Klotz — Insulin-mimetic stimulation of cellular signaling cascades by heavy metal ions

S4-3
M. Ziegler — NAD kinase levels control the NADPH concentration in human cells

Session 5 — **Human Applications**

July 8th 2008, 16:30–18:00
Chairs: A. Azzi, J. Cillard

S5-1
N. Kartal-Özer — Hypercholesterolemia and age related diseases

S5-2
K.H. Wagner — Oxidative stress and antioxidant responses after an ironman triathlon

S5-3
N. Breusing — Is iron a mediator in PDT-induced cytotoxicity?

Session 6 — **Protein oxidation and Proteolysis**

July 8th 2008, 16:30–18:00
Chairs: A. Simm, A. Taylor

S6-1
K. Rodgers — The misincorporation of oxidised amino acids into proteins as an experimental tool

S6-2
K. Davies — Inducibility of the proteasome and of the LON protease in oxidative stress, disease, and ageing

S6-3
T. Kueper — Advanced glycation endproducts – rather cause than consequence of human skin ageing

Workshops

Workshop 1 **Lipid Peroxidation and related diseases**
(COST B35 sponsored)

July 6th 2008, 11:30–13:00
Chairs: W. Siems, C. Spickett

WL1-1
G. Poli Oxidized lipids and vascular remodelling in atherosclerosis

WL1-2
F. Gueraud Lipid peroxidation products in foodstuffs and colon cancer

WL1-3
I. Wiswedel F2-Isoprostanes: sensitive biomarkers of oxidative stress

WL1-4
G. Mann Altered lipid metabolism and redox sensitive gene expression in fetal vascular cells in pre-eclampsia

WL1-5
C. Spickett Chlorinated and oxidized lipids in inflammation

Workshop 2 **Mitochondria and Aging**
(MIMAGE sponsored)

July 6th 2008, 11:30–13:00
Chairs: M. Breitenbach, H. Osiewacz

WL2-1
H. Osiewacz Manipulation of molecular pathways increasing the health span of the fungal ageing model Podospora anserina

WL2-2
N. Dencher Caloric restriction, oxidative stress and ageing: a proteomic view

WL2-3
M. Breitenbach Identification and physiological function of a yeast NADPH oxidase

Workshop 3 **Proteomics and Ageing**
(PROTEOMAGE sponsored)

July 7th 2008, 11:30–13:00
Chairs: B. Friguet, E. Gonos

WL3-1
S. Gonos Longevity assurance molecular pathways in human cells

WL3-2
B. Friguet
(COST B35 sponsored) Oxidized protein degradation and repair: implications in ageing and oxidative stress

WL3-3
F. Debacq-Chainiaux Role of IGFBPs in premature senescence

Workshop 4 **Biomarkers of Aging**
(MARK-AGE sponsored)

July 8th 2008, 11:30–13:00
Chairs: A. Bürkle, H. Griffiths

WL4-1
A. Bürkle MARK-AGE: Towards the establishment of biomarkers of human ageing

WL4-2
H. Griffiths Discovery of biomarkers of redox changes in vivo using proteomics

WL4-3
A. Simm Protein glycation – a biomarker of ageing or more?

Workshop 5 **Antioxidants: what are they good for?**
(BASF and GlaxoSmithKline sponsored)

July 8th 2008, 11:30–13:00

WL5-1
H. Biesalski (DSM sponsored), Antioxidants: what are they good for?
G. Rimbach,
T. Grune (COST sponsored)

Young Investigator Session

July 7th 2008, 14:30-16:00
Chairs: N. Breusing, R. Brigelius-Flohé

YIL-1 **T. Jäger**	Inhibitors of trypanothione synthetase: new drugs for neglected diseases
YIL-2 **C.F. Lourenco**	In vivo concentration dynamics of nitric oxide in anesthetized rat brain
YIL-3 **Y. Olmos**	FoxO3a and PGC-1α cooperate to regulate the oxidative stress response in endothelial cells
YIL-4 **S. Romão**	Peroxynitrite detoxification by Leishmania infantum tryparedoxin peroxidases: implications for parasite infectivity in mouse and human cells
YIL-5 **A.K. Samhan-Arias**	Plasma-membrane-bound cytochrome b5 reductase is associated with lipid rafts in cerebellar granule neurons in culture
YIL-6 **K. Brødbæk**	Urinary excretion of biomarkers of oxidatively damaged DNA and RNA in hereditary hemochromatosis (HH)
YIL-7 **A.C. Bulmer**	Improved resistance to serum oxidation in Gilbert syndrome: A mechanism for cardiovascular protection
YIL-8 **L. Rackova**	Is oxidative stress a central mechanism for glucose toxicity in neuronal cells in diabetes?
YIL-9 **E. Ilieva**	t.b.a.

Posters

Poster-Session 1 **Redox Biochemistry and Micronutrients**

P1-1
Influence of alpha-tocopherol in iNOS mRNA expression in UVA-stimulated HMEC-1
J. Bérczes, J. Wurster, D. Nohr

P1-2
A fish-oil rich diet reduces vascular oxidative stress in apoE-/-mice
K. Casos, M.P. Sáiz, N. Zarkovic, K. Zarkovic, M.T. Mitjavila

P1-3
Dynamics of accumulation of antioxidants and vitamin P in leaves and fruits of plants of stem Carya L. during vegetation
G.N. Chupahina, Y.D. Goryunova, T.S. Ivanova

P1-4
Contribution of hydrophilic fractions to the antioxidant activity in selected fruits
M. Ciz, V. Munilla-Saenz, O. Martin-Belloso, S. Gorinstein, A. Lojek

P1-5
NaHS-induced formation of reactive oxygen species in mice
H.W. Clement, P. Heiser, O. Sommer, D. Jargon, E. Schulz, U.T. Hopt, E. von Dobschuetz

P1-6
HPLC-MS analysis of antioxidant flavonoids obtained from Solidago species
L. Dobjanschi, M. Mureşan, A. Antonescu, M. Zdrinca, B. Simona, T. Jurca, M. Camelia

P1-7
H_2O_2 induction of PGC-1alpha as a model for ROS mediated signaling
S. Drori

P1-8
The potent vasodilator ethyl nitrite is formed upon reaction of nitrite and ethanol under gastric conditions
B. Gago, T. Nyström, C. Cavaleiro, B.S. Rocha, R. Barbosa, J.O. Lundberg, J. Laranjinha

P1-9
Hydrogen sulphide induces a large sensibilization of NMDA-receptor response to L-glutamate in cerebellar granule neurones
M.A. Garcia-Bereguiain, C. Gutierrez-Merino

P1-10
Silymarin on H_2O_2-induced toxicity and viability of rat primary mixed glial and rat glioma cells
D. Gezgin, S. Kabadere, R. Uyar

P1-11
The impact of therapy with alpha-lipoic acid and vitamin E in the oxidative stress associated with intermittent hypobaric hypoxia conditions
S. Ghibu, C. Craciun, C. Morgovan, C. Mogosan, A. Muresan

P1-12
DNA fragmentation assessed using a variant of sperm chromatin dispersion (SCD) test
R. Gutiérrez, P. Bécquer, A. Pandolfi, G. Cuevillas, J. Pupo, E.W. Hernández, G. Riverón, N. Pereira, E. Cuétara

P1-13
Antioxidant defence in Helicobacter pylori
K. Heller, L. Flohé, T. Jaeger

P1-14
Influence of antioxidant vitamins C and E on the expression of iNOS-protein in UVA-stimulated HMEC-1
S. Hirobe, J. Wurster, D. Schilling, D. Nohr

P1-15
4-hydroxy-2-nonenal inhibits SERCA1a function by its interaction with the ATP binding site
M.P .Hortigón-Vinagre, Y. Gutiérrez-Martín, Á.C. Román, F. Henao

P1-16
Myeloperoxidase dependent modulation of arachidonic and linoleic metabolites in vivo
L. Kubala, K.R. Schmelzer, B.D. Hammock, J.P. Eiserich

P1-17
Vitamin E and the vesicular transport
S. Nell, A. Kipp, R. Bahtz, A. Boßecker, S. Schumann, R. Brigelius-Flohé

P1-18
Physiological roles of hemoprotein nitrosyl complexes
A.N. Osipov, G.O. Stepanov, Y.A. Vladimirov

P1-19
Anthocyanins inhibit peroxynitrite-triggered endothelial cells toxicity by up-regulating cellular nitric oxide
J. Paixão, T. Dinis, L. Almeida

P1-20
Changes in the intracellular concentration of ROS and/or NO induced by passive stretching in skeletal muscle fibres from aged and dystrophic mice
J. Palomero, D. Pye, T. Kabayo, M.J. Jackson

P1-21
In vitro modulation of preservatives toxicity: hyaluronan decreases oxidative stress and apoptosis induced by benzalkonium chloride, phenoxyethanol and butylparaben
T. Pauloin, M. Dutot, J.M. Warnet, P. Rat

P1-22
Increased F2-isoprostanes and cholesterol oxidation products in colon of rabbits fed a high cholesterol diet. Influence of zinc supplementation
M. Pei-Ern Ng, B. Halliwell, A.M. Jenner

P1-23
Increased cholesterol oxidation products in colon of rats fed a westernised diet high in fat, cholesterol and heme
M. Pei-Ern Ng, W.Y. Ting, A.M. Jenner

P1-24
Differential effects of aqueous or ethanolic procyanidin solutions on hepatic redox status comparison to red wine
D. Pestana, A. Faria, I. Azevedo, C. Calhau, R. Monteiro

P1-25
The reaction of nitrone spin trap PBN with glutathiyl radical: probable antioxidant mechanism
D.N. Polovyanenko, E.G. Bagryanskaya, V.F. Plyusnin, V.A. Reznikov, V.V. Khramtsov

P1-26
Intracellular reactive oxygen species and nitric oxide generation in ageing muscle
D. Pye, J. Palomero, T. Kabayo, M.J. Jackson

P1-27
Ascorbic acid treatment leads to a decrease in NO levels in UV-irradiated human microvascular endothelial cells (HMEC-1)
S. Rodemeister, J. Wurster, D. Schilling, D. Nohr

P1-28
QSAR studies on di(hetero)arylamines derivatives of benzo(b)thiophenes as free radical scavengers
M.V. Rui, A. Abreu, C.F.R. Isabel, A. Ferreira, R.P. Maria-João, B. Queiroz

P1-29
Dependence of the peroxidation of membrane lipids on the physical proprieties of the membrane as studied in liposomes of different compositions
E. Schnitzer, I. Pinchuk, D. Lichtenberg

P1-30
Polyphenolic compounds protect and repair oxidative DNA damage in a neuronal cell model
J.P. Silva, A.C. Gomes, O.P. Coutinho

P1-31
Spin trapping of 1-palmitoyl-2-lineloyl-phosphatidylethanolamine free radicals generated under oxidative stress, using DEMPO, DMPO and mass spectrometry
C. Simões, M.R.M. Domingues, A. Reis, P. Domingues

P1-32
EPR-Imaging of reactive xygen species in mice
O. Sommer, H.W. Clement, E.von Dobschütz, P. Höfer, B. Fink

P1-33
Antioxidant and chemopreventive properties of Vicia faba extract and its flavonoid fractions
C. Spanou, N. Aligiannis, A.L. Skaltsounis, D. Kouretas

P1-34
Polyphenolic compounds of grapes, wines and their antimutagenic
P. Stopka, J. Krizova, J. Triska

P1-35
Short term exposure to aluminium decreases hepatic redox potential in mice
D. Viezeliene, E. Jansen, H. Rodovicius, P. Beekhof, J. Cremers, L. Ivanov

P1-36
Analysis of the mechanisms involved in intramacrophagic elimination of Leishmania
F. Teixeira, S. Romão, S. Carvalho, A.M. Tomás

Poster-Session 2 **Mechanisms of antioxidative defences**

P2-1
Protective effects of melatonin against uranium-induced nephrotoxicity in rats
M. Bellés, V. Linares, M.L. Albina, V. Alonso, J. Sirvent, D.J. Sánchez, J.L. Domingo

P2-2
Intestinal and intraperitoneal absorption and bioavailability of the antioxidants unconjugated bilirubin, biliverdin and bilirubin ditaurate in the rat
A.C. Bulmer, J.S. Coombes, J.T. Blanchfield, I. Toth, R.G. Fassett, S.M. Taylor

P2-3
A cinnamon-derived Michael acceptor for anticancer intervention: Cinnamic aldehyde impairs melanoma cell proliferation and tumor growth
C.M. Cabello, W.B. Bair 3rd, S.D. Lamore, S.M. Azimian, S. Ley, G.T. Wondrak

P2-4
Expression of smooth muscle-specific proteins in human gingival keratinocyte cell lines containing gp91phox homolog Nox1: possible involvement of MEF2B
W. Chamulitrat, A. Sattayakhom, Q. Sun, J. Backs, W. Stremmel

P2-5
The small Hsp ßCrystallin plays a key role in the resistance to oxidative stress determined by VEGF in skeletal myoblasts
I. Dimauro, N. Mercatelli, S.A. Ciafrè, M.G. Farace, D. Caporossi

P2-6
Effects of ochratoxin A exposure in HEK293 human embrionic kidney cell lines: A biochemical and molecular study
A. Dinischiotu, D. Marinescu, M.C. Munteanu, L. Postolache, M. Costache

P2-7
Mitochondria dysfunction and complex I dysfunction in a model of tolerance to nitroglycerin: an approach based on mitochondrial-targeted antioxidants
R. García, M. Rocha, A. Hernandez-Mijares, J.V. Esplugues, V.M. Víctor

P2-8
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L. Gille, A. Müllebner, A. Patel, W. Stamberg, T. Netscher, T. Rosenau

P2-9
Membrane-bound catechol-O-methyltransferase is involved in the metabolism of (-)-epicatechin in human umbilical vein endothelial cells
E. Kravets, Y. Steffen, T. Schewe, J. Sendker, P. Proksch, H. Sies

P2-10
Effect of complexes of precursors and modulator of coenzyme Q biosynthesis on bioenergetics and pro- to antioxidant balance under adriamycin-induced cardiomyopathy
O.B. Kuchmenko, D.M. Petukhov, G.V. Donchenko, L.S. Mkhitaryan, I.N. Yevstratova

P2-11
Changes in oxidative stress parameters in the brain of rats concurrently exposed to uranium and stress
V. Linares, M. Belles, L. Albina, V. Alonso, D.J. Sanchez, J.L. Domingo

P2-12
Triglyceride rich lipoproteins reverse the alterations in respiratory burst and PON2 activity in serum-deprived cells from the monocyte line U937
D.Lixandru, E. Heytens, L.I. Brasoveanu, B. Manuel-y-Keenoy

P2-13
Effects of parenteral lipid emulsions on the phagocytes in vitro
A. Lojek, M. Ciz, L. Kubala, J. Hofmanova, A. Kozubik

P2-14
Blood micromolar concentrations of kaempferol afford protection against ischemia/reperfusion-induced damage in rat brain
C. Lopez-Sanchez, F.J. Martin-Romero, F. Sun, L. Luis, A.K. Samhan-Arias, V. Garcia-Martinez, C. Gutierrez-Merino

P2-15
High glucose acutely enhances the respiratory burst of peripheral blood monocytes but not of differentiated cells from the monocyte line U937
B. Manuel-y-Keenoy, D. Lixandru, E. Heytens

P2-16
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A. Mestre-Alfaro, M.D. Ferrer, A. Sureda, P. Tauler, A.M. Proenza, I. Lladó, J.A. Tur, A. Pons

P2-17
First trimester increase in oxidative stress and risk of foetal growth restriction
V. Mistry, R. Singh, M.D. Evans, P.B. Farmer, J.C. Konje, N. Potdar, M.S. Cooke

P2-18
High fat diet-induced neuropathy of prediabetes and obesity: Effects of low fat diet and 12/15-lipoxygenase genedeficiency
I.G. Obrosova, O. Ilnytska, V.V. Lyzogubov, I.A. Pavlov, N. Mashtalir, U. Julius, J.L. Nadler, V.R. Drel

P2-19
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M. Ralser, M.M. Wamelink, E.A. Struys, B. Gerisch, G. Heeren, M. Breitenbach, A. Kowald, E. Klipp, C. Jakobs, H. Lehrach, S. Krobitsch

P2-20
Lymphocytes from porphyria variegate patients are more susceptible to suffer DNA damage induced by H_2O_2 treatment
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P2-21
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P. Thongphasuk, W. Stremmel, W. Chamulitrat

P2-22
1,4-Dihydropyridine derivatives as antioxidants and therapeutics. A review
G. Tirzitis, G. Duburs

P2-23
The effect of quercetin on menadione toxicity in rat primary mixed glial cells
P.O. Vatan, S. Kabadere, R. Uyar, Y. Altuner

P2-24
Fasting offers rapid and robust protection against ischemia reperfusion injury in mice
M. Verweij, M.van de Ven, S.van den Engel, T. Chu, J.I. Jzermans, J. Hoeijmakers, R. de Bruin, J.R. Mitchell

P2-25
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C.H. Yang, T.C. Tsai, C.F. Hsu, S.C. Tzeng, W.J. Yu, S.J. Chang

P2-26
Signs of oxidative stress persist in the gastric mucosa even after successful eradication of Helicobacter pylori
O. Yelisyeyeva, K. Semen, K. Zarkovic, A. Cherkas, D. Kaminskyy, N. Zarkovic

P2-27
Neuroprotective effects of polyphenols from diet: analysis of their antioxidant activities and intracellular targets
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Poster-Session 3 **Selenium and Sepsis**

P3-1
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P3-2
Influence of selenium on the accumulation of antioxidants in barley plants
G.N. Chupahina, L.N. Skrypnik

P3-3
Activation of the glutathione peroxidase 2 (GPx2) via the Wnt/ß-catenin pathway
A. Kipp, E. Göken, A. Banning, R. Brigelius-Flohé

P3-4
Glutathione S-transferase expression in upper urinary tract transitional cell carcinoma
M. Matic, A. Savic-Radojevic, M. Pljesa-Ercegovac, J. Mimic-Oka, T. Sasic, T. Simic

P3-5
Activities of GSH-replenishing enzymes inversely correlate with cleaved caspase 3 index in transitional cell carcinoma of urinary bladder
A. Savic-Radojevic, M. Matic, M. Pljesa-Ercegovac, D. Dragicevic, J. Mimic-Oka, T. Sasic, T. Simic

P3-6
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T. Simic, M. Pljesa-Ercegovac, T. Sasic, M. Matic, A. Savic-Radojevic, D. Dragicevic, J. Mimic-Oka

P3-7
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B. Speckmann, L. Alili, L.O. Klotz, H. Sies, H. Steinbrenner

P3-8
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A. Vasilijevic, B. Buzadzic, A. Korac, V. Petrovic, A. Jankovic, B. Korac

Poster-Session 4 **Oxidants and Signalling**

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N. Ale-Agha, L.O. Klotz

P4-2
PI3K/AKT dependent inactivation of Foxo3a mediates nitric oxide induced PGC-1a downregulation
S. Borniquel, I. Valle, Y. Olmos, E. Soria, S. Lamas, M. Monsalve

P4-3
Delayed ageing through damage protection by the Arf/p53 pathway. Role of oxidative stress
C. Borras, A. Matheu, A. Maraver, P. Klatt, I. Flores, I. Garcia-Cao, J.M. Flores, M.A. Blasco, J. Viña, M. Serrano

P4-4
Resveratrol suppresses peroxynitrite-triggered mitochondrial apoptotic pathway in endothelial cells by up-regulating intracellular levels of Bcl-2
P. Brito, N. Simões, L. Almeida, T. Dinis

P4-5
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P4-6
Mutual interactions among collagen, platelets and professional phagocytes
H. Cizova, I. Goshev, V. Jancinova, R. Nosal, A. Lojek, M. Ciz

P4-7
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A. Dinischiotu, L. Postolache, M.C. Munteanu, D. Marinescu, M. Costache

P4-8
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D. Dani, E. Schokraie, U. Warnken, M. Schnölzer, Y. Higami, I. Shimokawa, M. Sugawa, N.A. Dencher

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Role of Nerve Growth Factor in modulating the survival of myogenic cells exposed to free radicals
A. de Perini, I. Dimauro, L. Gatta, P. Parisi, D. Caporossi

P4-10
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L. Dong, J.C. Dyason, P.K. Witting, S.J. Ralph, J. Neuzil

P4-11
Heavy metal stress-induced modulation of FoxO signaling
A. Eckers, L.O. Klotz

P4-12
COX-2 expression is enhanced upon UVB irradiation in HaCaT keratinocytes: regulation at the posttranscriptional level
N.S. Fernau, L.O. Klotz

P4-13
The supramolecular organisation of OXPHOS complexes in different rat brain regions changes during ageing
M. Frenzel, F. Krause, H. Rommelspacher, M. Sugawa, N.A. Dencher

P4-14
Nutritional modulation of sirtuin expression is mediated by redox changes
J. Gambini, C. Borras, R. Lopez-Grueso, S.L. Valles, C. Gomez-Cabrera, J. Vina

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PLENARY SESSIONS

PL-1
Surprising links: membrane transporters and ROS-resistance

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We are mainly interested in the physiological importance of the cell membrane peptide transporter proteins PEPT1 and PEPT2. PEPT1 is expressed mainly in gut epithelial cells contributing to intestinal amino acid absorption whereas PEPT2 shows more widespread expression with the highest expression level in mammals in kidney tubules were it contributes to the renal reabsorption of filtered di- and tripeptides. We have analyzed *in extenso* the transporters function after expression in different target cells (Xenopus oocytes and various human cell lines) and have extended our research recently also to bacteria, yeast and non-mammalian models such as *C. elegans*. We also generated transgenic mouse lines deficient in either one of the peptide transporters. I shall be presenting data on *C. elegans* lacking the intestinal peptide transporter and on mice lacking PEPT2 in the kidney.

In assessing the alterations of a gene-deletion of the intestinal peptide transporter in *C. elegans*, we observed that the animals did show increased glutathione levels despite the fact that they generally have a reduced amino acid availability and impaired growth and development. Although these worms deficient of PEPT1 did not display an increased life-span, when crossed into a *daf-2* mutant background (insulin-receptor mutant) – which by itself doubles life-span – another extension of life-span to almost 4 times of that in wild-type was observed. The longevity in this line was associated with an almost complete resistance to a ROS-challenge in form of treatment with 150 mM paraquat. The ROS-resistance and the longevity phenotype were completely antagonized to wild-type when a *daf-16* mutant background was crossed in. This suggests that the daf-16 (mammalian FOXO transcription factor homologe) is centrally involved in mediating this stress-resistance. Moreover, we demonstrate that mTOR signalling also contributes to the stress resistance. The various transgenic lines have been submitted to a microarray-based transcriptome analysis, proteome analysis and metabolite profiling. Major alterations in adaptive stress response pathways were observed and amino acid as well as fatty acid metabolism and lipid storage showed alterations as well and those could partially be linked to changes in gene/protein expression and stress resistance.

In mice lacking PEPT2 no obvious phenotype was obtained and renal tissues did not show any impairment. However, profiling of kidneys by microarray, proteome and metabolite analysis revealed alterations in GSH metabolism and a prime role of PEPT2 in renal reabsorption of cysteinyl-glycine as the GSH degradation product. A challenge experiment with acetaminophen established finally severe tubular damage in animals lacking PEPT2.

In summary: Amino acid transport/homeostasis plays a prominent role in the organismic stress-resistance and the capability to cope with ROS.

PL-2
Does vitamin C matter? A quest for in vivo effects of vitamin C deficiency

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Vitamin C has long been recognized as an important dietary micronutrient based on its ability to prevent scurvy in humans. Moreover, over the past decades, ascorbate has been identified as a powerful redox modulator and named "the most important antioxidant in plasma". Several investigators have shown ascorbate to be an excellent biomarker of "oxidative stress" in a variety of biological settings from isolated cells to humans. However, in spite of the amazing redox powers of ascorbate, little evidence has been presented until now demonstrating that vitamin C deficiency results in any clinical manifestation beyond that of scurvy. Meanwhile, literally hundreds of millions of people worldwide can be diagnosed with hypovitaminosis C – i.e. a plasma concentration below 23 μmol/L – a condition that is typically chronic due to e.g. sustained malnutrition, smoking or disease. The magnitude of this potential problem has prompted discussions on the possible beneficial effect of supplementation to humans as a preventive measure but so far large clinical trials have shown no clinical relevance of antioxidant supplementation in general. Two possible pathological consequences of marginal vitamin C deficiency are discussed and supported by new in vivo evidence from animal studies. Apparently, vitamin C does matter...

PL-3

A transgenic approach to understanding the role of ROS in muscle ageing

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Tissues from old people and animals contain elevated levels of the end-products of lipid, DNA and protein oxidation, but direct evidence that this oxidative damage plays a key role in the ageing process has not been obtained. During ageing, loss of approximately 40% of skeletal muscle mass occurs in humans and this loss contributes significantly to the increasing frailty seen in older individuals. This pattern of loss of muscle mass is mirrored in ageing rodents and also associated with an increase in the content of markers of free radical activity in skeletal muscle [1]. In attempts to determine whether changes in free radical activity play a direct role in the loss of muscle mass, the effect of knockout or overexpression of key proteins regulating free radical activities in skeletal muscle on age-related loss of muscle mass and function has been examined in mice. A number of potential models of increased ROS activity have been examined, but only mice lacking CuZnSOD were found to show a phenotype of accelerated age-related loss of muscle mass and function [2], while protection against key aspects of age-related muscle mass were observed in mice overexpressing the inducible heat shock protein 70, apparently acting through a reduction in oxidative damage to the muscle [3,4].

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PL-4

Identification of new longevity-associated genes and their redox modulation

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A major aim of our laboratory is to identify longevity associated genes (LAG) and, importantly, given the difficulties encountered with gene therapy, to find ways of up- regulating their expression by physiological or nutritional manipulations.We have found that overexpression of p53/p16 increases longevity, on top of their role on cancer prevention [1]. Physical exercise increases antioxidant genes (another kind of LAG) and in this regard, we have proposed that exercise itself is an antioxidant [2]. Nutritional manipulations like food supplementation with soya also increase the expression of LAG. This is the mechanism by which phytoestrogens act as antioxidants (because they up-regulate antioxidant genes by interacting with specific receptors, an not because of their chemical phenolic structure) [3]. Sirtuins are another type of LAG [4]: we have found that they are tightly redox regulated, particularly by the NAD/NADH redox pairThe physiological implications of these facts will be discussed.

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PL-5

Tryptophan in physiology and pathophysiology

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Tryptophan is an essential amino acid that has 3 fates in mammals: (i) incorporation into protein; (ii) metabolism to 5-hydroxytryptamine; (iii) conversion to a range of biologically active molecules via the kynurenine pathway. The last-named accounts for around 90% of tryptophan metabolism. The first reaction in the kynurenine pathway, conversion of tryptophan to N-formylkynurenine, is catalysed by tryptophan dioxygenase or indoleamine 2,3-dioxygenase (IDO). The former is a constitutive enzyme found chiefly in liver and the latter can be induced in various tissues and cell types by the cytokine interferon-γ (IFNγ). Recently we discovered [1] the existence of another enzyme that catalyses the same reaction, termed IDO-2. The two genes, *IDO-1* and *IDO-2*, arose by a gene duplication event at least 350 million years ago. IDO-2 has a different protein expression pattern to IDO-1, being found chiefly in renal tubular epithelial cells, sperm and liver. The mechanisms that regulate IDO-2 expression have not yet been identified.

The kynurenine pathway yields numerous bioactive molecules, including the anthranilic acids, kynurenic acid (KA), quinolinic acid (QA), picolinic acid and NAD. Redox processes are relevant to the kynurenine pathway in several ways, for example:

i. 3-hydroxyanthranilic acid and 3-hydroxykynurenine have antioxidant activity [2];
ii. the expression and activity of IDO-1 are redox-regulated [3];
iii. nitric oxide inhibits the activity of IDO-1 by reacting with the haem moiety [4];
iv. IDO-1 activity requires an electron source, long believed to be superoxide but in fact likely to be cytochrome b_5 [5].

IDO-1 has been implicated in host anti-microbial defences and has immunoregulatory actions [6], most likely via local depletion of tryptophan. QA is a neuroexcitatory molecule, and KA inhibits this action. Changes in the kynurenine pathway have been implicated in several CNS diseases, for example AIDS-related dementia, meningitis and Huntington's Disease [7]. We have established its involvement in the pathogenesis of cerebral malaria (CM).

CM has been studied in a model system that mimics many features of the human disease, *Plasmodium berghei* ANKA (PbA) infection in mice, and in patient samples. At the stage when PbA-infected mice exhibited the characteristics of CM, the activity of IDO in the brain was increased 40-fold compared to uninfected mice. The ratio between the levels of QA and its antagonist KA in the brain also was significantly increased [8]. IDO-1 was strongly expressed in endothelial cells [9]. In the cerebrospinal fluid (CSF) of 261 Vietnamese adults with CM, the mean QA level and QA:KA ratio were significantly increased relative to UK controls [10]. In 83 African children with CM, the mean CSF QA level was significantly increased compared to UK controls [11]. In both clinical studies, increased CSF QA was statistically associated with fatal outcome. Inhibition of kynurenine 3-hydroxylase protects mice against PbA-induced CM [12].

Activation of the kynurenine pathway in the brain in malaria may be a host protective response, due to its antioxidant, anti-microbial and vasoregulatory actions. Furthermore, the kynurenine pathway yields NAD, which becomes depleted in brain tissue in murine CM. Changes in QA and KA levels might be significant factors in the pathogenesis of CM, especially where changes in levels of the neuroexcitotoxin are not compensated for by changes in the neuroprotectant.

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PL-6

Nitrite in nitric oxide biology in the stomach: Impact of red wine

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The stomach may be a source of new bioactive molecules with impact in physiologic and pathologic processes. By acting as a bioreactor, it affords chemical and mechanical conditions for the reaction between dietary components. Nitrite and red wine components, namely polyphenols and alcohol, reach high concentrations in the stomach and are prone to embark in mutual reactions, yielding an array of biologically relevant new molecules.

We demonstrate that:

1. wine polyphenolic fractions (procyanidins, flavonols, flavanols, and phenolic acids) dose- and pH-dependently promote the formation of nitric oxide (NO) when mixed with nitrite, and this reaction occurred in vivo in healthy volunteers. Mechanistically, the reaction involves the univalent reduction of nitrite, as suggested by the formation of NO and by the appearance of EPR spectra assigned to wine phenolic radicals;
2. under gastric conditions, ethyl nitrite forms from the reaction of red wine (alcohol fraction) or distilled alcoholic drinks with physiological amounts of nitrite. Ethyl nitrite acts as an NO donor and is able to induce relaxation of arteries and stomach tissues in a way dependent on guanylate cyclase.

Overall, these findings reveal a new pathway for the biological effects of dietary nitrite encompassing its interaction with red wine components (polyphenols and alcohol) and the production of nitric oxide and of ethyl nitrite with impact on human physiology.

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PL-7

Impact of apoE genotype on oxidative stress, inflammation and disease risk

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In Westernised societies the apoE4 genotype it is associated with increased morbidity and mortality, and represents a significant risk factor for cardiovascular disease, late-onset Alzheimer\\\'s disease and other chronic disorders. ApoE is an important modulator of many stages of lipoprotein metabolism and traditionally the increased risk was attributed to higher lipid levels in E4 carriers. However, more recent evidence demonstrates the multifunctional nature of the apoE protein and the fact that the impact of genotype on disease risk may be in large part due to an impact on oxidative status or the immunomodulatory/anti-inflammatory properties of apoE. An increasing number of studies in cell lines [1,2] targeted replacement rodents [3] and human volunteers [4,5] indicate higher oxidative stress and a more pro-inflammatory state associated with the epsilon4 allele.Information regarding the impact of apoE genotype on oxidative stress, inflammation and disease risk is often derived from observational studies or small intervention trials in which retrospective genotyping of the cohort results in small group sizes in the rarer E2 and E4 subgroups. Either larger well-standardised intervention trials or smaller trials with prospective recruitment according to apoE genotype are needed to fully establish the impact of diet on genotype-CVD associations and to establish the potential of dietary strategies such as reduced total fat, saturated fat, or increased antioxidant intakes to counteract the increased CVD burden in apoE4 carriers.

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PL-8

Prediction of carcinogenicity by oxidative DNA modifications

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Oxidative DNA modifications are among the most abundant modifications in DNA and occur also in other nucleic acids and in the nucleotide pool. There has been much dispute about the true levels that have been reported as high as 1 per 10,000 corresponding unmodified bases. The true levels are now believed to be about 1–5 per 1,000,000 corresponding unmodified guanines in DNA.
Measurement of oxidative stress to DNA can be performed by two distinct different methodologies:

1. Measurement of the level of oxidized lesions in a tissue, most often nuclear DNA
2. Measurement of the rate of oxidation of DNA, most often from urinary excretion

The level of oxidized lesions in e.g. nuclear DNA results from the balance between the formation rate of the lesion (oxidative stress) and the removal of the lesion (DNA repair). From a mechanistic point of view the higher the level the higher is the chance (risk) for the modification to result in a fixed mutation. Likewise, if the rate of oxidation of DNA bases is high, the higher is the chance (risk) that oxidation of that base will result in a fixed mutation.
Both measurements can be viewed as a biomarker, and the following criteria for a biomarker to predict disease can be set as the following points:

1. It should be predictive of development of the disease or condition under investigation
2. It should reflect biological event(s) that can be related to the pathogenesis of the disease
3. It should be stable over short time periods (weeks, months) in stable individuals.
4. It should produce identical results when the same sample is measured in different laboratories.
5. The sample from which it is measured should be stable on storage.
6. The biomarker should relate to immediate events within short periods of time, or should reflect integration of events over a well-defined time period.
7. Preferentially, the biomarker measurement should be non-invasive, or measurable in an easily available biological specimen (example: urine, sputum) or in minimally invasively obtainable biological specimen (example: blood or plasma).
8. The cost of sample analysis should be low and it should be possible to perform a large number of analyses within a reasonable time period.

For the most common oxidative DNA lesion, 8-oxodG, these points are fulfilled to a high degree. Furthermore changes in the urinary excretion of 8-oxodG in many intervention studies are in agreement with the outcome of trials with death or disease with endpoints.

PL-9

Life with Oxidative Stress

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Life depends on oxygen, and higher life forms utilize oxidative phosphorylation as major source of energy. Ground-state oxygen is a diradical, and partially reduced forms are of radical or nonradical nature. Oxygen can also be electronically excited to singlet molecular oxygen. Research revealed that all of these forms of oxygen serve in signaling events, modulating gene expression and protein function. However, the janus-faced side of oxygen and its metabolites is also known, inflicting damage to biomolecules. Thus, throughout evolution an armamentarium of antioxidant defense, enzymatic and non-enzymatic, was required to counteract oxidative stress. A major advance in recent years is the concept of redox control in biological organisation, i.e. the use of oxidative stress for purposes of regulation.Our currrent work focuses on responses in the human vascular endothelium, where there is an intricate interplay of superoxide and nitric oxide-dependent reactions. In studies on micronutirents such as flavanols and procyanidins, the picture emerged that, beyond direct antioxidant activities, specific target sites serving as modulator sites become of biological interest.

PL-10

Relationships between dietary carbohydrate, oxidation, proteostasis and increased risk for AMD and cataract

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The glycemic index is a measure of the rate at which consumption of specific foods results in a rise in blood glucose, as compared with intake of the same amount of a standard food or sugar. We recently detected lower levels of oxidatively modified, glycated proteins in lenses of mice fed lower as opposed to higher dietary glycemic index. Work in four different cohorts indicates that dietary glycemia, measured either as above the median for glycemic index or total carbohydrate is associated with increased risk for cataract or AMD and progress of AMD [1–6]. Associated with cataracts and AMD-related deposits is accumulation of damaged proteins, often including markers of sugar oxidation, or glycation, and ubiquitin conjugates. I will present the epidemiologic information to show the relationship between dietary glycemia and risk for cataract and AMD, and new mechanistic data which shows a dietary carbohydrate-related accumulation of glycated proteins in lens and retina as well as how proteolytic pathways which normally regulate removal of damaged proteins are compromised by glycation. This results in the enhanced levels of glycated and ubiquitinated proteins and is exacerbated by the compromised protein quality control that is associated with aging, cataract and AMD. From this work it would appear that diminishing the accumulation of glycated proteins would appear to offer a simple means to prolong retina and lens function. It is likely that cardiovascular disease and type II diabetes would also be diminished by such practice.

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PL-11

Selenium, glutathione peroxidases and cancer

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A low intake of selenium has been shown to correlate with a higher incidence of cancer and, therefore, chemopreventive functions have generally been attributed to selenium. The mechanism by which selenium might act anticarcinogenic, however, is far from being clear. In mammals, selenium is incorporated into selenoproteins as selenocysteine. About 30 selenoproteins exist, but functions are not yet know for all of them. Thus, it is not known either whether individual selenoprotein(s) contribute to cancer prevention or whether particular selenium compounds act independently from selenoprotein biosynthesis.Some information is available from mice in which individual selenoproteins have been deleted. Glutathione peroxidase 1 and 2 (GPx1 and GPx2) double knockout mice develop colitis and intestinal cancer [4] and GPx2 ko mice are more susceptible to UV-induced squamous cell carcinoma formation [7]. A protective role of GPx2 can be deduced from two observations: (i) its response to Nrf2 activators [1], which induce the expression of enzymes of the adaptive response, and (ii) its capability to counteract COX-2 expression in HT-29 cells [2]. On the other hand, blocking the Wnt pathway leads to a loss of GPx2 expression [6] and the GPx2 promoter is activated by β-catenin [5]. This might reflect a physiological role of GPx2 in facilitating proliferation, but also makes it a target of a dysregulated Wnt pathway. Cells in which GPx2 is stably knocked down by siRNA had an increased capability to migrate in a wound healing test, as well as an increased invasiveness in a transwell assay. However, siGPx2 cells did less grow anchorage-independently in soft agar and most interestingly, tumor development from siGPx2 cells was distinctly lower than from control cells when injected into nude mice [3]. The data show that GPx2 inhibits malignant behaviour of tumor cells such as migration and invasion but is required for the growth of transformed intestinal cells and, thus, may facilitate tumor cell growth.In conclusion, the selected example already reveals that selenoproteins depending on their physiological function may display both, prevention of carcinogenesis and promotion of tumor growth. Accordingly, the potential benefit of selenium will depend on the stage of tumorigenesis. Intensive research is therefore needed before a beneficial effect of selenium supplementation can be predicted.

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SESSIONS

SESSION 1 — REDOX BIOCHEMISTRY AND MICRONUTRIENTS

S1-1
Cell function and mitochondrial protein post-translational modifications

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Mitochondria generate second messengers, such as H_2O_2 and nitric oxide ($^{\cdot}NO$), which are involved in the regulation of redox-sensitive cell signaling through the mitogen-activated protein kinase (MAPK; *e.g.*, JNK) pathway, thus coordinating functional responses between mitochondria and other cellular processes. A complex crosstalk and interplay of cytosolic signaling pathways converge on the mitochondria, which ultimately function as an epicentral processor that determines the death/survival of the cell. The interaction between these two processes establishes a regulatory device that controls cellular energy levels and redox environment through specific mitochondrial protein post-translational modifications, including phosphorylation, tyrosine nitration, and cysteine S-nitrosation and S-glutathionylation. The role of these protein modifications in aging and age-related neurodegenerative diseases is established by an age-dependent increase in (*a*) the levels of $^{\cdot}NO$ – a consequence of increased neuronal nitric oxide synthase expression– leading to a specific pattern of mitochondrial protein nitration and (*b*) MAPK-dependent phosphorylation cascades in brain mitochondria leading to a deficient energy metabolism. In the former case, mitochondria – as the major cellular site of superoxide anion generation – set the platform for the specific nitration (and inactivation) of succinyl-CoA-transferase and F_1-ATPase. In the latter case, phosphorylation (inactivation) of the E_1? subunit of the pyruvate dehydrogenase complex brings about an energy deficit determined by the shift from aerobic- to anaerobic glucose metabolism. In a triple transgenic model of Alzheimer's disease these metabolic 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. This suggests limited substrate availability for mitochondrial energy metabolism.

S1-2
Redox systems biology: is there a redox code?

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Reversible sulfur switches control structure and function of proteins, and accumulating evidence indicates that oxidative stress represents a disruption of the normal function of these switches. If only 10% of proteins contain such elements, cells would have >2000 sulfur switches, indicating that organizing principles, i.e., a "redox code", could exist to integrate protein functions during the life cycle and in response to physiologic and toxicologic challenges. In an effort to understand these organizing principles, we have measured steady-state redox potentials of thioredoxins, GSH/GSSG and cysteine/cystine in different subcellular compartments and under different physiologic and toxicologic conditions. The results show that these central thiol/disulfide control nodes are maintained at stable but non-equilibrium states. The redox couples vary from reduced to oxidized in the following sequences: Trx > GSH > Cys and mitochondria > nuclei > cytoplasm > secretory pathway > extracellular fluids. The range of redox potentials is sufficient for regulation of protein functions by relatively shallow redox gradients, but the kinetics are too slow for this to occur without catalysis. These observations indicate that a first principle of the redox code is that subcellular compartments have distinct steady-state redox potentials. The data further suggest that within compartments, different subsets of protein cysteines are regulated separately by thioredoxin and GSH/GSSG systems, perhaps further delineated into networks coupled to catalytic oxidative mechanisms, i.e., specific peroxidases and oxidases. Better definition of the redox circuits and their regulation is needed for development of redox systems biology. The most critical needs are to delineate the electron transfer pathways controlling specific sulfur switches, to understand the insulation and communication of different redox compartments, and to discriminate orthogonal redox regulatory mechanisms which provide the basis for "cross-talk" between biologic processes and pathways.

S1-3

Are health attributes of carotenoids related to their antioxidant functions?

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Numerous epidemiological trials have associated the intake of high carotenoid-containing foods or blood carotenoid concentrations with reduced incidence of a number of chronic diseases such as cardiovascular disease and some forms of cancer. Some researchers have suggested that the antioxidant function(s) of carotenoids are responsible for this protection. Of all the carotenoids tested, lycopene has been demonstrated to be the most potent *in vitro* antioxidant, primarily acting as a singlet oxygen quencher. However, it is not clear whether lycopene is an important antioxidant *in vivo*. Lycopene intake and blood levels (mostly from tomato products) are each inversely related to prostate cancer incidence. Our laboratory has postulated that metabolic products of lycopene, lycopenoids [1], may be more biologically active than the parent molecule. A number of investigators have identified metabolites of lycopene in mammalian tissues. For example, we have found apo-8′- and apo-12′-lycopenals in rat liver following lycopene feeding [2]. We propose that lycopene is cleaved by the eccentric carotenoid monooxygenase cleavage enzyme, CMO-II, to initially produce lycopenals which could then be oxidized to lycopenoic acids, chain-shortened, or further metabolized. Lycopenoids, due to structural and polarity similarities to retinoids, may act as agonists or antagonists for a number of nuclear receptors such as RARs, RXRs, PPARs, LXRs, etc., or may activate response elements such as the ARE. Kiefer and coworkers [3] demonstrated in *E. coli* engineered to produce lycopene and expressing CMO-II exhibited a color shift while *E. coli* engineered to produce β-carotene and expressing CMO-II did not. Hessel et al. [4] showed in CMO-I KO mice that β-carotene was not converted to vitamin A, an observation that we have recently confirmed [5]. Moreover, we found in CMO-I KO mice fed lycopene that there was an altered tissue lycopene biodistribution and isomer pattern compared with wild-type mice. In a pilot study with CMO-II KO mice fed lycopene, many tissues had enhanced lycopene accumulation but unaltered isomer distribution [unpublished data]. Thus, there is emerging *in vitro* and *in vivo* evidence to suggest that lycopene is metabolized in mammalian tissues to produce retinoid-like compounds. These lycopenoids may be either agonists or antagonists for a number of nuclear receptors or other metabolic reactions. It is concluded that lycopenoids, or metabolites of other carotenoids, may be responsible for the chronic disease protection associated with carotenoids, not the antioxidant function of the parent molecules.

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S1-4

Redox signaling by nitrations and nitrosations

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Oxidative modifications at proteins have the potential to down- or upregulate enzyme activities and hence to serve in redox signaling. This applies to the oxidation of thiols to disulfides, sulfenic acids or S-nitroso derivatives, to the sulfoxidation of methionine residues,and the formation of tyrosine radicals or the nitration of tyrosine. We propose that a common primary step in redox signaling involves generation of superoxide anion (O_2^-) by four oxidases acting sequentially. Combination of O_2^- with nitric oxide (NO) to form peroxynitrite is an important second step by which all of the above mentioned posttranslational modifications can be explained. Selectivity in peroxynitrite action can be achieved by metal catalysis (e.g. zinc fingers, heme, manganese,copper). New data refer to the modification of peroxynitrite action by variation of O_2^- and NO fluxes which at a threefold excess of NO over O_2^- lead to S-nitrosations as a prerequisite in the formation of higher peroxynitrite levels. This is mediated by suppressing reductive pathways. According to such results we postulate that activation of endothelial cells by angiotensin II starts by NADPH oxidase assembly followed by a rise in 1–10 nM peroxynitrite levels which are sufficient to stimulate the cyclooxygenases with subsequent prostacyclin formation in the relaxation phase of smooth muscle.After pretreatment with endotoxin the endothelium builds up 50–100 nM peroxynitrite levels through O_2^- production by xanthine oxidase. This nitrates and inactivates prostacyclin synthase leading to prostaglandin endoperoxide mediated contractions of smooth muscle. These cells also contain prostacyclin synthase which is not nitrated even when high fluxes of NO and O_2^- are applied indicating a strong peroxynitrite reducing capacity in these cells but not in the endothelium. The rationale behind this is discussed in the context of the reversibility of oxidative signaling and the physiological implications.

S1-5

Biochemical flavonoid research – recent progress and perspectives

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Studies with human volunteers using high-flavanol cocoa intake identified nitric oxide metabolism of the vascular endothelium as major target of dietary flavanols [1], in particular of (–)-epicatechin [2]. This observation prompted us to study the metabolism of (–)-epicatechin in human vascular endothelial cells as well as the actions of (–)-epicatechin metabolites on processes linked to cellular NO metabolism. Human umbilical vein endothelial cells (HUVEC) convert (–)-epicatechin to 3′-*O*-methyl epicatechin and 4′-*O*-methyl epicatechin catalyzed by endothelial catechol-*O*-methyltransferase [3]. This metabolism leads to elevation of the intracellular levels of both NO and cGMP [4]. The effect was attributed to inhibition of endothelial NADPH oxidase activity in an apocynin-like fashion [3]. Besides epicatechin methyl ethers, a number of further flavonoids and other dietary polyphenols were identified as inhibitors of endothelial NADPH oxidase [3]. By blocking a major pathway of superoxide generation, these compounds attenuate prooxidant and nitrating processes mediated by peroxynitrite, as demonstrated for endothelial cells treated with oxidized LDL or angiotensin II [5].

While inhibition of endothelial NADPH oxidase may be responsible for the short-term effect of dietary flavanols occurring about two hours after intake, repeated daily intake leads to an adaptive longer-term effect involving other mechanisms [6].

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S1-6

Novel aspects of nitrogen monoxide action in the vasculature: modulation by nutritional flavonoids

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Atherosclerosis is the major cause for chronic vascular diseases. The key event in the pathogenesis of atherosclerosis is believed to be the dysfunction of the endothelium due to decreased nitric oxide (NO) bioavailability. Endothelium-derived NO plays a major role in vascular homeostasis and a decrease in NO-bioavailability accelerates the development of atherosclerosis. Given that endothelial dysfunction is at least in part reversible, the characterization of endothelial function as well as therapeutical approaches through lifestyle changes and dietary interventions have gained much attention over the past years. Recent studies demonstrated that especially the consumption of plant-derived foods rich in flavonoids can improve endothelial function in both compromised and healthy humans.

The benefits of flavanol-rich foods have been attributed to the antoxidative activity of their polyphenolic compounds. For many years it was accepted that this was the main mechanism by which flavanols mediate their beneficial effects. Recent studies in humans and animal models suggest that vascular effects of flavanols are due to an increased nitric oxide synthase activity and thus an augmented supply of bioactive NO. We could demonstrate that in individuals with cardiovascular risk factors or coronary artery disease, the consumption of a flavanol-rich cocoa drink leads to an increase of the circulating NO-pool. We have also shown earlier that in healthy young adults the ingestion of flavanol-rich cocoa was associated with acute elevations in levels of circulating NO-species. In smokers with endothelial dysfunction the consumption of a flavanol-rich cocoa drink increased the circulating NO-pool, and NOS inhibition by L-NMMA prevented these beneficial effects pointing towards an increased NOS activity and subsequently enhanced NO bioavailability. Just recently, we were able to demonstrate that regular daily intake of flavanol-containing cocoa acutely and chronically reverses endothelial dysfunction in medicated diabetic patients with severe atherosclerosis, highlighting the potential of flavanol-containing diets, and underscoring the potential health care benefit for reducing the risk of cardiovascular events.

Taken together, our results suggest that the circulating pool of bioactive NO is increased by intake of flavanols in an acute and sustained manner. The increase of circulating NO species may contribute to the potential health benefits of flavanol-rich foods and may have application for the treatment of diseases characterized by impaired regional NO production, such as peripheral and coronary artery disease.

SESSION 2 — MECHANISMS OF ANTIOXIDATIVE DEFENCES

S2-1
Products of oxidation as measurable indicators of oxidative stress in experimental animal studies

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Many techniques now exist that potentially allow the measurement of oxidative stress in experimental animal models and humans. The concept of measuring oxidative stress status in humans has not been approached as a real possibility in clinical practice because standardized methods are not yet established. To evaluate the available methodology for measuring oxidative stress with potential application to human studies, NIEHS/NIH has taken the lead in organizing and sponsoring the first national and international comprehensive comparative study for determining which of the available biomarkers of oxidative stress are most specific, sensitive and selective. Antioxidants and various oxidation products of lipids, proteins and DNA in plasma and urine of experimental animals with diverse oxidative exposures were measured as part of a comprehensive, international multilaboratory validation study searching for non-invasive biomarkers of oxidative stress in health and disease. The goal of the study was to find markers of oxidative stress that are applicable to different oxidative insults and measurable in stored specimens. The focus of this presentation will be on the findings from measurement of oxidative stress in different experimental animal models of CCl4 poisoning, ozone exposure and LPS treatment. The time and dose-dependent effects of all these oxidative insults on plasma and urine concentrations of lipid hydroperoxides, TBARS, malondialdehyde (MDA) and isoprostanes were investigated with different old and new techniques. Measures of oxidative products of proteins – protein carbonyls, methionine sulfoxidation, tyrosine oxidation products and DNA- strand breaks, 8-OHdG, M1G were carried out as well. In addition, the time- and dose-dependent effects of all exposures on plasma levels of alfa-tocopherol, coenzyme Q (CoQ), ascorbic acid, glutathione (GSH and GSSG), uric acid and total antioxidant capacity were investigated to determine whether the oxidative effects of diverse insults would result in decrease of plasma antioxidants.The pattern of oxidative stress biomarkers seen in these four exposures will offer insight into the specificity and sensitivity of the markers and will provide evidence that a given product of oxidation may be a marker for some type of oxidative stress but not others.

S2-2
Clinical diagnostic of redox balance an up-date

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The methodology for detecting oxidative stress status at clinical level is hardly to be found in the literature. There are some useful methods for investigating the oxidative profile but they are not applicable to the clinical diagnostic. Worldwide in spite of having a high prevalence of illnesses in which Reactive Oxygen Species are involved (cancer, diabetes, etc.) an integral diagnostic systems have not been developed. It is also insufficient the education and preparation of the professional personnel (doctors and related health personnel) to face the analysis of the data that contributes to a antioxidant/pro-oxidant diagnostic and to impact in the modification of life habits and other measure directed to correct the redox disruption.

In the CEIEB-IFAL-UH it was validate a group of analytic methods for the diagnosis of the oxidative stress. The validated analytic methodology was permit to establish the Reference values in normal populations, an individualized diagnostic as base to establish the adequate medical prescription, to fallow the evolution of chronic oxidative disorder and follow the effect of nutritional or pharmacological intervention. The methodology include clinical marker of biomolecule damage, antioxidant enzymes, concentration of low level antioxidant and indicators of total antioxidant status. The analytic methodology was adaptable to micro and ultramicro analytic systems and was validated according to the international recommendations. Low cost, high precision, fast analysis and integral evaluation of the redox system in the main characteristic of those methods.

The study of an extensive array of oxidative stress indices permitted the examination of the role of oxidative stress in diabetic patients with macroangiopatic complications, HIV subjects, patients with neurodegenerative disorders, dengue infected people, etc. Those results was publishes in international journals. An efficient introduction of those methods in clinic involves educational programs for physicians or health care personnel.

S2-3

Protein oxidation and proteasome inhibition during UVA irradiation

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Photoaging is the result of chronic damage to the skin caused by intense and chronic exposure to sunlight. It is also known as a premature aging phenomenon of the skin with regard to the formed wrinkles and pigments. Photoaging is characterized by a remodeling of the extracellular matrix, catalyzed at least partially by the matrix metalloprotease 1 (MMP-1). In the present study we investigated the expression of MMP-1 in human cultivated fibroblasts. We determined a correlation between protein oxidation, proteasome inhibition and MMP-1 expression after 0–60 J/cm^2 UVA irradiation in human dermal fibroblast cells. Interestingly, proteasome inhibition by lactacystin is also able to increase MMP-1 expression, modeling therefore the proteasome inhibition during UVA exposure. This indicates that proteasome inhibition has a key role in the increase of MMP-1 expression. We performed an analysis of transcription factors modulated by proteasome inhibition and tested their role in MMP-1 expression. All experiments are also confirmed with proteasome inhibitor treatment of the cells. Our results demonstrate that proteasomal inhibition might play a key role in the UVA-induced photoaging of the skin.

S2-4

Flavonoids antioxidant actions: starting at the membrane?

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Epidemiological evidence demonstrates that diets rich in fruits and vegetables promote health by attenuating or delaying the onset of various diseases, e.g. cardiovascular and neurodegenerative diseases, diabetes, and cancer. The chemical components involved, and the physiological and molecular mechanisms by which fruit and vegetables reduce the risk of disease, are matters of investigation. Flavonoids are polyphenols that are gaining acceptance as responsible for the health benefits offered by fruit and vegetables. Because of their chemical structure, flavanols and related procyanidins (PC) can scavenge free radicals, inactivate other pro-oxidants, and can also interact with a number of biological molecules. We have investigated in Jurkat and Caco-2 cells the effects of epicatechin (EC) and related procyanidins (PC) on cell oxidation. The obtained results indicate that the primary targets of EC and PC seems to be localized at the cell membrane, leading mainly to the regulation NADPH-oxidase activity, and the consequent decreased superoxide production. The proposed interactions of EC and PC with cell membrane (proteins) could better explain the health benefits of flavanols and other flavonoids by making compatible actual tissue levels with the observed antioxidant effects.

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S2-5

Potential role of antioxidants in protection against photodamage of skin

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Skin is the largest body organ that serves as an important environmental interface providing a protective barrier that is crucial for homeostasis. Because of this barrier function it is constantly exposed to both environmental (e.g. UV radiation) insults together with other contaminants such as dietary or pharmacological agents that are capable of altering its structure and function.

Many environmental pollutants are either themselves oxidants or catalyze the production of reactive oxygen species (ROS) directly or indirectly. ROS are believed to activate proliferative and cell survival signaling that can alter apoptotic pathways that may be involved in the pathogenesis of a number of skin disorders including photosensitivity diseases and some types of cutaneous malignancy.

ROS act largely by driving several important molecular pathways that play important roles in diverse pathologic processes including ischemia–reperfusion injury, atherosclerosis, and inflammatory responses. The skin possesses an array of defence mechanisms that interact with toxicants to obviate their deleterious effect. These include non-enzymatic and enzymatic molecules that function as potent antioxidants or oxidant-degrading systems. Unfortunately, these homeostatic defences, although highly effective, have limited capacity and can be overwhelmed thereby leading to increased ROS in the skin that can promote the development of dermatological diseases.

An approach to rebalance oxidative stress related injury is by the administration of antioxidant agents through functional foods in our diet. The aim of this study is therefore to demonstrate the effects oxidative stress has on skin homeostasis and to highlight the key areas of research using dietary antioxidants to improve skin health.

S2-6

A Transient increase in oxidative stress promotes health and extends life span: the concept of mitohormesis

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Calorie restriction is generally accepted to extend life span in a variety of model organisms, and exerts health-promoting metabolic alterations in humans. We here show that 2-deoxy-D-glucose-mediated impairment of glucose metabolism in *C.elegans* extends life span, while increased glucose availability decreases life expectancy of nematodes. The latter is associated with impaired mitochondrial respiration and increased body fat content resembling mammalian obesity, whereas glucose restriction increases oxygen consumption and reduces triglyceride content. While the NAD-dependent histone deacetylase Sir2.1 is here found to be dispensable for life span extension, we rather observe that genetic disruption of *aak-2,* a *C.elegans* homologue of mammalian AMP-dependent kinase (AMPK), abolishes both induction of respiration as well as extension of life span in states of chemically impaired glycolysis. Moreover, we find that reduced glucose metabolism promotes formation of reactive oxygen species (ROS), induces activity of catalase, and increases stress resistance as well as survival in states of exposure to paraquat or sodium azide, altogether suggesting a process named mitochondrial hormesis ('mitohormesis'). Accordingly, pretreatment of nematodes with either NAC or antioxidant vitamins, specifically ascorbic acid (vitamin C) and the alpha-tocopherol (vitamin E) derivative trolox unambiguously fail to extend life span in the absence of deoxyglucose, but abolish extension of life span in states of impaired glycolysis. In summary, these data indicate that glucose restriction promotes mitochondrial metabolism in an aak-2 dependent manner leading to increased ROS formation to cumulate in an antioxidant-sensitive, hormetic induction of stress resistance, and finally extension of eukaryotic life span.

SESSION 3 — SELENIUM AND SEPSIS

S3-1
Selenoproteins, the thyroid hormone axis and redox regulation

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The essential trace element selenium is required for adequate thyroid hormone biosynthesis and metabolism of thyroid hormones. The thyroid gland has one of the highest selenium contents per gram tissue in humans and several selenoproteins are expressed at high levels. Glutathione peroxidase 3 (pGPx) is secreted by thyrocytes across the apical membrane into the colloid lumen, where it is involved in degradation of excess H_2O_2. H_2O_2 is produced lifelong at the apical extracellular surface of thyrocytes by thyrooxidase (DUOX) and acts as essential cosubstrate for iodide oxidation and iodothyronine coupling during thyroid hormone biosynthesis, which is catalyzed by the hemoprotein thyroperoxidase (TPO) in the extracellular colloid lumen. Other selenoproteins are expressed in thyrocytes and might contribute to cellular redox control and antioxidative defense (e.g. thioredoxin reductases, Sep15) or to local thyroid hormone deiodination (DIO1 & DIO2). Inadequate selenium supply has been associated with thyroid gland dysfunction (myxedematous cretinism, goiter growth, autoimmune thyroid disease). Though all three deiodinases (DIO) are selenoproteins, nutritional selenium deficiency has no major impact on their function except under conditions of long-term parenteral nutrition or in association with a defect of the SBP2 gene, which is involved in selenoprotein biosynthesis. Selenium supplementation has been shown to markedly improve two forms of autoimmune thyroid disease, M. Hashimoto and postpartum thyroiditis, both affecting mainly females with high incidence during their reproductive age. Therefore, a well balanced supply with the essential elements iodine, selenium and iron is mandatory to prevent developmental impairments related to thyroid dysfunction and to maintain adequate thyroid hormone synthesis and status later on during life.

Less clear is the relationship between selenium status, selenoproteins and various forms of diseases with strong inflammatory components, such as non-thyroidal illness (also called euthyroid sick syndrome or low –T3 syndrome), sepsis or chronic inflammatory diseases. Here, impaired production of the active thyroid hormone T3 by (hepatic) DIO1 and accelerated degradation of T3 by DIO3, expressed de novo in activated macrophages and other cells, are not directly linked to inadequate selenium status. Rather, disease associated alterations of selenoprotein synthesis and function appear to be mediated by enhanced production of proinflammatory cytokines, altered expression of growth factors and specific changes of the biosynthesis machinery of selenocysteine containg proteins, the "selenosome". Animal experimental data mimicking sepsis (LPS administration), acute phase reactions or "oxidative stress" provide evidence for marked redistribution of tissue and blood selenium stores, altered expression and function of redox-regulating and anti-oxidative selenoproteins (e.g. SelS), changes in ER-stress response, and significant sex differences among these beneficial or pathological adaptations. These observations indicate the need for adequate nutritional supply with essential trace elements and intensified research on mechanistic links between nutrients, hormones and adaptive (patho-)physiological responses.

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S3-2
The role of selenium in the critically ill patient

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Selenium deficiency is commonly associated with critical illness and with poor outcome. Supplementation with selenium has been shown to improve antioxidant capacity, as demonstrated by increased GPx activity. Several randomized controlled studies have investigated the possible effect of selenium supplementation on outcome. However, most of these studies were performed on relatively small patient populations presenting with trauma, burns, sepsis, or acute pancreatitis and thus are underpowered to detect a treatment effect on clinically important outcomes.

Angstwurm et al. demonstrated a reduction in the incidence of acute renal failure in patients suffering from SIRS who received supplemental selenium, even if their creatinine concentration was elevated at admission. Selenium supplementation has been associated with earlier normalization of thyroid profile in surgical ICU patients, significant reduction in bronchpneumonia events and shorter hospital stays in burn patients, and reduced mortality in septic patients and patients with acute pancreatic necrosis.

In an older meta-analysis of randomized controlled studies ($n = 7$) in 186 patients, selenium supplementation (alone or in combination with other antioxidants) was associated with a trend towards a lower mortality ($p = 0.09$), while non-selenium antioxidants were found to have no effect on mortality. Studies using high doses of selenium (500–1000 μg/day) were associated with a trend towards a lower mortality ($p = 0.1$), whereas studies using lower selenium doses (< 500 μg/day) showed no effect on mortality. In a recent prospective, randomized placebo controlled, multicenter trial by Angstwurm and colleagues, 249 patients with severe SIRS, sepsis and septic shock were randomized to receive selenium or placebo. Patients in the study group received 1000 μg sodium selenite for 14 days. The primary end point of 28-day mortality was significantly reduced to 42.4% in the treatment group compared to 56.7% in the placebo group. In predefined subgroup analyses, the mortality rate was significantly reduced in patients with septic shock with disseminated intravascular coagulopathy as well as in the most critically ill patients with APACHE III scores > 102 or more than 3 organ dysfunctions. A more recent meta-analyisis that comprises this trial a statistical significant benefit for mortality reduction by selenium supplementation was found.

Selenium supplementation in critical illness and other disease states is promising; however, large randomized multicenter studies are needed to definitely confirm the beneficial effects of selenium supplementation in critically ill patients.

S3-3

Why selenium rather than sulphur catalysis in peroxidases? More we learn, less we understand it

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Hydrogen peroxide is both a byproduct of oxygen utilization during respiration and a physiological oxidant, build up to faith pathogens and act as a chemical signal in signal transduction cascades. Irrespective of its final use, hydrogen peroxide has also to be quickly removed to prevent collateral damage. A pivotal enzyme for this is the Selenocysteine (Sec)-containing enzyme Glutathione Peroxidase (GPx1), the first discovered of a family encompassing to date eight mammalian proteins. The amazing catalytic efficiency is provided by the reactivity with hydrogen peroxide of the selenol at the active site, in turn attributed to its low pK_a. This redox reaction is indeed much faster than the enzyme substrate interaction, giving rise to unusual non-saturation kinetics where the calculated rate constant for the oxidation of the active site selenol (k_{+1}) is around 10^8 M^{-1} sec^{-1}. This peculiar reactivity is assumed similar for the other SecGPxs, although careful kinetic data are available only for GPx1 and 4.

However, in the last years, new available information challenged the notion that the peculiar reactivity of GPxs can only be obtained when the redox moiety is selenol. First, a large number of GPxs have been identified in all living kingdoms, where the highly conserved active site contains a Cys residue in place of the redox-active Sec. As suggested by kinetic analysis of the *Drosophila melanogaster* variant (*Dm*GPx), these CysGPxs are predicted only marginally less reactive with the hydroperoxide than the SecGPxs. Similarly, in peroxiredoxins, the oxidation of the peroxidatic Cys by hydrogen peroxide takes place with a k_{+1} in the range of 10^7 M^{-1} sec^{-1}. Thus, apparently, when properly activated, a thiol can substitute for a selenol without affecting fast reduction of hydroperoxides. This raises the issue of the actual relevance of having a Sec residue in the active site.

Aiming to better define the constraints for Se or S activation at the active site of GPxs, we obtained an updated picture of the active site of these enzymes by analyzing more than 700 structures for sequence homology and molecular modeling. Data were implemented with activity measurement and kinetic analysis of the *Dm*GPx as a model, where some conserved residues were substituted by site directed mutagenesis. Our results suggest that the thiol or the selenol is activated by H bonding to the nitrogens of three strongly conserved residues, namely Gln 80, Trp 135 and Asn 136, while computational calculation of pK_a and kinetic analysis, suggest that the pK_a of the redox moiety is less relevant than H bonding. The deduced reaction mechanism suggests that, instead, the polarization of the peroxidic substrate and protonation of the hydroxyl-leaving group play a major role in accelerating the peroxidatic reaction. In conclusion, the previous notion that in nature Se is preferred to S just for a much faster reduction of hydroperoxides is not convincing anymore. Moreover, further complexity is added from phylogenetic analysis. While the ancestor of all GPxs is a CysGPx, and substitution of active site Cys with Sec is a relatively recent acquisition, for some GPxs an unexpected shift back to Cys is apparently taking place. In conclusion, from post-genomic acquisitions, unraveling the actual relevance about the use of Se rather than S in GPx catalysis, far from being clarified, appears more complex than before.

SESSION 4 — OXIDANTS AND SIGNALLING

S4-1

Signaling by alpha-tocopheryl phosphate

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Vitamin E is a typical antioxidant when studied in a test tube. However, like other antioxidants, in vivo this molecule has different properties, which are used to trigger cell information transfer and modulate gene transcription. We have shown that alpha-tocopherol (a-T) but not the very similar beta-tocopherol, is able to inhibit a master switch in cells, protein kinase C and consequently cell proliferation, inhibition of the NADPH oxidase assembly and inhibition of O2- production. Inhibition by a-T of the transcription of the gene coding for the scavenger receptor CD36 was the first observation which was followed by a number of studies that have shown that several genes are regulated by vitamin E and none of them codes for antioxidant enzymes. Such an expression would be expected as a compensatory mechanism as a consequence of vitamin E diminution, in case that its action where that of an antioxidant. All the above findings support the hypothesis that a-T does not act as an antioxidant. Recently, we have searched for an a-T derivative, more active than a-T. Alpha-tocopheryl phosphate (a-TP) has been found in normal tissues. a-TP is synthesized and hydrolyzed in animal cells and tissues; it modulates also several cell functions. While it is similar to a-T, a-TP appears to be more potent than a-T in inhibiting cell proliferation, down regulating CD36 transcription, inhibiting atherosclerotic plaque formation etc. a-TP does not act by liberating a-T; rather, the intact molecule appears to be more potent than a-T. Administration of a-TP to cells or to animals requires its transfer through membranes, an event that cannot occur by simple diffusion due to the size and the charge of the molecule but requires a transporter. Specific transport inhibitors, glybenclamide and probenecid showed no inhibition of cell proliferation or cytotoxicity. However, both compounds prevented, dose-dependently α-TP inhibition of cell proliferation. The data indicate that α-TP enters cells via a glybenclamide and probenecid-sensitive transport system. Both, members of the ABC transporter family and of the organic anion transporters (OAT), appear to be sensitive to these two inhibitors. However, since ABC transporters function to export cell solutes and α-TP is imported, a-TP transport may occur via an OAT family member. Finally, by GeneChip U133 Plus 2.0 Gene Array (Affymetrix) (further analyzed using the MEV software) 202 genes in THP-1 cells were found to be significantly regulated more than 2-fold by a-TP. Of 68 genes upregulated more than 5-fold, 44 uniquely regulated by a-TP and not by a-T.

S4-2

Insulin-mimetic stimulation of cellular signaling cascades by heavy metal ions

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Exposure of various different types of cultured mammalian cells to stressful stimuli such as redox-active or thiol-reactive metal ions (Cu^{2+}, Zn^{2+} and Cd^{2+}) resulted in phosphoinositide 3′-kinase (PI3K)-dependent activation of the Ser/Thr kinase Akt, followed by a PI3K/Akt-dependent phosphorylation of several Akt substrates, including transcription factors of the FoxO family. As a consequence of metal-induced phosphorylation the inactivation and nuclear exclusion of FoxO1a was observed in hepatoma cells. Furthermore, activity of a known FoxO-responsive promoter (the glucose 6-phosphatase promoter) was strongly impaired in cells exposed to Cu^{2+}. Stimulation of the PI3K/Akt cascade by heavy metal ions neither required the cellular generation of reactive oxygen species to propagate signals nor did it depend on the activation of a receptor tyrosine kinase (RTK), pointing to a direct interference of Cu^{2+} or Zn^{2+} with regulators of PI3K/Akt signaling downstream of RTK. Different from copper and zinc, the exposure of cells to Mn^{2+}, Fe^{2+}, Co^{2+}, Pb^{2+} did not affect phosphorylation of Akt. In line with this finding, no significant modulation of the activity of a FoxO-responsive promoter construct was observed. As with mammalian cells, the FoxO ortholog DAF-16 was inactivated in *C. elegans* worms exposed to Cu^{2+}, pointing to a conserved stress response machinery. In summary, exposure of cells to stressful stimuli imitates insulin signaling independent of a physiological stimulator (such as insulin) and independent of the generation of reactive oxygen species.

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S4-3

NAD kinase levels control the NADPH concentration in human cells

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NAD kinases (NADKs) are vital, as they generate the cellular NADP pool. As opposed to three compartment-specific isoforms in plants and yeast, only a single NADK has been identified in mammals whose cytoplasmic localization we established by immunocytochemistry. To understand the physiological roles of the human enzyme, we generated and analyzed cell lines stably deficient in or overexpressing NADK. Short-hairpin-RNA-mediated down-regulation led to similar (about 70%) decrease of both NADK expression, enzyme activity and the NADPH concentration, and was accompanied by increased sensitivity towards H2O2. Overexpression of NADK resulted in a 4–5 fold increase in the cellular NADPH concentration. No changes of NADP+ were observed, although the recombinant enzyme phosphorylated preferentially NAD+ suggesting that newly synthesized NADP+ is readily reduced by dehydrogenases.Surprisingly, NADK overexpression and the ensuing increase of the NADPH level only moderately enhanced protection against oxidant treatment. Apparently, the maintenance of the NADPH level for the regeneration of oxidative defense systems in human cells depends primarily on NADP-dependent dehydrogenases (which re-reduce NADP+), rather than on a net increase of NADPH. The stable shifts of the NADPH level in the generated cell lines were also accompanied by alterations in gene expression, for examples, of peroxiredoxin 5 and Nrf2. Since the basal oxygen radical level in the cell lines was only slightly changed, we propose that the redox state of NADP may be a major transmitter of oxidative stress.

SESSION 5 — HUMAN APPLICATIONS

S5-1
Hypercholesterolemia and age related diseases

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Hypercholesterolemia is a major risk factor for age related diseases such as atherosclerosis and Alzheimer's disease (AD). Changes in human plasma cholesterol levels results from the interaction between multiple genetic and environmental factors. The accumulation of excess cholesterol in blood vessels leads to atherosclerosis. During atherosclerosis, lipoproteins such as LDL become trapped at the site of lesion and are converted to oxLDL, which contains both oxidized proteins and lipids. In this process, scavenger receptors could play a critical role because of their ability to bind oxLDL and their function in transporting lipids and cholesterol into and out of the cells. Studies show that differential expression of oxidative stress proteins, lipid metabolism related enzymes and receptors response to atherogenic diet. Excess brain cholesterol has been associated with increased formation and deposition of amyloid-β peptide from amyloid precursor protein which may contribute to the risk and pathogenesis of AD. More than 50 genes have been reported to influence the risk of late-onset AD. Several of these genes might be important in cholesterol metabolism and transport. On the basis of these results, an *in vivo* study has been carried out. Rabbits were fed with cholesterol supplemented diet, after 4 weeks aortas and brains were removed. Atherosclerotic index calculated from the lesions stained by Sudan V. Scavenger receptor expression was measured in aortic pieces. On the other hand proteasome function, protein carbonylation, tau hyperphoshorylation and amyloid-β protein evaluated in the brain of hypercholestrolemic rabbits. The results indicate a cellular mechanism for hypercholesterolemia induced atherosclerosis and AD similar changes will be discussed.

S5-2
Oxidative stress and antioxidant response after an Ironman triathlon

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Background: There is overwhelming evidence that physical activity harvests a lot of beneficial physiological effects that improve physical fitness and plays a major role in the prevention of various chronic disease states. However, some data implicate that an exceptional high volume of exercise increases the risk of developing cardiovascular disease, probably due to accumulative oxidative stress [1]. Training appears to enhance endogenous antioxidant defences, but is unknown whether these adaptations are sufficient to prevent sustained oxidative damage in ultra-endurance athletes.
Objectives: The present study was aimed to examine changes in oxidative stress markers as well as antioxidant status and capacity following an Ironman triathlon (3.8 km swim, 180 km cycle, 42.2 km run) and whether there are indications for persistent oxidative stress such as oxidative modifications of low-density lipoproteins (LDL), and thus health consequences [2].
Methodology: Blood samples were taken from 42 well-trained male triathletes (mean $\pm$ SD: 35.3 $\pm$ 7 years, height: 180.6 $\pm$ 5.6 cm, weight: 75.1 $\pm$ 6.4kg, $VO_{2\,peak}$: 56.6 $\pm$ 6.2 ml kg^{-1} min^{-1}) 2 days (d) before an Ironman triathlon, then immediately post-race, 1, 5 and 19 d later. Blood was analysed for conjugated dienes (CD), malondialdehyde (MDA), oxidised LDL (oxLDL), oxLDL:LDL ratio, advanced oxidation protein products (AOPP), AOPP:total protein (TP) ratio, Trolox equivalent antioxidant capacity (TEAC), uric acid (UA) in plasma, and activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) in erythrocytes.
Results: The average completion time was 10 h 52 min $\pm$ 61 min. Immediately post-race there were significant increases in CD, AOPP, TEAC, UA (for all $P < 0.001$) and AOPP:TP ($P < 0.01$). MDA rose significantly ($P < 0.01$) 1 d post-race, while CD ($P < 0.01$), AOPP ($P = 0.01$), AOPP:TP ($P < 0.05$), TEAC ($P < 0.001$) remained elevated. OxLDL:LDL trended to increase, whereas oxLDL significantly ($P < 0.01$) decreased 1 d post-race. Except for GSH-Px ($P = 0.08$), activities of SOD ($P < 0.001$) and CAT ($P < 0.05$) significantly decreased post-race. All oxidative stress markers had returned to pre-race values 5 d post-race. Moreover, relationships between training status and oxidative stress markers, TEAC and antioxidant enzyme activities were noted.
Conclusion: The major finding of the current study was that there are no indications of persistent oxidative damage after an Ironman triathlon, despite a transient increase in most, but not all oxidative stress markers. Our data indicate that training- and exercise-induced antioxidant responses in well-trained athletes were capable to counteract long-term detrimental health effects due to oxidative stress.

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S5-3

Is iron a mediator in PDT-induced cytotoxicity?

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Photodynamic therapy is a promising antitumor treatment. It involves the administration of a photosensitizer which selectively accumulates in target cells followed by exposure of the cells to a light source. The reaction of the photosensitizer with light generates reactive oxygen species that cause cellular necrosis and apoptosis. However, antitumor effects are limited to just a few centimetres because of limited light penetration and due to an initiation of rescue responses of the tumour cell. This allows tumour cells to cope with oxidative stress. It is assumed that the induction of heme oxygenase 1 (HO-1) is a central part of the antioxidative defence in cells. In order to enhance the efficacy of PDT treatment our study was aimed to investigate the combination of PDT with inhibitors of HO-1 and to identify mediators of PDT-induced cytotoxicity. In a series of experiments human melanoma cells were loaded with the photosensitizer ALA and exposed to nonthermal light of 420–800 nm with doses between 0.54 and 5.4 J/cm^2. It was shown that inhibition of HO-1 activity by ZnPPIX increased PDT-induced cytotoxicity in a dose-dependent manner. Interestingly, the cytotoxic effects were not enhanced by the simultaneous application of the iron chelator desferrioxamine. These results show that iron does not act as a mediator of PDT-induced cytotoxicity. In further experiments the identification of possible mediators should be addressed in order to understand the mechanisms during PDT treatment and to enhance its efficacy.

SESSION 6 — PROTEIN OXIDATION AND PROTEOLYSIS

S6-1

The misincorporation of oxidised amino acids into proteins as an experimental tool

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The accumulation of oxidised proteins in cells and tissues is a feature of ageing and of many age-related diseases. The reasons why oxidised proteins accumulate and the functional consequences of their accumulation in cells are not fully understood.Protein oxidation in vivo commonly results in the in situ modification of amino acid side chains, generating new oxidised amino acid residues in proteins. We have shown that oxidised amino acids that are close structural analogues of protein amino acids can be (mis)incorporated into proteins by protein synthesis allowing oxidatively modified proteins to be generated in vitro to model those present in diseased tissues [1,2]. Using this novel approach, we can examine the intracellular metabolism of specific oxidised proteins and their impact on cell function.The introduction of oxidised amino acids into newly synthesised proteins generally results in accelerated protein turnover [1,2]. Certain oxidised amino acids however, such as DOPA (3,4 dihydroxyphenylalanine), can generate proteins that are resistant to degradation and accumulate in cells [1,3]. We show that the accumulation of DOPA-containing proteins in cells can cause apoptosis through a mechanism involving lysosomal membrane destabilisation and activation of caspase 3. When cells were incubated with D-DOPA (which is not incorporated into proteins) or with L-DOPA, under conditions where L-DOPA is not incorporated into proteins, there was no increase in apoptosis, indicating that DOPA-containing proteins are pro-apoptotic. Since proteins containing DOPA are present in many pathological tissues the accumulation of oxidised proteins in lysosomes could promote cell death by apoptosis in vivo by this mechanism. These results have important implications in Parkinson?s diseasewhere L-DOPA is the primary symptomatic treatment and can be incorporated into proteins in vivo.

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S6-2

Inducibility of the proteasome and of the lon protease in oxidative stress, disease, and ageing

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Proteins are major targets for oxidative damage and both the intracellular accumulation of oxidized proteins has been reported in many ageing and disease models. In young and healthy individuals, moderately oxidized soluble cell proteins are selectively and rapidly degraded by the Proteasome in the cell cytoplasm, nucleus, and endoplasmic reticulum. Inside mitochondria, the matrix proteinase called 'Lon' selectively degrades oxidized soluble proteins. Severely oxidized, aggregated, and cross-linked proteins, however, are poor substrates for degradation and actually inhibit the Proteasome and the Lon protease. In young cells, expression of the Lon protease, and of selected Proteasome subunits and regulators, can be induced by exposure to mild oxidative stress. Indeed, our recent work suggests that Lon, and certain proteasome subunits and regulators should be classified as true stress proteins. Induction of Lon/Proteasome expression provides significantly greater capacity to remove oxidized proteins, and such adaptive responses provide important, but transient, stress resistance. In older cells, however, both Lon and Proteasome activities decline, and adaptational responses become sluggish or even ineffectual. Studies in animals and humans suggest that declining Proteasome/Lon activities and, perhaps, declining responsiveness to stress, may contribute to the ageing process, and to various age-associated diseases.

S6-3

Advanced glycation endproducts – rather cause than consequence of human skin ageing

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In our approach we investigated if Advanced Glycation Endproducts (AGEs) do play a role in skin ageing. Therefore we developed a biological method using glyoxal to mimic the perennial modification of fibroblasts. These modifications result in a structural breakdown of the intermediate filament protein vimentin being the major target for glycation. The modification of vimentin is neither based on a slow turnover of this protein nor on an extremely high intracellular expression level, it is based on structural prerequisites. The collapse of vimentin results in a loss of the contractile capacity of fibroblasts – which is an essential ability to rearrange the structure of collagen and therefore required for the regeneration of tissues. Using our approach we were able to detect glycated, aggregated vimentin bundles in human skin of aged individuals and thus, linking the glycation reaction to skin ageing.

WORKSHOPS

WORKSHOP 1 — LIPID PEROXIDATION AND RELATED DISEASES

WL1-1
Oxidized lipids and vascular remodelling in atherosclerosis

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Inflammatory reactions in the arterial wall are unanimously recognized to play a crucial role in the progression of atherosclerosis and cardiovascular disease and appear as primarily triggered and sustained by lipid oxidation products. Indeed, lipid oxidation products (LOPs) such as oxidized cholesterol (oxysterols) and oxidized cholesterol esters (9-ONC), 4-hydroxynonenal (HNE), oxidized phospholipids (OxPLs) have been shown to consistently accumulate in atherosclerotic lesions, and to exert strong pro-inflammatory, pro-apoptotic and pro-fibrogenic effects. LOPs are most likely involved in all main steps of arterial wall remodelling due to atherosclerosis, such as endothelial dysfunction, adhesion and migration of circulating blood cells, vascular smooth muscle cell and fibroblast migration and proliferation, cell differentiation and foam cell formation, deposition of interstitial fibrous matrix and fibrous cap formation. As in the case of classic chronic inflammation characterized by granuloma, the key-players in both formation and expansion of the atherosclerotic lesion are cells of the macrophage lineage. Significant experimental evidence is already available in support of PLOs-mediated chemotactic attraction and differentiation of monocytic cells and eventual foam cell formation, in particular as induced by cholesterol, but only once the sterol is oxidized with consequent generation of oxysterols. Identification of main signalling pathways triggered and stimulated by oxysterols and other LOPs in macrophage-dependent vascular remodelling is now under progress.

WL1-2
Lipid peroxidation products in foodstuffs and colon cancer

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4-Hydroxynonenal (HNE), 4-hydroxyhexenal (HHE) and malondialdehyde are secondary oxidation products of omega-6 polyunsaturated fatty acids (PUFA), omega-3 PUFA, and PUFA bearing more than two double bonds, respectively. Those products are cytotoxic and genotoxic. HNE and MDA are present in tissues and fluids at a physiological range and their concentration is increased under numerous pathological conditions such as inflammatory diseases.As both PUFAs and oxidant compounds such as heme iron are present in foodstuffs, those secondary oxidation products of PUFAs can also be found in relatively high concentrations, particularly in meat and processed meat, but also in oxidized edible oils and infant milk formulas.In rats fed diets rich in omega-6 PUFA and heme iron, we have investigated the presence of HNE in the colon lumen and the consequences on colon cancer promotion, together with a urinary metabolite of HNE. We have also tested the cyto- and geno-toxic effects of HNE, HHE and MDA in vitro in immortalized mouse colon cell lines, bearing or not a mutation on the Apc gene, which represents an early event in human colon carcinogenesis.Secondary oxidation products of PUFA, and especially HNE, could be one of the missing link between heme iron content of the diet, luminal lipid peroxidation and increased risk of colon cancer development.

WL1-3

F2-Isoprostanes: sensitive biomarkers of oxidative stress

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Isoprostanes are prostaglandin-like compounds derived from the free radical-initiated peroxidation of arachidonic acid. They have been shown to represent highly specific and sensitive biomarkers of oxidative stress in a variety of experimental and clinical studies. We have analysed F2-isoprostanes by gas chromatography-mass spectrometry in different tissues (brain, lung and heart), in isolated rat brain mitochondria, in HaCaT keratinocytes, in microdialysates of human skin, in human plasma samples after application of flavonoid-rich cocoa drink and after exercise and in different human disorders (e.g. hemodialysis patients with end stage renal disease). Results from these studies reveal the significance of F2-isoprostanes as useful indicators of oxidative injury in vitro and in vivo. Data will be presented showing that F2-isoprostanes are associated with cyclooxygenase-mediated inflammation:

1. Low-dose UVB irradiation significally enhanced the concentrations of F2-isoprostanes and prostaglandins in vitro in human HaCaT keratinocytes and in vivo in the interstitial fluid of the dermis in a dose-dependent action. The application of non-toxic concentrations of diclofenac (non-steroidal anti-inflammatory drug; cyclooxygenase inhibitor) lowered not only the levels of prostaglandins, but of F2-isoprostanes, too.
2. In serum samples of hemodialysis patients, F2-isoprostanes were found to be markedly enhanced versus those of healthy control persons and they correlated to the levels of C-reactive protein indicating a link between oxidative stress and inflammation.

Furthermore, we have shown that in addition to arachidonic acid, isoprostane-like compounds (neuroprostanes) can also be formed from docosahexaenoic acid (C22:6 w-3).

WL1-4

Altered lipid metabolism and redox sensitive gene expression in fetal vascular cells in pre-eclampsia

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Endothelial dysfunction and activation are believed to be responsible for vascular abnormalities in the fetal and maternal circulation in pre-eclampsia (PE), with the ischemic placenta implicated as a source of reactive oxygen species (ROS) (see Afzal-Ahmed et al. 2007; Free Radic. Biol. Med. 42:1781–1790). PE is characterised by altered fatty acid metabolism, affecting triglyceride levels and metabolism of arachidonic acid (AA). In this context, we have reported increased Ca2+ responses to AA in human umbilical artery smooth muscle cells isolated from PE pregnancies, which are mimicked in cells from normotensive pregnancies by inhibition of either cyclooxygenase or lipoxygenase pathways (Steinert et al. FASEB J 2003;17:307–309). Reduced AA levels in PE placental tissue suggests an increased conversion of AA into vasconstrictor metabolites. Increased oxidative stress and generation of thromboxane by placental trophoblast cells during the progression of PE may underlie our finding that transcriptional regulation of the redox sensitive genes HO-1 and NQO1 via Nrf2 is significantly impaired in fetal endothelial and smooth muscle cells (see Mann et al. 2007; Acta Physiol. Sinica 59:117–127; Mann et al. 2007; Cardiovasc. Res. 2007;75:261–274). As these phenotypic changes in fetal vascular cells persist in culture, they may have implications for long-term programming of the fetal cardiovascular system. In summary, dyslipidemia together with sustained oxidative stress contribute significantly to the pathology of this pregnancy-associated disease.

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WL1-5

Chlorinated and oxidized lipids in Inflammation

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Inflammatory diseases involve activation of phagocytic cells to produce reactive oxygen species that can cause oxidative stress by attacking a variety of biomolecules. Myeloperoxidase is able to generate hypochlorous acid (HOCl), which can also cause chlorination of proteins and lipids. Polyunsaturated lipids are susceptible to free radical oxidant attack and generate a variety of lipid oxidation products, while HOCl can additionally attack monounsaturated lipids to form chlorohydrins, and plasmalogens to release ?-chlorofattyaldehydes. There has been considerable interest in the proinflammatory properties of oxidized phospholipids and oxysterols, and it is known that they can induce leukocyte-endothelial adhesion, chemokine production, and modulate cellular signalling pathways. Although there have been fewer studies on the biological effects of chlorinated lipids, there is also evidence that these can upregulate cell arterial adhesion molecule expression, mainly P-selectin, causing increased leukocyte-endothelial adhesion, as well as increasing the production of reactive oxygen species from phagocytes. One possible mechanism by which oxidized lipids could induce inflammatory responses involves interaction with the Toll-like receptors, which are present on many cells and recognize pathogen associated molecular patterns (PAMPs) in order to detect infection and damage to host tissues. TLRs have been shown to contribute to the progression of atherosclerosis and other inflammatory diseases. Studies of the role of TLRs in the bioactivity of oxidized lipids have proved controversial, but evidence is now accumulating to support the idea that oxidized phospholipids inhibit TLR responses to PAMPs, rather than activating the receptors themselves. Thus in some situations oxidized lipids may have antinflammatory effects, and overall it can be concluded that oxidized lipids have complex and pleiotrophic biological effects.

WORKSHOP 2 — MITOCHONDRIA AND AGING

WL2-1

Manipulation of molecular pathways increasing the health span of the fungal ageing model Podospora anserina

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The ascomycete Podospora anserina is a filamentous fungus with and limited life span which is controlled by environmental and genetic traits. Mitochondria play an important role in the network of pathways modulating both the life and the health span of this system. In particular, respiration and the generation of reactive oxygen species (ROS), ROS scavenging and maintenance of a healthy population of mitochondria are known constituents of the relevant molecular machinery. Experimental approaches towards the identification and modulation of pathways leading to an increased healthy period in the life time of the investigated system will be presented and discussed.

WL2-2

Caloric restriction, oxidative stress and ageing: a proteomic view

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Caloric restriction has been proved to be an effective intervention to combat ageing. Yet it is mystery how CR retards ageing and prolongs average and even healthy liveable lifespan. ROS mediated damage of macromolecules like DNA, proteins and lipids is considered as a root-cause behind the ageing processes. Mitochondria are not only prominent targets for ROS-induced damage but also the major source of ROS. Therefore, analysis of the protein profile of mitochondria and its age-dependent variation is a promising approach to unravel mechanisms involved in ageing and CR. We compared protein profiles of rat liver mitochondria to understand the interplay between ageing and caloric restriction. The combination of two powerful techniques employed, blue-native PAGE and fluorescence difference gel electrophoresis (fluorescence-DIGE), detected with high sensitivity and accuracy the changes in abundance as also in protein-protein interactions of both soluble and membrane proteins. We could differentiate the effects specific to aging and caloric restriction, which mainly involved energy transduction and detoxification pathways besides anti-oxidant and cell death mechanisms. In addition to the expected age-reversing effects of caloric restriction, which were short-term or long lasting, surprisingly ageing-analogous alterations were also observed.

An additional insight for the ageing process was obtained by analysis of the mitochondrial proteome of three brain areas and whole brain of rats. The ageing-associated decrease in abundance of MF_oF_1 ATP synthase besides the changes in its oligomeric status might be an important clue to understand the link between respiration and longevity. Equally interesting were the abundance changes in OXPHOS supercomplexes, the natural stoichiometric super-assemblies of respiratory complexes I, III, and IV, as observed in young and aged cortex tissue. With our knowledge of the 3D-structure of supercomplex $I_1III_2IV_1$ and of the respective electron carrier binding sites, we aim to understand the age-related changes in supramolecular architecture of OXPHOS complexes in context to the often described rise in ROS production.

The data discussed and the methodologies described are also of high relevance for elucidating the molecular basis of age-related diseases such as Alzheimer's and Parkinson's disease.

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WL2-3

Identification and physiological function of a yeast NADPH oxidase

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Superoxide radical ions are produced in eukaryotic cells by the mitochondrial electron transport chain, but also by specialized membrane bound NADPH oxidases. Superoxide and its reaction products have been discussed previously mainly as waste products that cause cellular aging and pathologies, as well as acting as defence against pathogenic microbes when produced by phagocytes. Recently, superoxide (and/or hydrogen peroxide) are believed to be physiological signaling substances, most probably transmitting a positive signal for growth and cell cycle progression.

NADPH oxidases were previously unknown in yeast cells. We are showing here that only one of the 9 yeast genes which show a significant sequence similarity to mammalian NOX is indeed a strong producer of superoxide. Consequently, we have named this yeast gene NOX1. Overexpression of NOX1 in yeast cells leads to slow growth and to cell death via apoptosis. Deletion of the yeast caspase, YCA1, prevents death induced by NOX1. However, in a strain deleted for NOX1 we did not find an obvious growth phenotype. NOX1 is under transcriptional control through the RAS/cAMP signaling system of yeast. However the strong ROS production in RAS2ala18, val19 is not completely compensated by deleting NOX1 or by making the cell respiratory deficient (rho-zero), or by deleting NOX1 in a respiratory-deficient (rho-zero) strain. This indicates that also other, still unknown sources of superoxide exist in the yeast cell.

WORKSHOP 3 — PROTEOMICS AND AGEING

WL3-1
Longevity assurance molecular pathways in human cells

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Ageing and longevity are two multifactorial biological phenomena whose knowledge at molecular level is still limited. We have developed a clonal senescence induced system and we have cloned several senescence associated genes. Analysis of the function of one of the isolated genes, encoding for Clusterin/Apolipoprotein J (CLU), suggests that it is a novel survival factor. CLU is found over-expressed in vitro under a variety of stress conditions and in vivo in samples from patients suffering from various age-related diseases as well as in primary tumours which have acquired chemotherapeutic drug resistance (Int J Cancer 120, 611–622, 2007). In addition, it has been demonstrated that inhibition of endogenous CLU expression by RNA interference induces growth retardation, higher rates of endogenous cellular death and sensitizes human cells to stress (Cancer Res 64, 1834–1842, 2004). Recent findings indicate that effective and sustained CLU depletion by siRNA induces late morphological alterations, growth arrest at the G_1/S checkpoint and activation of the mitochondrial axis of apoptosis that engages caspase-9. Moreover, CLU knock-down resulted in down regulation of the BH pro-survival (bcl-2 and bcl-X_L) proteins and activation of p53 and its downstream targets, namely $p21^{WAF1/CIP1}$ and bax.

We have also attempted an overall molecular and biochemical approach regarding proteasome function in replicative senescence and cell survival. We have observed reduced levels of proteasomal peptidase activities coupled with increased levels of oxidized and ubiquitinated proteins in senescent cells. We have found the catalytic subunits of the 20S complex and subunits of the 19S regulatory complex to be down-regulated in senescent cells. This is accompanied by a decrease in the level of both 20S and 26S complexes (J Biol Chem 278, 28026–28037, 2003). In support, partial inhibition of proteasomes in young cells by specific inhibitors induced a senescence-like phenotype. Stable over-expression of β subunits or POMP in human cell lines resulted in enhanced proteasome assembly and activities and increased cell survival following treatments with various oxidants. Moreover, stable over-expression of β_5 subunit delayed senescence in human fibroblasts (J Biol Chem 280, 11840–11850, 2005). Finally in search of natural compounds that may activate proteasome, we have identified that the main constituent of olives, oleuropein, exerts stimulatory effects on proteasome. Importantly, continuous treatment of human fibroblasts cultures with oleuropein delays senescence by approximately 15% (Rejuven Res 10, 1570172, 2007).

WL3-2
Oxidized protein degradation and repair: implications in ageing and oxidative stress

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Accumulation of oxidatively modified proteins is a hallmark of cellular ageing. However, not all proteins are equally sensitive to oxidative damage and evidence has been provided that certain proteins would be preferentially affected. Oxidized protein buildup with age may be due to increased protein damage, decreased elimination of oxidized protein (i.e. repair and degradation), or the combination of both mechanisms and increasing experimental evidence has indicated that failure of protein maintenance is indeed a major contributor to the age-associated accumulation of oxidized proteins. Oxidized protein degradation is mainly achieved by the proteasomal system in cytosol and nucleus while the Lon protease has been implicated in oxidized protein degradation within the mitochondrial matrix. We have previously reported that the age-related accumulation of oxidized and glycoxidized proteins in rat liver mitochondrial matrix is associated with an alteration of the ATP-stimulated Lon-like protease activity. It is now established that proteasomal function is generally impaired with age and declining proteasome activity with age has been attributed to either or both decreased proteasome subunits expression, inactivation upon alteration of proteasome subunits and accumulation of endogeneous inhibitors such as highly oxidized and cross-linked proteins upon formation of lipid peroxidation and glycoxydation adducts. To gain further insight in the mechanisms that might be implicated in the decreased activity of the proteasome with replicative senescence, the occurrence of proteins modified by glycoxidation and conjugation by lipid peroxidation products has been investigated in senescent cells.

Oxidized protein repair is limited to few modifications, such as methionine oxidation, that can be catalytically reversed within proteins by the methionine sulfoxide reductase enzymes, MsrA and MsrB. Msr function has been shown to be impaired with age and during replicative senescence of human diploid fibroblasts. To analyze the relationship between oxidative stress, protein oxidative damage and Msr, MsrA has been overexpressed in SV40 WI-38 human fibroblasts and MsrB2 has been overexpressed in Molt-4 lymphoblastoid cells. After hydrogen peroxide-induced oxidative stress, both MsrA- and MsrB-overexpressing cells exhibit lower reactive oxygen species production and protein oxidative damage indicating that the Msr system may play an important role in cellular defenses against oxidative stress by preventing the accumulation of oxidized proteins.

WL3-3

Role of IGFBPs in premature senescence

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Exposure of human proliferative cell types such as human diploid fibroblasts (HDFs) to acute stress with subcytotoxic concentrations of oxidative agents or DNA damaging agents results in Stress-Induced Premature Senescence (SIPS). Among the common biomarkers between replicative senescence and SIPS are the appearance of a senescent-like morphology of the fibroblasts, senescence-associated beta-galactosidase (SA beta-gal) activity, irreversible growth arrest in G1/S and similar mRNA steady-state levels of several senescence-associated genes. Transforming Growth Factor (TGF)-ß1 plays an essential role in premature senescence. Indeed TGF-ß1 activation is responsible of the appearance of several biomarkers of senescence [1, 2, 3]. In order to define the premature senescence phenotype, transcriptomic studies were achieved with low density DNA arrays [2, 3]. These studies revealed an overexpression of three members of the IGFBP family (IGFBP-3, IGFBP-5 and IGFBP-8 (also known as CTGF))in premature senescence of lung or skin HDFs induced by tert-butylhydroperoxide, ethanol or UVB [2, 3]. Western blotting and immunofluorescence showed that the protein abundance of these IGFBPs were also increased in premature senescence. In order to define the role of these IGFBPs in premature senescence, we used siRNA approach and showed that these IGFBPs regulate the appearance of several biomarkers of senescence [3]. The relationship between these IGFBPs and TGF-ß1 is also presented.

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WORKSHOP 4 — BIOMARKERS OF AGING

WL4-1

MARK-AGE: towards the establishment of biomarkers of human ageing

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The rate of ageing in humans is not uniform, due to genetic heterogeneity and the influence of environmental factors. Age-related changes in body function or composition that could serve as a measure of biological age and predict the onset of age-related diseases and/or residual lifetime are termed biomarkers of ageing. Many candidate biomarkers have been proposed but in all cases their variability in cross-sectional studies is considerable, and therefore no single measurement has so far proven to yield a useful biomarker of ageing on its own, probably due to the multi-causal and multi-system nature of ageing. Within the Seventh Framework Programme, the EU Commission has recently selected the MARK-AGE Project proposal for funding. MARK-AGE is a large-scale integrating project involving a total of 26 beneficiaries from 14 countries (www.MARK-AGE.eu). In the context of the project a population study (3,700 probands) will be conducted to identify a set of biomarkers of ageing which, as a combination of parameters with appropriate weighting, would measure biological age better than any marker in isolation. A wide range of candidate biomarkers will be tested, including (a) classical ones for which data from several smaller studies have been published; (b) new ones, based on recent preliminary data, as well as (c) novel ones, based on recent research on mechanistic aspects of ageing, conducted by project participants. Bioinformatics will be used in order to extract a robust set of biomarkers of human ageing from the large amounts of data to be generated and to derive a model for healthy ageing.

WL4-2

Discovery of biomarkers of redox changes in vivo using proteomics

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Ageing associates in a shift in the cellular and cytoplasmic redox balance towards a more oxidising environment. Redox-sensitive transcription factors are more sensitive to activation under these conditions and the expressed proteome is likely to be correspondingly modulated. Using 2-dimensionsal electrophoresis based proteomic separation of plasmas, we have shown that supplementation of healthy subjects with vitamin E (400 iU/d for 4 weeks) significantly enhances plasma levels of the HDL associated pro-apolipoprotein A1 protein. Ongoing studies comparing the proteomes of 2 healthy cohorts of male subjects with mean ages of 24 and 57 years of age respectively has revealed an age-dependent differential in protein expression. Th identity of these proteins are under investigation as potential novel biomarkers of ageing.

WL4-3

Protein glycation – a biomarker of ageing or more?

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Advanced Glycation Endproducts are formed in vivo by a non-enzymatic reaction of proteins with carbohydrates. AGEs can harm tissue by 1) changing protein function due to the modification, 2) change protein turnover, 3) crosslinking proteins leading to tissue stiffening, 4) inducing radical formation and 5) the induction of an inflammatory response after binding to specific AGE receptors like the receptor for AGEs, RAGE. The AGE accumulation depends on AGE formation, mainly by reactive carbohydrates and elimination by the kidneys by a still unknown process. Therefore, diseases like diabetes (accelerated AGE formation) or end stage- renal disease (less elimination) leads to increased AGE blood levels. On the other hand, AGEs will accumulate in tissues independent of diseases just by age. The age-related accumulation of AGEs was shown in pericardial fluid and correlate inversely with heart function. Protein glycation is discussed to be responsible for many age- and diabetes related diseases. Therefore, AGE modifications were discussed to be a biomarker of ageing and related diseases. On the other hand, glyoxal induced AGE formation in human trabeculae can reduce the ischemia reperfusion injury in vitro independent of age. As a result, it seems to be an open question if protein glycation can act comparable to other posttranslational modifications as a regulatory mechanism as well.

WORKSHOP 5 — ANTIOXIDANTS: WHAT ARE THEY GOOD FOR?

WL5-1
Antioxidants: what are they good for?

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For several years the usage of antioxidant was claimed to be helpful and related to health promoting effects. This was based on large population based studies that high vitamin or antioxidant intake as a consequence of healthy nutrition may protect from a couple of diseases. This lead to the conclusion, that a supplementation with single or several antioxidative compounds might have health promoting effects on the overall population and might be important for the prevention of several diseases including cardiovascular events, cancer or neurodegenerative symptoms. As a consequence a large number of trials were started to evaluate whether antioxidant treatment would have health promoting effects. Until now the evidence for this are rather sporadic. Furthermore, recently published meta analysis claimed an increase of mortality after antioxidative treatment. Although, the conclusions of such analyses are rather obscure, it seems to be important to analyze in more several questions related to the treatment with antioxidants. Some of these questions will be addressed in this workshop and discussed in a wider context. These questions will include the following ones:

- Are antioxidants able to do more then compensating a nutritional deficiency?
- Are antioxidants supplemented in an appropriate dosage? Are they giving additional antioxidative protection?
- Is the endpoint of the study related to the inadequate status and do the supplemented vitamins cover all or only a part of the vitamins or co-factors involved in the pathologies? Was and if yes, why was the supply at start of the study inadequate in the study group?
- What do we know about bioavailability of antioxidants in clinical studies?
- Is the dosage exceeding the upper level?

YOUNG INVESTIGATOR SESSION

YIL-1
Inhibitors of trypanothione synthetase: new drugs for neglected diseases

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The most unique feature of the trypanosomatids is their ability to transform glutathione into the spermidine derivative trypanothione and, in contrast to their mammalian hosts, to use this redox mediator instead of glutathione for the detoxification of hydroperoxides, heavy metals, drugs and hydroxyaldehydes. In T. brucei trypanothione synthesis is achieved by trypanothione synthetase (TryS). Even partial suppression of TryS by dsRNA interference is sufficient to impair viability in T. brucei and rapidly kills the parasites under mild oxidative stress. TryS thus is a validated drug target and TryS inhibition is considered to be a particularly attractive strategy to fight trypanosomal infections. Further aspects supporting the use of TryS as drug target are low abundance and uniqueness of sequence. Our mechanistic studies on TryS had suggested that TryS, like glutathione synthetase, belongs to the group of ATP-grasp enzymes. These proteins undergo substantial conformational changes upon binding of the co-substrate ATP. Similar conformational changes upon binding of ATP or ATP-homologous inhibitors have been reported for protein kinases. In view of this mechanistic analogy, we screened a diversified compound library that had been designed for a protein kinase inhibition strategy and comprised all major types of kinase inhibitors in order to identify lead compounds active against TryS. The screen yielded 20 compounds that reasonably inhibited TryS. Starting from these findings, we initiated a drug design project, that provided a novel series of paullones that specifically inhibit TryS with IC50s < 100 nM. As expected for ATP-homologous inhibitors of ATP-grasp proteins, inhibition is neither affected by ATP nor by GSH. Accordingly, these compounds may be considered as promising leads for the development of useful trypanocidal drugs.

YIL-2
In vivo concentration dynamics of nitric oxide in anesthetized rat brain

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In the brain, nitric oxide (NO) synthesis by neuronal isoform of nitric oxide synthase coupled to N-methyl-D-aspartate (NMDA)-subtype glutamate receptors is implicated in mechanisms of synaptic plasticity, learning and memory but also in pathological events underlying neurological disorders, including Alzheimer?s and Parkinson?s diseases. The direct measurement of NO in brain tissue in vivo and in real time is critical to know its concentration dynamics and bioactivity. We have used selective carbon fiber microelectrodes, coated with ®Nafion and o-phenylenediamine (o-PD), coupled with electrochemical techniques to measure NO concentration dynamics in rat brain. NO production was evoked by local pressure ejection of both NMDA and glutamate, in hippocampus and striatum. It is quantitatively evidenced: 1) the dynamics of NO production and decay, as modulated by intraperitoneal injection of both, glutamate receptors antagonists and nitric oxide synthase inhibitors; 2) an heterogeneous NO concentration pattern along the trisynaptic loop in hippocampus upon stimulation of glutamate receptors, being significantly lower in dentate gyrus (additionally, NO responses are compared between hippocampus and striatum) 3) the kinetics of NO decay following local application of exogenous NO in the brain regions. Overall, these results help establishing a quantitative conceptual framework for NO activity in the brain.

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YIL-3

FoxO3a and PGC-1α cooperate to regulate the oxidative stress response in endothelial cells

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Increased production of ROS is thought to be one of the key events in the pathogenesis of endothelial damage or dysfunction, an early indicator of atherosclerosis and its clinical complications. We have recently shown that the transcriptional co-activator PGC-1α positively modulates the expression of the mitochondrial detoxification system in endothelial cells, preventing endothelial dysfunction and apoptotic cell death in oxidative stress conditions. As PGC-1α cannot bind directly to the DNA, we have sought to identify the transcription factor that mediates the PGC-1α effect on the mitochondrial ROS protection system.

The FOXO transcription factors have been recently shown to play a key role in endothelial homeostasis; they regulate the expression of genes involved in metabolic control, stress resistance, cell cycle arrest and apoptosis. FoxO3a has been shown to be a direct modulator of two key antioxidant enzymes at the transcriptional level, Mn-Superoxide Dismutase and Catalase. Importantly, these two enzymes are also targets of PGC-1α activity.

We hypothesized that PGC-1α regulation of the mitochondrial antioxidant defense system could be mediated by Foxo3a, a transcriptional factor capable of binding to specific sequences in the promoter region of the genes belonging to the mitochondrial antioxidant system. This idea was supported by the observation that mutation of the functional FOXO site in the MnSOD promoter abrogates the transactivational activity of PGC-1α.

We have therefore investigated if PGC-1α requires FoxO3a to regulate the oxidative stress protection system in endothelial cells, and if this regulation is mediated by their direct interaction in the context of the target promoters. We are also evaluating how this interaction impacts the cellular response to oxidative stress.

Overall, our results suggest that Foxo3a and PGC-1α work together to regulate the expression of the mitochondrial antioxidant system. We propose that PGC-1α and FoxO3a are key members of the complex that coordinately regulates this detoxification system. Further investigation of the functional interaction between FoxO3a and PGC-1α may provide significant insight into the mechanisms of cell response to oxidative stress and the life/death decision under stress conditions.

YIL-4

Peroxynitrite detoxification by Leishmania infantum tryparedoxin peroxidases: implications for parasite infectivity in mouse and human cells

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Leishmania infantum, the causative agent of visceral leishmaniasis in the Mediterranean areas, needs to deal with toxic reactive oxygen and nitrogen species derived by their host cells during an infection. Promastigote phagocytosis triggers NADPH oxidase assemblage on the macrophage membrane and, consequently, it induces O2?- production. On the other hand, macrophage activation by Th1 cytokines such as IFN-gamma, can stimulate iNOS expression and, therefore, generate NO. A concomitant production of O2?- and NO leads to the formation of peroxynitrite a strong oxidant and a cytotoxic molecule. As other trypanosomatids, Leishmania possess ROS/RNS detoxification cascades dependent on trypanothione, the trypanosomatid's specific thiol. The last components of these cascades are peroxiredoxins of the typical two-cysteine subtype, designated as tryparedoxin peroxidases. We have verified that Leishmania recombinant TXNPxs are able to reduce both H_2O_2 and peroxynitrite. Based on those observations, we have generated L. infantum parasites overexpressing a cytosolic tryparedoxin peroxidase and we show that they can detoxify peroxynitrite in the context of the parasite. In order to see whether these tryparedoxin peroxidases would play a role in parasite resistance to macrophage produced ROS/RNS, murine peritoneal macrophages were used to compare wild type and TXNPx overexpresser parasites in terms of infectivity. The recombinant parasites showed higher infectivity rates. Since there is a strong controversy about the ability of human macrophages to generate NO, and consequently peroxynitrite, we are also testing the recombinant parasites in these cells. We verified that human macrophages can generate O2?- but so far we could not detect iNOS expression or NO production. Like some authors argued, it might be that human macrophage defences differ from the ones observed in the murine model. In an attempt to clarify this issue, experiments are underway to compare the infectivity rates of the TXNPx overexpressers in human andse phagocytes.

YIL-5

Plasma membrane-bound cytochrome b5 reductase is associated with lipid rafts in cerebellar granule neurons in culture

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Plasma membrane redox centres play a major role in neuronal defence against oxidative stress and survival. In cerebellar granule neurons in culture (CGN) a large pool of the flavoproteins are associated with the plasma membrane, and the intensity of CGN green/orange autofluorescence correlated with the levels of expression of cytochrome b5 reductase. Fluorescence resonance energy transfer (FRET) demonstrated a large compartmentation of cytochrome b5 reductase in the plasma membrane of CGN. To this end we have used CGN fixed with paraformaldehyde. Cytochrome b5 reductase was stained with fluorescent antibodies, and dyes forming good donor/aceptor FRET pairs were used to label major \\\'lipid rafts\\\' markers, like cholera toxin B, caveolin and flotillin. To further assess the tight association of cytochrome b5 reductase with \\\'lipid rafts\\\', these were isolated and analyzed by western blotting. This study unravels that cytochrome b5 reductase form a major network of redox centres largely enriched at interneuronal contact sites in the neuronal soma and associated with \\\'lipid rafts\\\' of the CGN plasma membrane. We also show that cytochrome b5 reductase makes a large contribution to the NADH oxidase activity and to the red-shifted flavines fluorescence of purified rat brain synaptic plasma membranes.

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YIL-6

Urinary excretion of biomarkers of oxidatively damaged DNA and RNA in Hereditary Hemochromatosis (HH)

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Criteria for validation of a biomarker include disease predictability, an elucidated biological mechanism, applicable methodology and cost. These criteria are fulfilled for urinary excretion of 8-oxodG. We used this biomarker to study if untreated HH patients with high iron overload had increased oxidatively damaged DNA.20 HH patients and 20 matched controls had conventional blood biochemistry measured and spot urine was colleted for measurement of 8-oxodG, 8-oxoGuo and 8-oxoGua by LC-MS/MS, and creatinine. A diagnostic liver biopsy was also used for measurement of liver iron concentration and non-transferrin bound iron. The patients were then treated with phlebotomy until blood biochemistry parameters for iron status had normalised and urine collection was repeated.All patients had clear signs of iron overload.The urinary excretion of the RNA oxidation product 8-oxoGuo was 2.5 fold increased ($p < 0.05$) compared with controls. The urinary excretion of the DNA oxidation product 8-oxodG did not differ between HH patients and controls.

After normalisation of iron status the excretion of the RNA oxidation product 8-oxoGuo returned to control values and the excretion of the DNA product 8-oxodG was reduced to 50% ($P < 0.05$). There was a strong linear correlation between plasma ferritin and 8-oxoGuo excretion ($r = 0.92$).Untreated HH patients have increased risk of cancer. The findings of oxidative stress to nucleic acids in HH patients and the normalization with normalization of iron status, strongly suggest that oxidative stress to nucleic acids is part of the pathogenesis. Further the data suggests that an unidentified antioxidant mechanism protects nuclear DNA to some degree, and that this mechanism persists after the treatment.

YIL-7

Improved resistance to serum oxidation in Gilberts syndrome: a mechanism for cardiovascular protection

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Bilirubin is a potent antioxidant, however, uncertainty surrounds its physiological importance. Individuals with Gilberts syndrome (GS) have increased circulating bilirubin and a reduced prevalence of cardiovascular disease (CVD). The aim of this study was to investigatemechanisms that may link bilirubin to protection from CVD seen in GS by examining markers of antioxidant and oxidative stress status andthe susceptibility of serum to oxidation. Nine individuals with GS and twelve controls, matched for age, height and weight, were assessed forplasma antioxidant status, red blood cell antioxidant enzyme activities, plasma malondialdehyde, the susceptibility of serum to copper (Cu^{2+}) induced oxidation and blood lipid profile. Individuals with GS had significantly elevated unconjugated bilirubin (GS: 26.0 ± 6.4; control:9.7 ± 3.0 umol/L; $P < 0.001$), increased trolox equivalent antioxidant capacity (GS: 1.59 ± 0.07; control: 1.52 ± 0.07 mmol/L trolox Equ; $P = 0.035$) and ferric reducing ability of plasma (GS: 1.09 ± 0.16; control: 0.92 ± 0.14 mmol/L Fe^{2+} Equ; $P = 0.024$). The lag phase of serumoxidation was significantly longer in the GS group (GS: 121.4 ± 10.5; control: 106.8 ± 14.6 min; $P = 0.020$) and was positively correlatedwith the bilirubin concentration ($r = 0.451$, $P = 0.040$). A trend toward elevated HDL:LDL ratio was observed in GS (GS 0.96 ± 0.31; control: 0.73 ± 0.21; $P = 0.072$). In summary, individuals with GS have an increased circulating antioxidant status and an improved resistance to serumoxidation which may partially explain their reduced prevalence of CVD.

YIL-8

Is oxidative stress a central mechanism for glucose toxicity in pancreatic beta cells?

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The excessive formation of oxygen radicals derived from increased glucose metabolism represents the well-established model explaining toxic effects of hyperglycemia in diabetes. Besides retina, kidney and vascular tissue, insulin producing β cells and neurons represent one of the major targets of damage by excessive glucose. In this study, the effects of exposure to high glucose were evaluated in pancreatic INS-1 β cells in view of the metabolic oxidative stress and also in comparison to the hyperglycemia effects in neuronal HT22 cells. The exposure to 10-fold higher glucose concentration showed more toxic effects in INS-1E than in HT22 cells. These were accompanied by an increase of reactive oxygen species generation, and moderately increased protein carbonyl levels, along with inhibition of proteasome activity in both types of cells. Alterations in cytosolic versus nuclear distribution of proteasome, protein carbonyls and AGE-modified proteins were observed in high glucose treated cells. Citrate, a glycolysis inhibitor, phloridzin, a blocker of glucose transporter and an indole-type antioxidant, diminished, with the comparable efficacy, oxidative stress markers and reduced toxic effects of high glucose to INS-1E and HT22 cells. However, treatment with glucose antimetabolite, 2-deoxy-D-glucose, even enhanced intracellular oxidative stress and abolished the cell proliferation. In conclusion, these results indicate that metabolic oxidative stress can participate in hyperglycemia damage to insulin producing β cells and neuronal tissue suggesting also that influencing the steps in glycolytic pathway, as well as, antioxidant treatment may provide a possible protection against glucose toxicity in diabetes.

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YIL-9

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POSTERS

SESSION 1—REDOX BIOCHEMISTRY AND MICRONUTRIENTS

P1-1
Influence of alpha-tocopherol in iNOS mRNA expression in UVA-stimulated HMEC-1

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UV irradiation leads to the induction of oxidative stress in various cell types, including endothelial cells. As one consequence, iNOS is upregulated leading to an enhanced concentration of NO that finally can end up in nitrosative stress. Studies in our lab had shown protective effects of antioxidants (alpha-tocopherol and ascorbic acid) on iNOS protein and NO expression in UVA-irradiated human microvascular endothelial cells (HMEC1; see abstracts by Hirobe et al. and Rodemeister et al.), i.e. a supplementation of the cells with VE prior to UV-irradiation resulted in an attenuation of the normally enhanced iNOS protein expression after irradiation. To further clarify this effect, it was the aim of this study to investigate possible changes also in the expression of iNOS mRNA due to UV irradiation with or without prior supplementation with alpha-tocopherol (alpha-toc).
Cells were either supplemented with antioxidative alpha-toc (35 μM and 45 μM) or UV-irradiated (25 J/cm^2) or supplemented before irradiation to determine the iNOS mRNA expression by quantitative real time PCR. alpha-toc was applied as a water-soluble formulation (solubilisate) in micelles that consist of polysorbate.
Supplementation with alpha-toc at 30 μM had no influence on the expression of iNOS mRNA, the higher concentration (45 μM) led to a decrease of about 30% below control levels. UV irradiation led to an unchanged iNOS concentration 6h pUV followed by an increase (80%) at 12h pUV. At 18h pUV there was a downregulation below control value (40%); the final concentration at 24h pUV was comparable to control values. Supplementation with alpha-toc prior to irradiation led to a loss of amplitude of the curve, i.e. at all time points nearly equal concentrations were found that were comparable to control levels. At 45 μM concentration, an increase (130%) of iNOS mRNA was found at 6h pUV that decreased to control levels at 24h. Finally, first few results indicate an involvement of polysorbate as carrier of alpha-toc in the iNOS pathways, however without interfering with the antioxidant effects of alpha-toc. Our data further complete the series of effects of UV-irradiation with or without alpha-toc supplementation on the expression of iNOS mRNA and protein, supporting the view that UV-induced upregulation can be attenuated by antioxidant vitamins. Further investigations on the additional effects of ascorbic acid and a combination of both vitamins have to be performed.

P1-2
A fish-oil rich diet reduces vascular oxidative stress in apoE-/- mice

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Oxidative stress is involved in atherosclerosis development and is attributed to a decreased nitric oxide (NO) bioavailability due to and increased superoxide anion ($O_2^{\bullet}$) production in blood vessels. It is accepted the long-chain (n-3) PUFA are cardioprotective and also improve endothelial function and lower blood pressure. The (n-3) PUFA are easily oxidized in vitro. However, their susceptibility to oxidation in vivo is a matter of debate and we postulated the involvement of NO and the decrease of oxidative stress to explain the beneficial effects of long-chain n-3 PUFA against atherosclerosis.
Thus, we fed apoE-/- mice that spontaneously develop atherosclerosis, with a fish oil- or a corn oil-rich diets for 8, 14 or 20 weeks and we evaluated parameters related to NO and $O_2^{\bullet -}$ and also the hydroxynonenal as a product of lipid peroxidation in the aorta.
We observed that fish oil increased NO production and endothelial NO synthase expression and lowered the expression of the inducible form of NO synthase while $O_2^{\bullet -}$ production and p22phox expression were also decreased. This modulation was essentially observed after 14 and 20 weeks of diet. Besides, peroxynitrite, an adduct from the reaction between NO and $O_2^{\bullet -}$ with oxidizing capability, was also reduced. As a consequence, the hydroxynonenal presence in the aortic tissue was decreased.
All these results suggest that FO is able to counteract the oxidative stress induced changes in atherosclerosis. Our findings provide important insights into the mechanism involved in the beneficial effects of long-chain (n-3) PUFA against atherosclerosis.

P1-3

Dynamics of accumulation of antioxidants and vitamin P in leaves and fruits of plants of stem Carya L. during vegetation

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Plants organisms possess sufficient resistance to oxidizing damages which arise only at sharp change of a physiological condition of a plant or influence of various external factors (light, temperature, etc.). It is caused by existence in a vegetative cell effective antioxidant systems. The purpose of work was studying dynamics of accumulation of water-soluble antioxidants and vitamin P in leaves and fruits of plants of stem Carya L. (C. tomentosa and C. cordiformis) during vegetation in ecological conditions of the Kaliningrad area.

Researches were spent from May till October, intensity of light and temperature of air was considered. Dynamics of accumulation of antioxidants in leaves of plants of stem Carya L. Has shown, that at the studied kinds in the beginning of vegetation (the end of May – the beginning of June) has noted been the highest level of antioxidants. In the beginning of summer (June) their content decreased, however, from the end of July, the pool of antioxidants again raised. The maximal content of antioxidants during the autumn period has been fixed on 20-th week of research. By the end of vegetation (last weeks of October) the level of antioxidants in leaves decreased to following values: at C. tomentosa – with 17,97 up to 12.95 mg/g, and at C. cordiformis – with 16.93 up to 10.08 mg/g. The same high values antioxidant activity were characteristic to young leaves in the first week of vegetation.

During ripening fruits (18-th – 23-rd weeks of research) C. tomentosa and C. cordiformis the level of antioxidants in them changed within the limits of 2.84 – 3.23 mg/g and 2.45 – 4.15 mg/g accordingly. The content of antioxidants in fruits was below, than in leaves.

Thus, a level of antioxidants in leaves of plants of stem Carya L. during vegetation changed as follows: in the beginning vegetations (May) the pool raised and in the summer – decreased and only in the autumn, as well as in the spring, the increase in the content of antioxidants has noted been. In fruits the level of antioxidants always was below, than in leaves. Positive correlation between the content of antioxidants in leaves and ripening fruits of the studied kinds and intensity of light, and also temperature it has not been revealed.

Maximum level rutin in leaves of representatives of stem Carya L. In the beginning of the period of vegetation it was marked on 2-nd week of research and has made (36.8 and 40.53 μg/g for C. tomentosa and C. cordiformis accordingly). In the summer decrease in a pool of vitamin P was observed. In the middle of October (on 23 week of research) the content rutin was minimal: C. tomentosa – 4.8 μg/g, and at C. cordiformis – 6.4 μg/g.

During all period of ripening of fruits (18–23 week), investigated kinds were characterized by decrease in a level rutin which changed within the limits of 4.8–9.6 μg/g. The maximal content of vitamin P in fruits C. tomentosa has been revealed on 20-th week, and at C. cordiformis on 19-th week of research, i.e. in the end of September.

Thus, representatives of stem Carya L. (C. tomentosa and C. cordiformis) were characterized by similar dynamics of accumulation rutin in leaves. In the beginning of the period of vegetation (the middle of May-the beginning of June) was marked its raised content, in the further the pool rutin decreased down to the end of vegetation. Dynamics of accumulation of vitamin P in fruits, also as well as in leaves, was characterized by decrease in a level rutin in process of their ripening.

The statistical analysis of data has shown, that between intensity of light and the content the rutin in leaves has been revealed positive correlation dependence ($r = 0.67$; $r = 0.65$ for C. tomentosa and C. cordiformis accordingly). Also positive correlation between accumulation of vitamin P in leaves and fruits of plants of stem Carya L. (for C. tomentosa $r = 0.62$ has noted been; C. cordiformis $r = 0.86$).

P1-4

Contribution of hydrophilic fractions to the antioxidant activity in selected fruits

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Fruits and vegetables provide our diet with a complex mixture of natural substances, especially lipophilic phenolic compounds, with well documented antioxidant properties. The major aim of the present study was to evaluate the antioxidant activity of the hydrophilic fraction in pulp, peel, grain and juice of various types of fruits.

The selected fruits (lemon (Citrus limon), orange (Citrus sinensis), grapefruit (Citrus paradise), apple (Malus domestica), pear (Pyrus communis), and peach (Prunus persica) were portioned into pulp, peel, and grain samples and hydrophilic extracts of the samples were prepared. Juices from all types of fruits were also prepared. A luminol-enhanced chemiluminescence method was used to follow up the ability of studied samples to scavenge peroxyl radicals produced by thermal decomposition of 2,2′-azobis(2-amidinopropane).

The highest antioxidant activity was observed in juices of individual fruit samples. This observation is in a good concordance with a high antioxidant activity detected in pulp samples. A lower antioxidant activity was seen in peel samples and the lowest antioxidant activity was recorded in grains of the fruits studied. Irrespective of the parts of fruits, the absolutely most efficient antioxidant activity was observed in lemon samples. Generally, the citrus fruits (Rutaceae family (lemon, orange, and grapefruit)) showed higher antioxidant activity when compared to the fruits of Rosaceae family (apple, pear, and peach). Interestingly, some apple samples (pulp and juice) exerted antioxidant activity comparable to that of citrus fruits.

It can be summarized that hydrophilic antioxidants contribute to the total antioxidant activity of fruits to a great degree.

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P1-5

NaHS-induced formation of reactive oxygen species in mice

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Introduction: Nitric oxide (NO), carbon monoxide (CO) and hydrogensulfide (H_2S) are endogenously formed vascular gases active in the multilevel regulation of pathophysiological functions in mammalian cardiovascular tissues. H_2S produced by cystathionine-ß-synthase (brain) or cystathionine-gamma-lyase (systemic) dilates blood vessels by opening muscle K^+ATP channels and H_2S is involved in corticotropin-releasing-hormone formation in the hypothalamus. H_2S levels are decreased in the brains of Alzheimer's patients and promotes central long-term potentiation by enhancing sensitivity to NMDA receptors. H_2S is systemically enhanced in septic shock, pancreatitis and other diseases with a systemic inflammatory reaction. Up to now there are no data available about sulphide and reactive oxygen species (ROS) which induce or are involved in inflammatory processes. Aim of the investigation was to analyse mouse striatal reactive oxygen species after local and systemic sulphide application.
Methods: Microdialysis probes were stereotactically implanted into the striatum of balb/c mice. NaHS was co-infused in ringer solution (1.5µl/min) via microdialysis probe together with the spin label CMH to measure ROS. ROS were detected by electron spin resonance spectroscopy. Central nervous glutamate formation was analyzed by Tandem MR Spectroscopy. For analyzing the systemic inflammatory reactions plasma sulphide content, ROS content in blood, myeloperoxidase activity in lung and duodenum, as well as NADPHox-activity in liver were investigated 1h after i.p. injection of NaHS (1mg/100 µl).
Results: Systemic application of NaHS (100 µg) induced a significant decrease in blood born ROS and an increase in plasma H_2S (8 vs. 25 µg/ml) of mice. The application of NaHS (100 µM) in liver homogenat showed a biphasic and concentration dependent formation of ROS. After 100µM of NaHS enhanced ROS to 200%, while with 1mM NaHS a decrease to 5% of the initial value was obtained. The i.p. injection of NaHS results in a highly significant increase in lung MPO (control: 3 mU, NaHS: 19 mU/mg tissue). Striatal application of NaHS (0.6µg/min) results in a diminished ROS formation (−15%).
Conclusions: H_2S is a new vascular mediator with probably interactive reaction with NO and CO. Still many questions arise about ROS formation and glutamate release in the brain.

P1-6

HPLC-MS analysis of antioxidant flavonoids obtained from Solidago species

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The goal of our study was to identify and quantify the flavonoid components obtained from the Solidago species. The species we studied were: Solidago virgaurea, Solidago gigantea and Solidago canadensis. Solidago species are well known in the literature for their saluretic, diuretic, antiinflammatory, spasmolythic but also cytostatic and antioxidant properties [1,2]. The antioxidant properties are due to the flavonoid components [3]. That is why we wanted to isolate, identify and determine the main flavonoid components, using HPLC-MS [4].
We analysed the upper part of the plant (herba) from the three species of Solidago: S. virgaurea, S. gigantea şi S. canadensis. The analysis of phenolic components from the 3 species was performed using high performance liquid chromatography associated with mass spectrometry (HPLC-MS) [5,6,7,8]. HPLC analysis in conjuction with mass spectrometry were used to analyze extracts obtained from Solidago species revealed the following results.
In the S. Virgaurea extract the most important flavonoid was represented by rutozide (291.3 µg/ml) folowed by *isoquercitrine* (69.1 µg/ml) and *hiperozide* (24.16 µg/ml). The free flavonoid aglicones are represented by *kempferol* (1.45 µg/ml), *lutheoline* (0.8 µg/ml and *apigenine* (1.23 µg/ml). The free phenylpropanic componuds are represented by *caftaric acid* in reduced quantities and *clorogenic* and *cafeic acids* in high percentage. These compounds appear together and their identity was confirmed with MS.
Flavonoid heterozides from the *S.gigantea* extract are represented by *quercitrine* as a major component (450.56 µg/ml) followed by *hiperozide* (120.0 µg/ml), *izoquercitrine* (82.49 µg/ml) and *rutozide* (45.54 µg/ml), As a free aglicone we identified *kempferol* (8.5 µg/ml). The free phenylpropanic componds are represented by *chlorogenic and cafeic acids* confirmed by MS.
Flavonoid heterozides from the *S.canadensis* are represented *rutozide* as a major component (400.89 µg/ml) followed by *isoquercitrine* (67.75 µg/ml) and *hiperozide* (7.7 µg/ml), Free aglicones present in high concentrations are: *quercetol* (60.25 µg/ml) and *kempferol* (7.62 µg/ml). Phenyl propanic compounds are represented by *cafeic and chlorogenic acids* (nr.3.4 din fig.105) identified by MS.
HPLC-MS analysis of flavonoid aglicons resulted after acid hidrolysis revealed the following facts: In the *S. Virgaurea* sample *quercetol* represents the main flavonoid aglicon (64.73 µg/ml), followed by *kempferol* (9.95 µg/ml) and traces of *apigenine* (1.12 µg/ml) and *luteoline* (0.77 µg/ml). Phenylpropanic analysis revealed that *chlorogenic and cafeic acids* are major components whereas *ferulic and p-cumaric acids* are present in reduced quantities. *Caftaric and gentisic acids* were identified only by MS.
In the *S. gigantea* sample *quercetolul* was identified as the main aglicon component (124.56 µg/ml), followed by *kempferol* (10.25 µg/ml) and *apigenine* (1.02 µg/ml). Phenyl propanic compounds are represented by *ferulic acid* (2.93 µg/ml) and *p-cumaric acid* (1.42 µg/ml). C*afeic and chlorogenic acidsi* are present in large quantities, whereas *caftaric and gentisic acids* were confirmed only by MS.
The *S. canadensis*, sample contained *quercetolul* as a major component (144.95 µg/ml), followed by *kempferol* (25.38 µg/ml). Phenyl propanic compounds are represented by *ferulic* (6.24 µg/ml) and *p-cumaric acids* (1.29 µg/ml), and *cafeic and chlorogenic acids*, confirmed only by MS. HPLC-MS allowed us to identify and determine the quantity of the flavonoid components from the upper part of Solidago species plants. HPLC revealed that each Solidago species has a characteristic flavonoid: rutozide for *S. virgaurea* and *S. canadensis* and quecetolul for *S. gigantea*. Other flavozides are represented by hiperozide and isoquercitrine in various percentages, and *S. canadensis* contains free quercetol. Flavonoid aglicons from *Solidago* species are represented by quercetol as a major component in all species, and kempferol.

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P1-7

H_2O_2 induction of PGC-1alpha as a model for ROS mediated signaling

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Reactive Oxygen Species (ROS) were traditionally considered to be unavoidable byproducts of aerobic respiration. It has been gradually appreciated that certain ROS derivatives, such as hydrogen-peroxide (H_2O_2), can act as a second messenger under selective physiological conditions. However, H_2O_2 mediated signaling pathways and mechanisms are poorly understood. Recently, we identified PGC-1alpha (PPARgamma coactivator 1alpha) as a key regulator of anti-oxidative defense program and cellular ROS homeostasis. PGC-1alpha knock-out cells and mice have reduced expression of ROS detoxifying enzymes and are more sensitive to oxidative stress and MPTP induced neurodegeneration, respectively.

PGC-1alpha is induced upon H_2O_2 stimulation and its induction is necessary for the anti-oxidative defense program (St-Pierre, J., Drori, S., et al. Cell 2006). Induction of anti-oxidative defense program is a critical response to cellular ROS elevation. Therefore, the inner connection between cellular H_2O_2 levels and PGC-1alpha activation is important for further understanding H_2O_2 mediated signalling.

Toward this aim, a real-time cellular system was developed. Both fibroblast and cancer cells were transfected with a fluorescent indicator (pHyper, Evrogen) that reads H_2O_2 relative concentration in live cells. These cells were also transfected with either PGC-1alpha homologous recombinant promoter fused to PGC-1alpha-GFP (indication of protein levels) or fused to GFP alone (transcript levels). This cellular system monitors simultaneously both PGC-1alpha levels and H_2O_2 changes using dose and time dependent stimuli in live cells. Finally, we can use this real-time system to further analyze key elements that participate and affect H_2O_2 mediated signalling.

P1-8

The potent vasodilator ethyl nitrite is formed upon reaction of nitrite and ethanol under gastric conditions

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By acting as a bioreactor, affording chemical and mechanical conditions for the reaction between dietary components, the stomach may be a source of new bioactive molecules.

Using gas chromatography-mass spectrometry we here demonstrate that, under acidic gastric conditions, ethyl nitrite is formed in μM concentrations from the reaction of red wine or distilled alcoholic drinks with physiological amounts of nitrite.

Rat femoral artery rings and gastric fundus strips dose-dependently relaxed upon exposure to nitrite:ethanol mixtures. Mechanistically, the relaxation effect was assigned to the generation of nitric oxide (NO), as supported by the measurement of NO release from ethyl nitrite and the absence of relaxation in the presence of the soluble guanylyl cyclase inhibitor, ODQ in both tissues. NO released from ethyl nitrite diffuses trough the stomach wall as followed by NO detection outside the whole stomach, suggesting direct effects on the stomach tissue and possible systemic diffusion trough the stomach local circulation.

In conclusion, these results suggest that ethanol in alcoholic drinks interacts with salivary-derived nitrite in the acidic stomach leading to the production of the potent smooth muscle relaxant ethyl nitrite. These findings reveal a new reaction pathway for the effects of dietary nitrate and nitrite involving NO metabolism with impact on gastric physiology and pathophysiology.

P1-9

Hydrogen sulphide induces a large sensibilization of NMDA-receptor response to L-glutamate in cerebellar granule neurones

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Hydrogen sulphide concentration can be maintained in cell cultures within the range reported for rat brain by repetitive pulses of sodium hydrogen sulphide. Less than two hours exposure to hydrogen sulphide concentrations within 50 and 120 micromolar, i.e. within the upper segment of the reported physiological range of this gas in rat brain, produces a large shift of the intracellular calcium homeostasis in cerebellar granule neurones (CGN) in culture leading to a large and sustained increase of cytosolic calcium concentration. L-type Ca^{2+} channels antagonists nimodipine and nifedipine block both the hydrogen sulphide-induced rise of cytosolic calcium and the cell death. In addition, partial depolarization of the CGN plasma membrane largely increase the sensitivity of CGN towards deregulation of cytosolic calcium by hydrogen sulphide. The N-methyl-D-aspartate receptor antagonist MK-801 affords a nearly complete protection against hydrogen sulphide-induced CGN death and attenu ates the rise of cytosolic calcium. Thus, the sustained rise of cytosolic calcium reaches the neurotoxic cytosolic calcium range, leading to glutamate-induced excitotoxic CGN death. CGN treated with hydrogen sulphide display a strong enhancement of the response to L-glutamate, pointing out that this neuromodulator induced a large potentiation of the NMDA receptor. This effects of hydrogen sulphide are mimicked by extracellularly added disulphide reductants like DTT and TCEP, suggesting that hydrogen sulphide reacts with the well known redox centre of the NMDA receptor.

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P1-10

Silymarin on H_2O_2-induced toxicity and viability of rat primary mixed glial and rat glioma cells

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Silymarin, a standardized extract from milk thistle, family Asteraceae, is a kind of flavonoid that contains approximately 70–80% flavonolignans and 20–30% non-identified polyphenolic compounds. Primarily known as a hepatoprotective and antioxidant, silymarin has anti-mitotic effects against lung, breast, kidney, skin, pancreas and prostate cancer models. We intended to determine whether silymarin protects against H_2O_2-induced toxicity and/or affects viability of mixed glial cells and rat glioma (C6) cells.

We studied the effect of 1, 10, 50, 75 and 100 μM silymarin concentrations on survival of primary glial cells that were obtained from 1–3 day old rat whole brain and C6 cells for 24 or 48 hr *in vitro*. Combined with 100 μM H_2O_2 that kills 75% of the cells, silymarin at the same doses were applied to the cells for 3 hr. Later, the medium was removed and refed for 24 hr. The number of surviving cells was determined by using MTT method and statistical significance was ascertained by one way analysis of variance, followed by Tukey multiple comparison test.

The treatment of glial and C6 cells with respective doses of silymarin for 24 or 48 hr reduced the cell number down to 73%. After a 24 hr of exposure, IC50 of silymarin for C6 cells was calculated as 50 μM, and for the glia cells as 79 μM. The calculated IC50 dose of silymarin for C6 is 43 μM and for the glia 67.5 μM for 48 hr. Silymarin is more effective on decreasing cell survival of C6 cells than primary glia. On the other hand, silymarin could not show any protective effect against toxicity of H_2O_2 on glia and C6 cells.

P1-11

The impact of therapy with alpha-lipoic acid and vitamin E in the oxidative stress associated with intermittent hypobaric hypoxia conditions

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It is known that the conditions of hypoxia-reoxygenation represent an important source of reactive oxygen species.
The purpose of this study was to investigate the oxidative stress (OS) induced by intermittent hypobaric hypoxia (IHH) and the impact of the associated antioxidants therapy. The rats devised in six groups (10 rats/group) were treated intraperitonelly with NaCl 0.9% (CH, CN), 50 mg/kg/day alpha-lipoic acid (LA) (LAH, LAN) and intramuscularly with 50 mg/kg/day vitamin E (EH, EN), for 14 days. A week after the start of the treatment, CH, LAH, EH groups were exposed for 7 days to hypobaric hypoxia equivalent to 2500m altitude, followed by a daily sequence of reoxigenation. During this period CN, LAN, EN groups were maintained in normoxia (N) conditions. Subsequently, the plasma and cardiac OS was determined by the measurement of lipids and proteins oxidation and by the antioxidant status.
After IHH, malondialdehyde (MDA) and plasma proteins carbonyl content was higher ($p < 0.05$) at CH as compared to CN. For all three groups kept in IHH, the antioxidant status (measured as serum hydrogen donor ability) was lower ($p < 0.01$) than the one for the equivalent groups maintained in normoxia. The cardiac OS was less obvious in our experimental conditions. The plasma MDA was reduced with $\sim 36\%$ by vitamin E and with $\sim 23\%$ by LA, as compared to CH. The treatment associated with IHH did not influence the plasma or cardiac protein carbonyl content.
The results obtained suggest that the OS occurs in IHH conditions and that the LA and vitamin E therapy attenuates essentially plasma lipid peroxidation.

P1-12

DNA fragmentation assessed using a variant of Sperm Chromatin Dispersion (SCD) test

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Background: Infertility affects approximately 15% of all couples trying to conceive, male factor infertility plays a role in approximately 50% of infertile couples. Men with normal semen analysis are reported to have a higher fraction of sperm with chromatin defects and DNA breaks than fertile controls, which can be a major cause of undiagnosed/unexplained infertility. In this work, we estimate the level of damage DNA, using a modified protocol of the Sperm Chromatin Dispersion (SCD) test.
Methods: We performed a DNA controlled denaturation to generate restricted single-stranded DNA (ssDNA) motifs from DNA breaks. Later, nuclear proteins were removed to form nucleoids with a central core and a peripheral halo of dispersed DNA loops. Such DNA halos are absent or extremely small in nucleoids with nonfragmented DNA. To demonstrate the sensitivity of the SCD test, aliquots of sperm samples, from two different subjects were exposed to increasing concentrations of the nitric oxide donor sodium nitroprusside (SNP), for 1 hour at room temperature, to induce DNA damage. To estimate interobserver variability in sperm cells scoring, three technicians analyzed 500 sperm cells from each sample, three times a day. Means of DNA fragmentation level obtained by the observers of each sample were compared.
Results and conclusions: The modified protocol of SCD test was sensible to detect DNA fragmentation from 60 μM of SNP. Analysed samples showed a dose-response effect by SNP. There wasn't significant differences (Pearson Test, $P > .05$) in the mean of sperm cells with fragmented DNA observed, among the observers. SCD is a simple, fast, cheap and reproducible technique does not require complex or expensive equipments. Sperm DNA damage can be determined with accuracy using this assay, in laboratories with a basic instrumentation. The analysis can be carried out with conventional bright-field microscopes.

P1-13

Antioxidant defence in Helicobacter pylori

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Helicobacter pylori is a wide-spread and important human pathogen that causes diseases starting with strong inflammations through to stomach cancer. Nearly half of the world's population is infected with H. pylori. Patients are usually treated with a cocktail of antibiotics but the growing antibiotic resistance induces the search for alternative therapeutic strategies and a better understanding of bacterial defence systems.

Like other pathogens H. pylori relies on a variety of protective antioxidative mechanisms to resist the oxidative burst of the host's innate immune response. Referring to the mammalian defence system, H. pylori is equipped with only one of the two major intracellular disulfid-reducing systems, the thioredoxin (Trx) system, and lacks the glutaredoxin/glutathione system. The peroxide metabolism of the pathogen has for long been recognized to depend on catalase and superoxide dismutase but deletion of the gene encoding catalase did not affect the viability of the organism. Accordingly, bacterial survival and virulence are attributed to the two peroxiredoxin-type peroxidases, alkylhydroperoxide reductase (AhpC) and thiol peroxidase (TPx), which show acivity to detoxify a wide range of hydroperoxides. While in most bacteria AhpC is reduced by the flavoprotein AhpF, H. pylori lacks an ahpF homologue. Instead, the pathogen possesses the redox protein thioredoxin, Trx1, and the associated enzyme thioredoxin reductase, TrxR1, that together form a reductase system for AhpC and TPx. Besides H. pylori expresses another peroxiredoxin, Bcp, a second thioredoxin, Trx2, and thioredoxin reductase, TrxR2, whose functions still remain unclear.

In short, the antioxidant defence system of H. pylori differs substantially from that of the mammalian host.

Its constituents may therefore be regarded as potential drug targets. Selective inhibition of the pathogen's defence systems will enhance the efficacy of the innate immune response without adversely affecting the host.

P1-14

Influence of antioxidant vitamins C and E on the expression of iNOS-protein in UVA-stimulated HMEC-1

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Aim of the study was to analyze impacts by ascorbic acid (AA), vitamin E (VE) and the combination of both on the expression of iNOS protein by UVA-stimulated human endothelial cells (HMEC-1). Studies in our lab had shown effects of AA on NO (decrease in sham cells as well as after UVA) as well as on iNOS mRNA (decrease in sham cells and attenuation of increase after UVA). No objective data on the effects on the iNOS protein expression were available. Therefore, sham and UVA irradiated cells were supplemented with antioxidative vitamins (AOX) in 2 concentrations each to determine the iNOS protein expression in correlation with iNOS mRNA and NO. VE was applied as a water-soluble formulation (solubilisate) in micelles of polysorbate.

HMEC-1 were supplemented with AOX or irradiated with 25 J/cm^2 UVA-light with or without preincubation with AOX. Paraformaldehyde-fixed cells were incubated with polyclonal iNOS antibody. Immunoreactions were visualized using a Cy3-labeled, species-specific secondary antibody. Fluorescence signal was quantified either by subjective rating or by image analysis (imageJ).

Supplementation with AA in general led to no significant differences compared to controls except an increase at 6h for AA 100 μM and at 24 h for AA 50 μM. VE led to significant increases at 36 h for VE 45 μM and at 42 h for VE 30 μM. Irradiation led to a significant increase of iNOS protein immediately and 6h after irradiation that dropped at 12h to re-increase after 18 h. Preincubation with AOX led to a decrease of iNOS protein after irradiation, a combination of AA and VE appearing to be most effective. These data are in line with previous observations showing an increase of NO in irradiated cells that could be attenuated by AOX. iNOS mRNA concentrations decreased by 50% at 6h to increase at 12 to 150% of controls and to decrease again at 18h and 24h (see abstract by Berczés et al.).

Thus, our results filled the gap between irradiation-induced iNOS mRNA and NO production by describing iNOS protein contents in equally treated cells.

P1-15

4-hydroxy-2-nonenal inhibits Serca1a function by its interaction with the ATP binding site

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Lipid peroxidation in cells is associated with degradative processes induced by oxidative stress. 4-hydroxy-2-nonenal (HNE) is a major product from lipid peroxidation that can have a causal role in citotoxicity. The structure of HNE includes three functional groups, a C1 carbonyl group, a double bound in C2 and a C4 hydroxyl group which contribute to the high reactivity and cellular toxicity of this compound. It has been shown that HNE directly interacts with several amino acid residues of proteins, mainly cysteine, histidine and lysine leading to the structural modification and functional impairment of proteins. On the other hand, the Ca^{2+}-ATPase of sarcoplasmic reticulum from skeletal muscle (SERCA 1a) is a useful model for studying protein damage induced by oxidative stress.

Here we reported that the exposure of sarcoplasmic reticulum membranes to HNE resulted in the inhibition of the SERCA 1a activity and its associated Ca^{2+} transport. This inhibition was modulated by the concentration of ATP and the amount of membrane protein. The high affinity Ca^{2+} binding sites of the SERCA were not alterated by HNE. Also we observed a loss of free SH groups in the enzyme after treatment with HNE. When SR vesicles were premixed with Mg-ATP, before exposure to HNE, a protection of the SERCA function was observed without a recovery in the content of free SH groups. Competition studies with FITC revealed that HNE interacts with Lys515 on the nucleotide binding pocket of the SERCA.

Our results suggest that the alteration of the SERCA function by HNE is mediated by a direct attack on the ATP binding site located in the nucleotide domain of the SERCA.

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P1-16

Myeloperoxidase dependent modulation of arachidonic and linoleic metabolites in vivo

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Acute inflammation is common sign of many life-threatening pathologies such as septic shock. A characteristic feature of acute inflammation is formation of bioactive lipid peroxidation products of polyunsaturated fatty acids that play significant regulatory roles. Myeloperoxidase (MPO) is an abundant phagocyte-derived hemoprotein released during phagocyte activation.

Herein, a role of MPO in modulation of the biologically active arachidonic (AA) and linoleic acid (LA) metabolites during acute inflammation was evaluated. Comprehensive arrays of LA and AA oxidation products in plasma of wild-type and MPO-deficient mice exposed to endotoxin for 24 hours were characterized. Significantly decreased levels of the epoxides of LA and corresponding fatty acid diols of both LA and AA were detected in MPO-deficient mice compared to wild-type mice.

Similarly, hydroxy intermediates of AA and LA, hydroxyeicosatetraenoic and hydroxyoctadecadienoic acids, were significantly decreased in MPO-deficient mice. In contrast, significantly higher levels of cysteinyl-leukotrienes with well-known pro-inflammatory properties were observed in MPO-deficient mice. *In vitro* experiments with isolated polymorphonuclear neutrophils confirmed the *in vivo* observations.

Our results reveal that MPO, either directly or indirectly, modulates the balance of pro- and anti-inflammatory lipid mediators during acute inflammation with a consequent efficacy to control acute inflammatory diseases.

P1-17

Vitamin E and the vesicular transport

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Vitamin E is considered the most important lipid-soluble, chain-breaking antioxidant within biological membranes. Considering the novel non-antioxidant functions of vitamin E, specific emphasis is given to a-tocopherol, the most abundant member of the vitamin E family in mammalian tissues, and its role in the regulation of gene expression. Global gene expression profiling of liver of mice fed diets differing in a-tocopherol content using microarray technology identified a-tocopherol sensitive genes involved in vesicular trafficking and exocytosis. A role of a-tocopherol in exocytosis was demonstrated by the in vitro ß-hexosaminidase release assay in two secretory cell lines, PC12 and RBL-2H3 cells (Nell et al. 2007).

Subsequent detailed studies revealed a 2.5-fold increase in the PMA/ionomycin-stimulated degranulation of ß-hexosaminidase after 24 h but also after 1 h of incubation with 100 μM a-tocopherol. Induction of genes involved in the degranulation was not observed at any time point. Thus, the stimulation does not require a direct gene-regulatory effect of a-tocopherol. Since the same effects were observed with ß-tocopherol but not with trolox, a water-soluble analog of vitamin E, it was hypothesized that a-tocopherol might affect degranulation from the membrane. During exocytosis, a class of proteins termed SNAREs mediate the fusion of secretory granules with the plasma membrane. These SNARE proteins have been reported to associate with lipid rafts, membrane microdomains enriched with cholesterol and glycosphingolipids. Mast cell stimulation increases the amount of raft-associated SNARE complexes (Puri & Roche 2006). Therefore, the distribution of a-tocopherol in lipid rafts was analyzed in RBL cells. It was found that a-tocopherol was enriched in lipid rafts isolated from plasma membrane vesicles. An increased recruitment of SNARE proteins in lipid rafts by a-tocopherol could explain the observed effects.

P1-18

Physiological roles of hemoprotein nitrosyl complexes

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Nitric oxide and its compounds possess unique physiological functions: they can control blood vessel relaxation, apoptosis etc. Among the NO compounds the key role can be attributed to the hemoprotein nitrosyl complexes that can bind and release free NO. Hemoprotein nitrosyl complexes possess photosensitivity and can be decomposed upon irradiation with visible light.

This paper deals with some physiological effects of NO that can be monitored when hemoglobin or cytochrome c nitrosyl complexes are subjected to laser radiation. In our experiments we observed that if hemoglobin in erythrocytes contains nitrosyl complexes than laser irradiation can induce decomposition of these complexes and subsequent NO release. If this irradiation takes place in vivo than blood vessel relaxation can be monitored. Another example of this principle is the modulation of cytochrome c peroxidase activity that is involved into the apoptosis development. It is well known that cytochrome c can acquire peroxidase activity on the interaction with anionic phospholipids (say, cardiolipin). This activity can be inhibited when nitric oxide forms nitrosyl complexes with cytochrome c. These complexes can be destroyed upon laser irradiation. This decomposition is accompanied with the increase of the cytochrome c peroxidase activity.

Finally, we can conclude that hemoprotein nitrosyl complexes can serve as a temporal depot of the NO and that NO can be released from this depot upon visible light irradiation, moreover formation/decomposition nitrosyl complexes is the instrument to control the enzymatic activity of hemoproteins.

P1-19

Anthocyanins inhibit peroxynitrite-triggered endothelial cells toxicity by up-regulating cellular nitric oxide

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Several epidemiological evidences have shown that diets rich in fruit and vegetables can confer protection against several diseases including vascular disorders. Some of the major contributors for these actions are anthocyanins, a group of flavonoid pigments widely distributed in the diet mentioned above but also in red wine.

Thus, the aim of the present work was mainly to compare the protection afforded by four anthocyanins (Ac), malvidin- (Mv3glc), cyanidin- (Cy3glc), delphinidin- (Dp3glc), and pelargonidin-3-glucoside (Pg3glc), against peroxynitrite-promoted endothelial cells toxicity, correlating such effects with their antioxidant activities and evaluating how their substitution patterns in the B ring of the aglycon influence their activities. Pre-incubation of bovine aortic endothelial cells (BAEC) with these compounds, at very low micromolar concentrations, protect them from death promoted by peroxynitrite exposure, in a concentration-dependent way. Our results indicate that Pg3glc and Mv3glc, both with a monophenol structure, have the highest protective action while Dp3glc and Cy3glc, both with an ortho-dihydroxyl group in the B ring, are the less efficient, pointing that their cytoprotective effects are not well correlated with their antioxidant activities. This may be explained, in a first approach, by the higher cellular concentrations attained by Pg3glc and Mv3glc compared with those of Dp3glc and Cy3glc, after pre-incubation of cells with each Ac, as evaluated by the HPLC analysis of cellular extracts.

In trying to accomplish our main goal, recent data suggest that the protection afforded by the Ac is related to the increase of nitric oxide (NO) production as revealed by the results of the Griess reaction in the presence and absence of an eNOS inhibitor. Research is under way to explore the underlying mechanisms, namely the enhancement in eNOS activity or in its expression.

Despite the physiological and molecular mechanisms by which Ac reduce the risk of disease still remain to be fully elucidated, our data support the view that their importance as cardiovascular protectors goes far beyond their antioxidant capacities, being the attention now focused on their interaction with crucial cell signalling cascades and gene regulation.

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P1-20

Changes in the intracellular concentration of ROS and/or NO induced by passive stretching in skeletal muscle fibres from aged and dystrophic mice

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Skeletal muscle constantly produces reactive oxygen species (ROS) and nitric oxide (NO) which may play an important role in signalling and regulatory pathways. Isolated muscles in vitro release NO to the extracellular space [1] and passive stretching of muscle increases the release of NO from rat skeletal muscle in vitro [2]. Recently, we have developed a model to study the generation of ROS and NO in real time in isolated single muscle fibres subjected to a protocol of passive stretching [3].

The aim of this study was to evaluate the effect of passive stretching on the intracellular concentration of ROS and NO in single muscle fibres from young and old mice and in an animal model of Duchenne muscular dystrophy, the mdx mouse. We used young (2–4 month-old) and old (26–28 month-old) C57BL/6 mice and mdx mice. Muscle fibres were isolated from the Flexor Digitorus Brevis and attached to a flexible silicone membrane which had been previously coated with a collagen Matrigel? matrix. Fibres were loaded with different fluorophore probes: DCFH (general detector of ROS), hydroethidine (detector of superoxide) and DAF-FM (detector of NO). The passive stretching protocol was applied to fibres using the FX-4000? Flexercell® system. Using fluorescence microscopy, fluorescence emission from fibres at different time points was quantified by image analysis in order to detect the intracellular concentration of ROS and/or NO. Results from positive control experiments indicated that the technique is able to detect changes in the intracellular concentration of ROS and/or NO.

However, the changes of fluorescence observed in the fibres subjected to the protocol of passive stretching were lower and less evident, which indicates that the generation of ROS and NO induced by passive stretching is relatively low.

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P1-21

In vitro modulation of preservatives toxicity: hyaluronan decreases oxidative stress and apoptosis induced by benzalkonium chloride, phenoxyethanol and butylparaben

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Objective: Benzalkonium chloride (BAK), phenoxyethanol (PE) and butylparaben (BP) are chemicals used as preservatives in cosmetic and pharmaceutical products. They are suspected to induce toxic effects. Hyaluronan (HA), a linear biopolymer, is involved in several biological processes. The aim of this work is to in vitro investigate if HA is able to decrease preservatives toxicity.

Methods: A sensitive human epithelial cell line was treated first with BAK, PE and BP, then treated with HA (HA 1.6 kDa, 0.2% w/v). Flow cytometry, fluorescence microscopy and microplate cytofluorometry were performed to evaluate expression of CD44 receptor, cell viability, oxidative stress, mitochondrial mass, chromatin condensation and plasmic-membrane permeability.

Results: HA induces neither oxidative stress nor apoptosis. HA significantly decreases oxidative stress, apoptosis and necrosis induced by BAK, PE and BP. We suggest that HA interacts with cell plasmic-membrane and inhibits cell death receptors.

Conclusion: High molecular weight HA (1.6 kDa, 0.2%) is an effective protective agent against three different toxic preservatives.

P1-22

Increased F2-isoprostanes and cholesterol oxidation products in colon of rabbits fed a high cholesterol diet. Influence of zinc supplementation

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Colon cancer is the second most common cause of cancer deaths in more affluent western societies. Epidemiological studies indicate that colon cancer is ultimately linked to diet for the majority of cases [1]. In particular, diets low in fibre, high in calories, high in animal fats, high in red and processed meat are associated with increased colon cancer risk. Rat diets supplemented with heme have previously been shown to increase colonocyte proliferation, fecal water cytotoxicity and pathophysiological changes in colon crypts [2, 3]. Therefore we wished to further examine mechanisms of oxidative damage in the colon of heme fed rats and investigate the influence of high fat diet in order to explain why western diets are associated with higher risk of colon cancer.

Groups of 5 rats were fed 4 different purified diets containing either heme diet (2.5% w/w hemoglobin), high fat diet (18% w/w fat, 1% w/w cholesterol), western diet (combination of hemoglobin 2.5% and 18% fat, 1% cholesterol) or control diet. After 4 weeks colons were excised and washed gently before immediately freeze clamping. Specific lipid peroxidation biomarkers were measured by gas chromatography-mass spectrometry (GC-MS) with heavy isotope dilution. Colonic lipids were extracted using the Folch extraction, hydrolysed in anaerobic conditions and fractionated using a single anion exchange solid phase extraction procedure developed in our laboratory. Total F2-isoprostanes and cholesterol oxidation products were derivatised and analysed by different GC-MS methods.

Histochemical examination demonstrated that increased fat and/or heme in rat diet caused significantly altered colon crypt morphology and colonocyte mucin secretion. Compared to control fed animals, several cholesterol oxidation biomarkers were significantly elevated by all 3 supplemented diets, particularly western diet. Control colon levels of F2-isoprostanes were not significantly elevated by any diet.

Our results indicate that elevated levels of heme or fat in diet induce colon damage and cholesterol oxidation. Feeding rats westernised diet may be a useful model to study the influence of diet on colon disease.

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P1-23

Increased cholesterol oxidation products in colon of rats fed a westernised diet high in fat, cholesterol and heme

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A constant flux of complex mixtures of dietary chemicals is delivered into the intestinal lumen. Therefore the colon is exposed to many agents, including toxins, antioxidants and pro-oxidants that can generate reactive oxygen species. Epidemiological studies indicate that colon cancer is ultimately linked to diet for the majority of cases [1]. In particular, diets high in animal fats are associated with increased colon cancer risk.

We have investigated an animal hypercholesterolemia animal model to examine the effect of high cholesterol diet on colon and the influence of zinc. New Zealand White Rabbits were fed a high cholesterol 1% (w/w) diet with or without zinc (1g/kg diet) supplementation for 8 weeks. Controls were fed normal diet.

Distal colon was washed gently and sections were immediately freeze clamped. Specific lipid peroxidation biomarkers were measured by gas chromatography-mass spectrometry (GC-MS) with heavy isotope dilution. Colonic lipids were extracted using the Folch extraction, hydrolysed in anaerobic conditions and fractionated using a single anion exchange solid phase extraction procedure developed in our laboratory. Total F2-isoprostanes and cholesterol oxidation products were derivatised and analysed by different GC-MS methods.

Histochemical examination demonstrated that cholesterol fed rabbits had significantly altered colon crypt morphology and colonocyte mucin secretion.

Control colon levels of F2-isoprostanes (1.2 ± 0.3 ng/g tissue, mean $\pm$ S.D. $n = 5$) were significantly elevated ($p < 0.05$), together with increases in cholesterol oxidation, in rabbits fed high cholesterol diet. Treatment with zinc significantly protected against the increases of all lipid peroxidation biomarkers, indicating an antioxidant effect.

Our results indicate that high cholesterol diet induces colon damage and may be significantly influenced by levels of metal ions in the diet.

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P1-24

Differential effects of aqueous or ethanolic procyanidin solutions on hepatic redox status comparison to red wine

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Oxidative stress, defined as the imbalance of the organism redox state (oxidant/antioxidant), has been seen as an important element in several pathologies, including cardiovascular diseases, cancer, neurodegenerative pathologies, and aging itself. Moderate red wine (RW) consumption has been related to improved health and the antioxidant potential of the beverage has been implicated. Procyanidins (P) are abundantly found in RW and studies are attributing part of the effects of RW to these compounds, namely metal quelation, free radical scavenging or interference with cellular enzyme systems. The content of ethanol in RW seems also very important for the effect of the beverage, as evidenced by studies that compare the effect of RW with alcohol-free RW or grape juice.

For this reason, we intended to evaluate the effects of RW or grape seed P solutions in the presence of absence of ethanol on hepatic redox status. Twenty-five male Wistar rats were divided into 5 groups and treated with 1) RW, 2) aqueous P solution (PW, 200 mg/L) or 3) ethanolic P solution (PE, 200 mg/L in 13% ethanol) and appropriate water (W) or 13% ethanol (E)-drinking controls were included. After 8 weeks of treatment, animals were perfused and plasma and livers were collected. Damage to lipids, proteins and DNA, levels of glutathione (GSH-reduced form and GSSG-oxidized form) and glutathione dependent enzymes, as well as the activity of catalase and superoxide dismutase were assessed as hepatic oxidation biomarkers. NFkB transcription activity was measured as well as TNFa expression. Enzyme indicators of hepatic damage were assessed in the plasma.

Although no change was seen in hepatic lipid and DNA oxidation levels in either group, protein oxidation was only significantly decreased in PE-treated animals and in RW-treated animals. The low GSSG/GSH ratio levels observed in E-treated animals was reverted in PE and RW groups, to values similar to those found in W control. Measurements of antioxidant enzyme activities showed a significant increase of superoxide dismutase in RW animals. Regarding catalase, treatments with E and PW significantly decreased its activity. NFkB activation was present in RW-treated animals when compared to both control groups, but these changes were not accompanied by an increase in TNFa transcription levels. Only in PE-treated rats a significant decrease in the expression of this cytokine was observed. In respect to hepatic damage markers measured in the plasma, there were no differences between treatment groups.

In conclusion, RW alters hepatic redox state, part of these effects being similar to those obtained after P treatment. The presence of ethanol together with P seems to intensify the effect of the later revealing some kind of interaction between the two RW components. Despite NFkB activation, the meaning of redox changes is still unclear and deserves further attention.

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P1-25

The reaction of nitrone spin trap PBN with glutathiyl radical: probable antioxidant mechanism

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Glutathione, GSH, is considered to be a major intracellular antioxidant. However, the product of one-electon oxidation of GSH, glutathiyl radical, GS*, may contribute in oxidative damage processes, e.g. via formation of highly reducing glutathione disulfide radical anion. N-tert-Butyl-alpha-phenylnitrone, PBN, in numerous conditions demonstrates neuroprotective, anti-inflammatory and anti-ageing effects but the mechanisms of therapeutic activity of PBN are poorly understood. The primary role of the radical trapping mechanism in PBN therapeutics is considered doubtful particularly taking into account extremely low trapping efficiency of the superoxide radical. On the other hand, the rate constant of GS* scavenging by PBN has not been reported.

In this work we measured the rate constant of GS* scavenging by PBN using direct detection of GS* by laser flash photolysis and indirectly by competitive EPR detection of the spin adducts of PBN and another spin trap, DMPO (5,5-dimethyl-1-pyrroline N-oxide).

The rate constants of glutathiyl radical scavenging by PBN and its fluorinated analogue, fPBN, have been found to be equal to $(6.7 \pm 1.5) \times 10^7$ 1/(Mxs) and (4.3 ± 0.8)x10^7 1/(Mxs), respectively. The reverse reaction, release of GS* radical from the PBN/GS* adduct, has been unambiguously proved by the analysis of EPR and NMR spectra of reaction products.

The rate constant of the GS* release from PBN and fPBN were found to be equal to (1.7 ± 0.2) 1/s and (0.9 ± 0.2) 1/s, correspondingly. Diamagnetic, EPR-invisible products of PBN adduct degradation were studied by 1H NMR and 19F NMR using fPBN. This latter approach allowed us to observe non-radical addition of GSH to PBN with the formation of corresponding hydroxylamine.

The observed monomolecular decomposition of the paramagnetic adduct, ST/GS*, back to GS* and the parent nitrone, and decomposition of the corresponding reduced diamagnetic adduct, ST/GSH, back to GSH and parent nitrone, result in recycling of both nitrone spin trap and glutathione. In combination with high trang efficiency of GS*, the observed reactions may contribute to the biological activities of the nitrones. Being diffusible inside the cells, PBN reacts with GS* radical with extremely high rate constant. At low physiological rates of GS* formation, the recombination of GS* is less probable than its trapping by PBN. The corresponding antioxidant efficiency of the PBN, is determined by significantly lower oxidizing potential of PBN/GS* adduct compared with that for highly oxidizing GS* radical. Note also that the competition between monomolecular decomposition and reduction of PBN/GS* adduct might be affected by intracellular redox state influencing PBN functionality.

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P1-26

Intracellular reactive oxygen species and nitric oxide generation in ageing muscle

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An important characteristic of ageing is loss of muscle strength which is caused by the depletion of muscle fibres and atrophy of the remaining fibres. By age 80, humans have lost about 40% of their muscle mass [1]. Reactive oxygen species (ROS) are constantly produced by skeletal muscle and play a critical role in the ageing process demonstrated by increased levels of markers of oxidative damage to DNA, proteins and lipids in ageing muscles [2].

The aim of this study was to further elucidate the roles of ROS in ageing by examining the real-time generation of intracellular ROS and nitric oxide in single mature skeletal muscle fibres isolated from young and aged mice.

Single muscle fibres were isolated from the Flexor Digitorum Brevis muscles of young (2–4 months) and aged (26–28 months) mice and intracellular ROS/RNS generation was detected with the use of three fluorescent probes, DCFH, hydroethidine and DAF-FM. Fibres were loaded with the appropriate probes and subjected to resting or contracting protocols and their intracellular ROS/RNS generation was analysed by fluorescence microscopy. Fibres subjected to electrical stimulation displayed increases in DAF-FM and ethidium fluorescence compared with rested fibres, however there was no difference in the rate of change in fluorescence between young and aged muscle fibres during the stimulation period. The baseline rate of change in DCFH fluorescence was higher in aged muscle fibres compared with young muscle fibres. Furthermore, an increase in the rate of change of fluorescence was observed in young muscle fibres during electrical stimulation. However, this rise was not evident in aged muscle fibres. These data provide further evidence that there is increased generation of some ROS during the ageing process and indicates that intervention in this process may provide an approach to attenuation of the muscle loss observed in ageing.

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P1-27

Ascorbic acid treatment leads to a decrease in NO levels in UV-irradiated human microvascular endothelial cells (HMEC-1)

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In several cell types UV light induces the synthesis of inducible nitric oxide synthase (iNOS) by activating a couple of transcription factors, first of all AP-1 and NF-κB. In the presence of molecular oxygen and other cofactors, iNOS converts the proteinogenic amino acid L-arginine to L-citrulline. A further product of this reaction is the free radical nitric oxide (NO), which reacts with reactive oxygen species (ROS) to yield reactive nitrogen species (RNS). RNS trigger nitrosative damage on proteins, lipids and even DNA and thus can lead to cellular dysfunction and death. Ascorbic acid (AA), as the main water-soluble antioxidant vitamin can limit the formation of ROS. Thus, also a subsequent reduced creation of RNS and a decrease of ROS-iNOS-mediated NO production should be the consequence. Furthermore the NO concentration should be reduced by a direct interaction of the free radical NO with AA as a scavenger.

Human microvascular endothelial cells (HMEC-1) were used as a model system for simulating UV-induced oxidative stress, e.g. like sunburn in the skin. Cells were supplemented with AA in two concentrations (50 and 100 μM) and subsequently irradiated with UV-A (25 J/cm^2) to investigate if this preventive supplementation can attenuate the increased NO levels in only UV-irradiated cells. NO concentration was visualized using the fluorescent probe DAF-2 DA on a life cell imaging system. Quantification was done using TILLVision software.

While NO concentrations – as expected – increased in UV-irradiated cells, the preventive supplementation with AA dose-dependently attenuated this increase. In addition, AA supplementation without subsequent irradiation led to decreased NO levels in the cells, indicating a direct interaction independent from ROS.

In conclusion, an adequate supply of ascorbic acid seems to protect endothelial cells against oxidative and nitrosative stress and its consequences at least in vitro. Maybe, the known effects of high-dosis AA infusion in burn patients to reduce water loss can be explained by comparable pathways.

P1-28

QSAR studies on di(hetero)arylamines derivatives of benzo(b)thiophenes as free radical scavengers

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Synthetic antioxidants are widely used in food industry, but because of toxic and carcinogenic effects revealed by some compounds such as BHA and BHT, their use is being restricted. The pursuit for novel compounds with antioxidant properties gained higher significance, since these compounds may contribute for the prevention of diseases in which free radicals are implicated. As reported in our previous papers, different series of novel di(hetero)arylamines derivatives of benzo(b)thiophenes were synthesized and studied as free radical scavengers [1,2]. In this study, a quantitative structure activity relationship (QSAR) model was developed to guide the synthesis of new potential radical scavengers. To increase the predictability of the QSAR model, DDPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of 14 di(hetero)arylaminobenzo(b)thiophenes was assayed, and the results were pooled together with the DPPH radical activity results of 12 di(hetero)arylaminobenzo(b)thiophenes already reported by us. The 26 compounds used were divided in training set (18 compounds) and validation set (8 compounds). The antioxidant activity was correlated with 4 molecular descriptors calculated using DRAGON software tool, and the QSAR model was built using the partial least squares projection of latent structures (PLS) method. This QSAR model ($n = 18$; $r^2 = 0.958$; $q2 = 0.919$; $r2\text{pred} = 0.943$) properly predicted pEC50 values for the validation set of benzo(b)thiophene derivatives, and proved to be a useful tool for the screening of new potentially better di(hetero)arylaminobenzo(b)thiophenes free radical scavengers.

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P1-29

Dependence of the peroxidation of membrane lipids on the physical proprieties of the membrane as studied in liposomes of different compositions

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Free radicals, formed via different mechanisms, induce peroxidation of membrane lipids. This process is of great importance because it modifies the composition, hence the physical properties and the function of the membranes. Much research effort has therefore been devoted to the understanding of the factors that govern peroxidation, including the composition and properties of the membranes and the inducer of peroxidation. In view of the complexity of biological membranes, many studies addressed the latter issues in simplified model systems, mostly lipid vesicles (liposomes). Lipid peroxidation in these model membranes may be very different from peroxidation in biological membranes. Yet, the results obtained in model membranes may be used to advance our understanding of issues that cannot be studied in biological membranes. Nonetheless, in spite of the relative simplicity of peroxidation of liposomal lipids, these reactions are still quite complex because they depend in a complex fashion on both the inducer of peroxidation and the composition and physical properties of the liposomes. This complexity is the most likely cause of the apparent contradictions between the results of different studies. We have studied the peroxidation of liposomal oxidizable phospholipids in liposomes made of various compositions with respect to the fraction of oxidizable polyunsaturated fatty acid residues, surface charge and added cholesterol. The main conclusion of this study is that all our results, as well as most, if not all, of the published results can be understood on the basis of the physico-chemical properties of the liposomes. Specifically: (1) The kinetics of peroxidation induced by an??external?? generator of free radicals (e.g. AAPH) is governed by the balance between the effects of membrane properties on the rate constants of propagation (kp) and termination (kt) of the free radical peroxidation in the relevant membrane domains, i.e. in those domains in which the oxidizable lipids reside. Both these rate constantsend similarly on the packing of lipids in the bilayer, but influence the overall rate in opposite directions. (2) Peroxidation induced by transition metal ions depends on additional factors, including the binding of metal ions to the lipid?water interface and the formation of a metal ions-hydroperoxide complex at the surface. (3) Reducing agents, commonly regarded as??antioxidants??, may either promote or inhibit peroxidation, depending on the membrane composition, the inducer of oxidation and the membrane/water partitioning. All the published data can be explained in terms of these (quite complex) generalizations. More detailed analysis requires additional experimental investigations.

P1-30

Polyphenolic compounds protect and repair oxidative DNA damage in a neuronal cell model

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The excessive intracellular accumulation of reactive oxygen species (ROS) can cause a disturbance in the cells natural antioxidant defence systems, resulting in damage to all biomolecules, including nucleic acids. In fact, oxidative DNA damage is sometimes difficult to repair, being described as the type of damage most likely to occur in neuronal cells.

In this study, the protective effects of three polyphenolic compounds, luteolin, quercetin and rosmarinic acid, against oxidative DNA damage induced in PC12 cells, a neuronal cell model, were investigated by the Comet assay.

Although luteolin and quercetin prevented the formation of strand breaks to a greater extent than rosmarinic acid, this last one presented the highest capacity to repair strand breaks formation. In addition, rosmarinic acid was the only compound tested that increased the repair of oxidized nucleotidic bases induced with the photosensitizer compound Ro 19–8022. The activity of repair enzymes was indicated by the in vitro base excision repair assay, using a cell-free extract obtained from cells previously treated with the compounds to incise DNA. The quantification of the expression of OGG1 and APE1 repair genes by real time RT-PCR indicated a regulation, at the level of OGG1, by rosmarinic acid.

The data obtained is indicative that the effect of rosmarinic acid seems to be more specific for DNA repair mechanisms, rather than acting directly on ROS scavenging, as it is the case for luteolin and quercetin. Therefore, these results suggest the importance of these polyphenols, and in particular rosmarinic acid, as protecting agents against oxidative stress-induced DNA damage that commonly occurs in neurodegenerative diseases.

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P1-31

Spin trapping of 1-palmitoyl-2-lineloyl-phosphatidylethanolamine free radicals generated under oxidative stress, using DEMPO, DMPO and mass spectrometry

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Mass spectrometry is a method that has been used in the study of free radical spin adducts of lipids and phospholipids, allowing the detection and structural characterization of both carbon and oxygen centered radical adducts (1, 2). This approach give complementary and additional information, namely the identification of the specific location of the radical, when compared with EPR, the most common used method for the detection of spin adducts. However, the detection of radical adducts by mass spectrometry is not so vast when compared with EPR, and there are no studies published dedicated to the identification of spin adducts of radicals generated from phosphoethanolamine oxidation, using mass spectrometry.

In this study, spin trapping and electrospray (ESI) mass spectrometry (MS) was used to identify and characterize the free radical species formed from 1-palmitoyl-2-lineloyl-phosphatidylethanolamine (PLPE), when in presence of the hydroxyl radical (Fenton reaction). The spin traps used were DMPO and DEPMPO. DMPO was used in other spin trapping studies of phosphatidylcholine oxidation and allowed the detection of both carbon and oxygen centered radicals. The spin trap DEPMPO has not been used, to our knowledge, for the detection of phospholipid radicals, using MS.

In the ESI-MS spectra it was possible to observe the DMPO and DEPMPO adducts, as $(M^{+}Na)^{+}$ ions, of both carbon and oxygen centered radicals. It were also observed adducts formed by insertion of one DMPO or DEPMPO molecules combined with insertion of one, two, three, four and five oxygen atoms, and adducts resultant from insertion of two DMPO/DEPMPO molecules combined with insertion of one, two and three oxygen atoms. The fragmentation observed in the ESI-MS/MS spectra allowed to confirm the presence of both traps linked to PLPE radicals, as well as the presence of hydroxyl and peroxyl radicals, inferred by the observation of product ions formed by loss of the spin trap plus one and two oxygens, as well as the ions $(\text{spin trap} + O + Na)^{+}$ and $(\text{spin trap} + 2O + Na)^{+}$. The identification of the location of the radicals in the unsaturated sn-2 fatty acyl chain was pinpointed by product ions formed by cleavage of C-C bond adjacent to the location of the spin trap.

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P1-32

EPR-Imaging of reactive xygen species in mice

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Introduction: Formation of reactive oxygen species one of the key factors for different physiological and pathological conditions such cell growth, exercise, cancer, aging, cardiovascular diseases. The detection as well as the imaging of reactive oxygen species does still required further clarifications, development of applications and techniques. Our aim was to provide the information about novel successful visualization of oxidative stress in vivo by focusing on production of ROS in internal organs.

Methods: For the imaging of ROS we used C67/blc mice. Animals were anesthetized with ketamin/xylazine (4 mg/200 μg). The new synthesized spin probe NOX-C224 solution containing 25 μM of deferoxamine and 5 μM DETC was injected intraperitoneal. The 2-d imaging was performed using the L-Band EPR system ELEXSYS E540 (Bruker BioSpin GmbH) equipped with newly developed L-Band microwave bridge (power: 300 mW; microwave frequency 1.1 GHz; tune range:1–100 MHz), which was able to reduce or compensate breathing or heart beat interferences. Measurements were performed 20, 35, 50, and 120 min after injection of the spin probe.

Results: We observed the decay of colour intensity of images representing the formation of ROS from high to low in the liver, followed by kidney and bladder. We were not able to detect ROS in colon or small intestine. The attenuation of colour intensity in liver and other tissue correlate with decrease of spin probe concentration from 280μM to 1μM to the end of experiment.

Conclusions: Application of this imaging method will allow in the near future long-term vision in living animals and patients clarifying pathophysiological conditions involving oxidative stress e.g. the early detection of blood vessels at risk from atherosclerosis or thrombosis, long before the event takes place. Thereby the measured parameters could be used for preventative medicine or as a direct read-out of successful therapy.

P1-33

Antioxidant and chemopreventive properties of Vicia faba extract and its flavonoid fractions

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The last decade, remarkable progress has been made in the development of chemoprevention strategies, started by research of chemopreventive agents derived from plants and functional food which constitute integral part of human diet. Legumes which play an important role in Mediterranean diet, known for many beneficial health effects, constitute a good source of phytochemical compounds which can be considered as possible chemopreventive agents. Various Leguminosae family plants cultured in Greece were collected and screened using in vitro assays for possible chemopreventive agents. Initially, various methanolic and aqueous extracts were tested for their antioxidant capacities.

From the results obtained, the methanolic extract derived from the aerial plant parts of Vicia faba plant exhibited significant radical scavenging capacity and protective activity against free radical-induced DNA damage and was chosen for further research in order to uncover its active principles.

Ten flavonoid fractions, which are mainly mixtures of two compounds, were isolated and identified in the methanolic extract of Vicia faba and screened for their $DPPH^X$ radical scavenging capacity and their protective activity against ROO^X radical and OH^X radical-induced DNA damage. All these fractions exhibited significant radical scavenging capacities and very potent protective activity against both ROO^X and OH^X radical induced DNA damage. The activities of the fractions were more potent than that of the initial extract implying that the polyphenolic compounds present in them may constitute some of the extract components responsible for the observed properties of the extract. These results also indicate mechanisms by which these phenolic compounds isolated from Vicia faba plant extract may act as possible chemopreventive agents.

P1-34

Polyphenolic compounds of grapes, wines and their antimutagenic

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Antioxidative capacity and free radicals in various parts of Vitis vinifera plant were studied *in vivo* using EPR spectroscopy. The phenolic substances, frequently called polyphenols, are secondary plant metabolites that are distributed throughout the plant kingdom and they include many classes of compounds ranging e.g. from phenolic acids, colored anthocyanins, simple flavonoids and complex flavonoids. Polyphenolic compounds present in vine grapes and in products made of them (grape juice, wine) can gain ground in the mechanism of mutagenesis and of carcinogenesis as chemopreventive factors. It is mostly the case of ability of some chemical substances of inducing conjugative enzymes of second phase of metabolism (glutathion-transferase, N-acetyl-transferase), eventually of inhibiting enzymes dependent on cytochrome P450, taking part in the first phase of metabolic change. During this phase of metabolism a number of environmental procarcinogens (PAHs, aflatoxin B1) is activated. It is quite clear from the above mentioned short literature survey, that it is absolutely necessary for the successful research in this area to measure free radicals and antioxidative capacity of grapevine and wine. Reactive oxygen species (ROS) are constantly formed in the human body and removed by antioxidant defenses. Antioxidants can act by scavenging biologically important reactive oxygen species. Free radicals in different parts of the Vitis vinifera plants were studied in vivo using EPR spectroscopy. During vinification the content of the phenolic acids (gallic and caftaric), catechins, resveratrol, piceid and pterostilbene in some cases was measured not only in the wines, but also in a must, rachis, pedicels, yeast and rape. In the selected wine samples the antioxidative capacity was measured using EPR spectroscopy. Testing of phenolic compounds adsorption during the final wine processing, e.g. clarification of the white wines and sorption capacity of the various sorbents and their adsorption ability for different types of phenolic compound. The relationship between the structure of the adsorbent and the phenolic compounds are studied.

Methodology for the EPR spectroscopy studies, especially *in vivo* studies and testing of antimutagenity was developed. The antioxidative capacity and the presence of free radicals in wine samples was measured and evaluated. The measurements of antioxidative capacity, free radicals, antimutagenic activity will continue and some hypothesis regarding the content of phenolic compounds and their antioxidative capacity and antimutagenic activity will be tested.

The main purpose is to find the mechanisms which influence the distribution of phenolic compounds in all parts of Vitis vinifera plants and their transportation to the red wines during vinification processes and further to answer the question how the dryness and irrigation as a stress factors could influence the content of phenolic compounds in the Vitis vinifera plants and subsequently in the wines. Very important additional information will be the antioxidative capacity and free radicals measurements (in vivo at the Vitis vinifera plants and in wine samples) and antimutagenic activity of the wine samples. Also very important results are expected from the experiments of white wine finishing, e.g. clarification using different sorbents. This last question was solved mostly empirically based on the professional experience more than on the scientific results up to now. The obtained complex results will be very interesting from the practical point of view. As a very important results we expect beside good publication also some general recommendations for the wine producer how to maintain high level of phenolic compounds in the final wines and simultaneously to keep the production of the high quality wines, especially Moravian ones.

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P1-35

Short term exposure to aluminium decreases hepatic redox potential in mice.

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Introduction: A number of environmental factors have been suggested as possible contributory causes of Alzheimer's disease (AD). For aluminium (Al) there is only circumstantial evidence linking this metal with Alzheimer's disease but no causal relationship has yet been proved.

The major pathohistological findings in the AD brain are the presence of neuritic plaques containing β-amyloid as a result of oxidative stress. So, chronic Al administration might be responsible for oxidative cell damage, interfering with mitochondrial functions inducing β-amyloid accumulation and neurodegenerative damage.

In this preliminary study the short-term exposure of Al and interaction of Al with zinc (Zn) and selenium (Se) has been studied in relation to the redox status of liver, brain and kidney.

Methods: Balb/c mice ($N = 6$) have been exposed to Al (25 mg Al^{3+}/kg), Zn (1.56 mg/kg), Se (1.25 mg/kg), Al + Zn and Al + Se for 16 hr. Liver, kidneys, and brain were removed and rapidly cooled on ice. Organs were carefully weighed and homogenized. Homogenates were centrifuged at 1,000 g for 10 min at 4 °C. Supernatant was saved and pellets were discarded. The supernatants were centrifuged again at 12,000 g for 15 min at 4 °C, filtered, poured into Eppendorf tubes and frozen at − 80 °C.

Total glutathione (GSH_{tot}) and oxidized gluthation (GSSG) have been measured after deproteinization by using glutathione reductase and DTNB. GSSG is determined after derivatization of GSH by 2-vinylpyridine. Glutathion (GSH) and the ratio GSH/GSSG have been calculated.

Results and discussion: The short term exposure to Al, Zn and/or Se resulted in changes of the redox status of the liver mitochondria, not in brain or kidney samples. Changes in LDH were not observed. Most pronounced changes have been observed in GSH_{tot}. Control samples showed a GSH_{tot} of 1.5 mmol/L, whereas exposure to Al decreased the GSH_{tot} to 0.7 mmol/L. Exposure to Zn and especially Se had a positive influence on GSH_{tot} and co-exposure with Al + Se or Al + Zn can restore the decrease of Al exposure.

The ratio between GSH/GSSG was decreased by exposure to Al from 5.1 to 2.0 and can be restored to 4.8 by co-exposure to Al + Se.

From these preliminary experiments it can be concluded that short-term exposure to Al decreases the glutathione status in the postmitochondrial supernatant of the liver, which can be prohibited or restored by co-exposure with Zn or Se.

P1-36

Analysis of the mechanisms involved in intramacrophagic elimination of Leishmania

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Activation of murine macrophages infected with Leishmania species results in intracellular parasite killing. Although it is known that this only occurs in the presence of a Th1 immune response, it is not clear which are the molecular mechanisms responsible for parasite destruction. Hydrogen peroxide, nitric oxide and peroxynitrite are all toxic to axenic Leishmania and, indeed, studies from different authors have supported these molecules as important agents in the elimination of this pathogen. However, others provided evidence suggesting that it is N-Hydroxy-L-Argininie (NOHA), an intermediate formed during the synthesis of NO, the molecule used by macrophages to kill Leishmania. The pathway that results in NO production depends on L-Arginine availability. All living organisms need L-Arginine to replicate and grow, and Leishmania is no exception. This amino acid is metabolized by arginase to ornithine, the building block for polyamine formation. Being a potent inhibitor of arginase, NOHA produced by macrophages would inhibit parasite arginase (in addition to its own), starving Leishmania from essential polyamines and leading to their death. In this work we have produced L. infantum strains expressing higher levels of arginase. The parasites were seen to present an enhanced capacity to metabolize L-arginine. We are now comparing the infectivity rates of recombinant and control parasites. A higher infectivity of recombinant cells suggest that either NOHA is a major microbicidal mechanisms or that these parasites present increased capacity to remove L-arginine from the hosted by biochemical and histological alterations, and oxidative damage. Mel administration partially restored these adverse effects.

POSTER-SESSION 2 — MECHANISMS OF ANTIOXIDATIVE DEFENCES

P2-1
Protective effects of melatonin against uranium-induced nephrotoxicity in rats

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The effects of uranium (U) exposure have been widely studied in mammals. One of the most important adverse effects of U is nephrotoxicity.
In this study, we investigated modifications on endogenous antioxidant capacity and oxidative damage in renal tissue, suggesting an oxidative stress-like mechanism for U toxicity. Likewise, the protective effects of melatonin (Mel) were investigated. Biochemical and histopathological changes were also evaluated. Rats were given single doses of uranyl acetate dehydrate (UAD) at 5 mg/kg (sc), Mel at 10 and 20 mg/kg (ip), UAD (5 mg/kg) plus Mel (10 and 20 mg/kg), or vehicle (control group).
The results showed a significant effects of Mel in some urinary and serum parameters when UAD was administered. At 20 mg/kg of Mel, an increase of U excretion accompanied by a significant decrease in the renal content of U was detected. Moreover, Mel administration also reduces the severity of the U-induced histological changes. On the other hand, UAD produced oxidative tissue damage, as evidenced by increases in SOD activity and TBARS levels. Concurrent administration with Mel reduced these values, but not significantly. In erythrocytes, the reduction in SOD activity and the increase in TBARS levels induced by UAD were significantly restored by Mel.
In plasma, only the GSSG/GSH ratio was decreased with Mel. The present results suggest that exposure to UAD caused renal toxicity evidenced by biochemical and histological alterations, and oxidative damage. Mel administration partially restored these adverse effects.

P2-2
Intestinal and intraperitoneal absorption and bioavailability of the antioxidants unconjugated bilirubin, biliverdin and bilirubin ditaurate in the rat

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The bile pigments, bilirubin and biliverdin, possess antioxidant and anti-inflammatory properties and their exogenous administration protects against a wide array of models of inflammation and damage. Despite the encouraging and versatile therapeutic potential of bile pigments, very little is known about their in vivo parenteral or enterologic absorption after exogenous administration.
This study aimed to investigate the absorption and pharmacokinetics of bile pigment administration after intravenous, intraperitoneal and intraduodenal administration. Anaesthetized Wistar rats had their bile duct, jugular and portal veins cannulated. Sodium bilirubinate, bilirubin ditaurate and sodium biliverdinate were infused and the circulating bile pigment concentrations and their biliary excretion were measured over 180 minutes.
When sodium bilirubinate, sodium biliverdinate and bilirubin ditaurate (1 mg; 2.7 mg/kg body weight) were administered intravenously, their plasma concentrations decreased exponentially over time and the native and metabolized compounds subsequently appeared in the bile. When administered intraperitoneally (1 mg; 2.7 mg/kg body weight), their absolute bioavailabilities equaled 17.1, 16.1 and 33.1%, respectively and correspondingly 40, 30 and 37% of the same bile pigment doses were excreted in the bile. Intraduodenal sodium bilirubinate and bilirubin ditaurate administration (10 mg; 27 mg/kg body weight) increased their portal and systemic concentrations and their systemic bioavailability equaled 1.0 and 2.3%, respectively. Correspondingly, 2.8 and 4.8% of the doses were excreted in the bile. Sodium biliverdinate was not absorbed. Bile pigments are significantly absorbed from the intestinal and peritoneal cavities, demonstrating potential routes of administration for the study of these potentially efficacious antioxidant compounds.

P2-3

A cinnamon-derived Michael acceptor for anticancer intervention: cinnamic aldehyde impairs melanoma cell proliferation and tumor growth

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Altered redox signaling and regulation in cancer cells represent a chemical vulnerability that can be targeted by selective prooxidant redox intervention. Dietary constituents that contain an electrophilic Michael acceptor pharmacophore may therefore display promising chemopreventive and chemotherapeutic anti-cancer activity.

Here, we demonstrate that the cinnamon-derived dietary Michael acceptor trans-cinnamic aldehyde (CA) impairs melanoma cell proliferation and tumor growth. Guided by our earlier work that demonstrated CA-induction of the cellular electrophilic Nrf2/Keap1 stress response pathway, we tested CA for inhibition of melanoma cell proliferation and viability in vitro and in vivo.

Feasibility of therapeutic intervention using high doses of CA (120 mg/kg, p.o., q.d., 10 days) was demonstrated in a human A375 melanoma SCID-mouse xenograft model, where significant reduction of tumor growth and PCNA immunohistochemical staining were observed. Low micromolar concentrations (IC50 < 10 μM) of CA suppressed proliferation of human metastatic melanoma cell lines (A375, G361, LOX) with concomitant elevation of intracellular ROS production. CA-induction of cell cycle arrest in G1 phase was confirmed by bivariate flow cytometric analysis of BrdU-incorporation and 7-AAD DNA staining in A375 cells. At higher CA doses (25 μM), induction of apoptosis was observed as confirmed by annexinV-FITC/PI staining, procaspase-3 activation, and PARP cleavage. Using RT-PCR array expression analysis (Human Stress and Toxicity Pathway Finder, Superarray) followed by immunoblot confirmation in A375 cells, it was demonstrated that CA strongly upregulates HMOX-1 (heme oxygenase-1) and CDKN1A (p21), a key mediator of G1 phase arrest. These findings support a previously unrecognized role of CA as a dietary anticancer factor.

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P2-4

Expression of smooth muscle-specific proteins in human gingival keratinocyte cell lines containing gp91phox homolog Nox1: possible involvement of MEF2B

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Transdifferentiation of human epithelial cells and fibroblasts to myoepithelial cells and myofibroblasts respectively has been recognized one of the steps in neoplastic progression of breast cancer. It has been shown that low levels of oxidants (H_2O_2) can induce transdifferentiation of human fibroblasts to myofibroblasts. The role of oxidants (H_2O_2) on generation of myogenic phenotypes from human gingival (pre)malignant epithelial cells has not been investigated.

Here, smooth muscle-specific proteins were measured by Western analysis in human immortalized gingival keratinocytes. We have previously generated cell lines (NuB1, NuB2, NuB3, FuB1, and FuB2) by overexpression of gp91phox homolog Nox1 (J Invest Dermatol. 127, 2171, 2007). Without Nox1 transfection, one so-called "non-differentiating" line (lacking involucrin) was also generated which later expressed Nox1 when they reached proliferative state. Here, we showed that vimentin-positive fibroblast-like (elongated and spindle-shaped) lines, i.e., "non-differentiating" line, NuB1, and FIB cells expressed higher levels of alpha-smooth muscle actin and phospho-caldesmon than epithelium-like or cobblestone cells, i.e., NuB2, NuB3, FuB1, FuB2 and EPI cells. By using Boyden chamber for invasion assay, we concomitantly found that elongated and spindle-shaped cells migrated through filters (indicating motility) in a much greater extent than those cobblestone cells. It is known that myocyte enhancing factor (MEF) 2B is a potent transactivator expressed in early myogenic lineages, and that MEF2B regulates Nox1 in vascular smooth muscle cells (FEBS J 274, 5128, 2007).

In nuclear extracts, cobblestone cells expressed MEF2A, 2C, and 2D; while elongated and spindle-shaped cells expressed MEF2A and 2B indicating an association of MEF2B with elongated phenotype. When cobblestone NuB2 cells were overexpressed with MEF2B, morphological changes including cellular elongation and parallel striated features were observed; concomitantly, there were increases in alpha-smooth muscletin and phospho-caldesmon expression compared with plasmid transfection. Herein, we have demonstrated that MEF2B may mediate myogenic induction of human gingival keratinocytes which contain Nox1. This process was related to increased cellular plasticity and motility. These spindle-shaped human gingival keratinocytes exhibiting dual fibroblast and myocyte characteristics are likely in an increased step of neoplastic progression.

P2-5

The small Hsp β-crystallin plays a key role in the resistance to oxidative stress determined by VEGF in skeletal myoblasts

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Although the role for Vascular Endothelial Growth Factor (VEGF) expression in non-endothelial cells has not been clearly identified, recent studies suggest that this growth factor and their receptors could have protective potential, as for example in preventing neuronal cell death from ischemia and promoting neurogenesis in vitro and in vivo. Indeed, it is well known that in different cellular types, as vascular smooth cells, keratinocytes, rat heart endothelial cells, VEGF expression is triggered upon the influence to different injuring factors, e.g., hypoxia, UV light, reactive oxygen species (ROS) or mechanical injury. Regarding this hypothesis, we have already seen that myogenic cell line, stably transfected with hVEGF165 cDNA (C2C12VEGF) and releasing high level of VEGF in culture medium, have an enhanced cell survival and resistance to apoptosis after exposure to cytotoxic concentrations of H_2O_2 (100–700 μM). Moreover, we identified alpha b-crystallin (b-Cry), a member of the small Hsp family, as possible mediator of the anti-apoptotic effect exerted by VEGF.In this work, we additionally investigated about the role of b-Cry in the resistance to apoptosis induced by H_2O_2 in the myogenic C2C12 cells exposed to VEGF, and the molecular pathways involved in this process.

To this aim, we first analysed the cellular and molecular response towards H_2O_2-induced apoptosis in C2C12 myoblasts exposed to different concentrations of exogenous VEGF (0–10 ng/ml), and then we verified if the specific silencing of b-Cry by RNA interference (RNAi) could modify the apoptotic response of the C2C12VEGF cell line.

The supplementation of culture medium with VEGF (1–10 ng/ml) determined a decrease of susceptibility to apoptosis induced in C2C12 myoblasts by cytotoxic concentration of H_2O_2 (up to 50% reduction in respect to control, $p < 0.01$). This reduction was paralleled by an increase in the expression of the b-Cry protein, but not of the anti-apoptotic proteins Bcl-2 and Bcl-XL, which levels were not significantly modified. In addition, after specific b-Cry silencing in the C2C12 VEGF, cell line, the anti-apoptotic effect of VEGF disappeared, with a rescue of apoptosis susceptibility back to control levels. Together, our data provide that VEGF, beside its angiogenic properties, is also involved in protecting muscle cells against apoptotic stimuli caused by free radicals. Moreover, we demonstrate that the small Hsp b-Cry plays a key role in the molecular mechanism underlying the anti-apoptotic effect of VEGF in myoblasts.

P2-6

Effects of ochratoxin A exposure in hek 293 human embrionic kidney cell lines: a biochemical and molecular study

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Introduction: Ochratoxin A (OTA) is a widespread mycotoxin contaminating feed and food, known for its carcinogenicity, nephro-, geno- and immuno-toxicity, which has been related to its property to interfere with cell oxidative status as well as other processes. Little is known about OTA effects on Bcl-2 protein family members. Pro-survival protein Bcl-2 is known to protect the cell against diverse cytotoxic signals that trigger the mitochondrial apoptotic pathway and Bax is the typical pro-apoptotic member of the Bcl-2 protein family. In this study, OTA mediated induction of oxidative stress, cytotoxicity, modulation of reduced glutathione and modification of Bcl2/Bax gene expression were investigated in Hek293 cell line.

Methods: The MTT test was used to assess cell viability after Hek 293 cells treatment with several concentrations of OTA for different periods of time. The levels of total reduced glutathione and lipid peroxidation (LPO) were assessed after 24, 48 and 72 hours of cells treatment with 5μM OTA. The gene expression of Bcl2 and Bax was analyzed by quantitative real time PCR (qRT-PCR) after 6, 12 and 24 hours of exposure to OTA.

Results: After 24, 48 and 72 hours of incubation, viability of Hek 293 cells was strongly decreased by OTA concentrations higher than 5 μM. Reduced glutathione levels were not significant, even if an increase after 72h of exposure, compared to control, was noticed and it was not correlated with the glutathione reductase and glucose 6 phosphate dehydrogenase specific activities. The lipid peroxidation was increased significantly after 24 and 48h and insignificantly after 72h compared to control. Our work also showed that Bcl2/Bax mRNA levels were increased in Hek293 cells after 12 and 24 hours of exposure to OTA.

Conclusions: According to our results, the oxidative stress and lipid peroxidation in Hek293 cells might represent important factors in the chain of cellular events leading to renal cells carcinogenicity of OTA.

P2-7

Mitochondria dysfunction and complex I dysfunction in a model of tolerance to nitroglycerin: an approach based on mitochondrial-targeted antioxidants

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Nitroglycerin (GTN)-tolerance was induced in vivo (rats) and in vitro (rat and human vessels). Electrochemical detection revealed that the incubation dose of GTN (5×10^{-6} mol/L) did not release NO or modify O_2 consumption when administered acutely. However, development of tolerance produced a decrease in both mitochondrial O_2 consumption and the Km for O_2 in animal and human vessels and endothelial cells in a non-competitive action. GTN tolerance has been associated with impairment of GTN biotransformation through inhibition of ALDH-2, and with uncoupling of mitochondrial respiration. Feeding rats with mitochondrial-targeted antioxidants (mitoquinone, MQ), and in vitro co-incubation with MQ (10^{-6} mol/L) or glutathione ester (GSH ester, 10^{-4} mol/L) prevented tolerance and the effects of GTN on mitochondrial respiration and ALDH-2 activity. Biotransformation of GTN requires functionally active mitochondria and induces ROS production and oxidative stress within this organelle, as it is inhibited by mitochondrial-targeted antioxidants and is absent in HUVECrho0 cells.

Experiments analyzing Complex I-dependent respiration demonstrate that its inhibition by GTN is prevented by mitochondrial-targeted antioxidants. Furthermore, in presence of succinate (10^{-2} mol/L), a Complex II electron donor added in order to bypass Complex I-dependent respiration, GTN-treated cells exhibited O_2 consumption rates similar to those of controls, thus suggesting that Complex I was affected by GTN.

We propose that, following prolonged treatment with GTN in addition to ALDH-2, Complex I is a target for mitochondrially-generated ROS. Our data also suggest a role for mitochondrial-targeted antioxidants as therapeutic tools in the control of the tolerance that accompanies chronic nitrate use.

P2-8

Differential inhibition of the mitochondrial respiratory chain by natural and synthetic vitamin E-related compounds

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A major structural feature of vitamin E compounds is their chroman ring structure substituted by the antioxidative hydroxyl group. While the benefit of vitamin E compounds to prevent excessive oxidative stress in biological systems by radical scavenging is widely known, their structural similarity to the potent mitochondrial cytochrome bc1 complex inhibitor stigmatellin (a chromanon) was not taken into account so far. Therefore, we studied the inhibitory properties of alpha-/gamma-tocopherol (alpha-/gamma-Toc), their p-benzoquinone-like oxidation products alpha-/gamma- tocopheryl quinone (alpha-/gamma-TQ), and corresponding model compounds without phytyl side chain. In addition, two synthetic chroman structures 6-hydroxy-tetramethyl-chroman-2-on (TMC2O) and 6-hydroxy-tetramethyl-chroman-4-on (TMC4O) were investigated.

The ubiquinol: cytochrome c oxidoreductase activity of isolated cytochrome bc1 complex in the presence of those compounds revealed that not fully substituted chromans and quinones, such as the gamma-congeners of Toc and TQ, exhibited stronger inhibitions than alpha-Toc-derived compounds. Likewise, these compounds inhibited the oxygen consumption of bovine heart submitochondrial particles. At the isolated mitochondrial bc1 complex the strongest inhibiting natural and synthetic compounds were gamma-TQ and TMC2O, respectively. Mechanistic studies by stopped flow photometry and low temperature EPR spectroscopy revealed that TMC2O binds to the Rieske iron-sulfur protein in the cytochrome bc1 complex thereby inhibiting the electron transfer to cytochrome c1.

These data suggest that small structural modifications can turn an antioxidant molecule into a mitochondrial inhibitor, highlighting the differences in the physiological effects of naturally occurring alpha- and gamma-congeners of vitamin E and corresponding tocopheryl quinones.

P2-9

Membrane-bound catechol-O-methyltransferase is involved in the metabolism of (-)-epicatechin in human umbilical vein endothelial cells

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Background: Human umbilical vein endothelial cells (HUVEC) were shown to convert the dietary flavanol (-)-epicatechin to B-ring monomethyl ethers, which, in turn, inhibit endothelial NADPH oxidase activity and elevate the intracellular levels of nitric oxide and cGMP [1,2]. These effects were attenuated by 3,5-dinitrocatechol, an inhibitor of catechol-O-methyltransferase (COMT).
Objectives: (i) Detection and distribution of COMT isoforms in HUVEC; (ii) characterization of the reactions of isolated COMT isoforms with (-)-epicatechin; (iii) comparison of the regiospecificity of the methylation of epicatechin in intact cells and with isolated COMT isoforms.
Methodology: (i) Immunocytochemical detection of COMT in HUVEC, and Western blotting in cell lysates. (ii) Methylation of (-)-epicatechin by recombinant porcine S-COMT and human liver microsomes as detected by TLC and LC/MS-MS.
Results: (i) COMT protein is expressed in HUVEC. (ii) The 30 kDa membrane-bound isoform (MB-COMT) predominates over the 25 kDa soluble isoform (S-COMT). (iii) Both MB-COMT and S-COMT as well as intact HUVEC convert (-)-epicatechin preferentially to 3'-O-methyl epicatechin, whereas 4'-O-methyl epicatechin is a minor product.
Conclusion: COMT-dependent methylation of (-)-epicatechin, and possibly of other catechol-type polyphenols, occurs not only in intestinal cells and liver but also in endothelial cells, which are a potential target of polyphenol actions.

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P2-10

Effect of complexes of precursors and modulator of coenzyme Q biosynthesis on bioenergetics and pro- to antioxidant balance under adriamycin-induced cardiomyopathy

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Introduction: Adriamycin is an anticarcinogenic drug application of which leads to significant cardiotoxicity, hepatotoxicity etc. This may cause adriamycin-induced cardiomyopathy (AC). Coenzyme Q administration is an effective countermeasure that reduces AC effects. The aim of the present work was to study the effect of complexes of precursors and modulator of coenzyme Q biosynthesis (alpha-tocopherol acetate, 4-hydroxybenzoic acid, methionine without or with dimethylsulfoxide-C1 and C2 respectively) on bioenergetics and pro- to antioxidant balance in heart and liver of rats during AC.
Methods: AC was modeled by intraperitoneal infusion of adriamycin daily for 8 days in dose of 2.2 mg/kg of body weight.
Results: Under AC Q content decreases in liver homogenates and increases in heart and liver mitochondria. C1 and C2 administration leads to increase in Q content. NADH-Q-oxidoreductase activity in liver and heart mitochondria does not change under AC as well as C1 and C2 correction. Activity of succinate-Q-oxidoreductase and cytochrome-c-oxidase decreases in liver and heart mitochondria under AC. C1 and C2 administration normalizes their activities in liver and heart mitochondria. C1 and C2 administration leads to normalization of free radical lipid (content of conjugated dienes, TBA-reactive products) and protein peroxidation and activity of antioxidant enzyme systems (catalase and superoxidedismutase) in heart and liver which are changed under AC. Vitamin E content does not change in liver and heart homogenate and mitochondria under AC. *Conclusions*: The results obtained provide ground for application of said complexes in cancer chemotherapy for reducing the incidence of the cardiac toxicity of adriamycin.

P2-11

Changes in oxidative stress parameters in the brain of rats concurrently exposed to uranium and stress

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Uranium, a heavy metal, is found widespread in nature, always in combination with other elements. Metal toxicity may be associated with increased rates of reactive oxygen species (ROS) generation within the central nervous system (CNS).

The aim of this study was to investigate the changes on endogenous antioxidant capacity and oxidative damage in several areas of the brain of U-exposed rats. Eight groups of adult male rats received uranyl acetate dihydrate (UAD) in the drinking water at 0, 10, 20, and 40 mg/kg/day for 3 months. Rats in four groups were concurrently subjected to restraint stress during 2 h/day throughout the study. Cortex, hippocampus and cerebellum were removed and processed to examine the following stress markers: reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), thiobarbituric acid reactive substances (TBARS), as well as U concentrations.

The results show that UAD exposure promoted oxidative stress in all cerebral tissues studied. In cortex and cerebellum, TBARS levels were positively correlated with the U content, while in cerebellum GSSG and GSH levels were positively and negatively correlated, respectively, with U concentrations. In hippocampus, CAT and SOD activities were positively correlated with U concentration.

Our results suggest that chronic oral exposure to UAD can cause progressive perturbations on physiological brain levels of oxidative stress markers. Although at the current UAD doses restraint scarcely showed additional adverse effects, its potential influence should not be underrated.

P2-12

Triglyceride rich lipoproteins reverse the alterations in respiratory burst and PON2 activity in serum-deprived cells from the monocyte line U937

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In diabetes mellitus the higher morbidity and mortality from atherosclerosis is related to abnormalities in serum lipids, in particular the persistence of elevated triglyceride-rich lipoproteins (TRL) in the postprandial state. To gain insight into the underlying mechanisms we compared the impact of lipoproteins rich and poor in triglyceride on the oxidant-antioxidant balance of monocytes.

Cells from the monocyte line U937 (differentiated to exert a respiratory burst, RB) were cultured for 24 hours in culture medium with (S+) or without serum (S-) containing either a triglyceride-rich lipoprotein (VLDL) or LDL (100μg protein/3 E5 cells). The capacity to release pro-oxidants and to neutralize them was determined by measuring respectively the RB by chemiluminescence and the activity of the intracellular antioxidant enzymes superoxide dismutase (SOD) and paraoxonase2 (PON2) by spectrophotometry.

The RB of serum-deprived cells (S-) was significantly lower (0.033 ± 0.006 versus 0.092 ± 0.016 peak RLU in S+, $p < 0.001$). Activity of PON2 was higher (0.524 ± 0.061 versus 0.277 ± 0.029 U/mg protein in S+, $p = 0.003$) and that of SOD lower (3.851 ± 0.737 versus 6.685 ± 0.857 U/mg protein in S+, $p = 0.02$). Addition of VLDL normalized the RB (to $82 \pm 18\%$ of the S+), the PON2 (to 0.298 ± 0.048 U/mg protein) and SOD (to 4.921 ± 0.950 U/mg protein). In contrast, when LDL was added, the RB remained significantly lower ($61 \pm 12\%$ of S+, $p = 0.03$), PON2 higher (0.580 ± 0.030 U/mg protein, $p = 0.003$) and SOD lower (3.543 ± 1.022 U/mg protein, $p = 0.04$).

These results show that VLDL, but not LDL, can reverse the abnormalities in respiratory burst and antioxidant enzyme capacity that are induced by serum deprivation. Further investigations will identify the lipoprotein components involved.

P2-13

Effects of parenteral lipid emulsions on the phagocytes *in vitro*

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Parenteral lipid emulsions (PLE) are widely used in clinical practice to maintain or improve the nutritional status of patients. The aim of the study was to investigate the influence of five PLE composed of long-chain triacylglycerols (LCT) only (ClinicOleic, Nutralipid P, Intralipid, Deltalipid, Omegaven) and two PLE containing also medium-chain triacylglycerols-MCT/LCT (Nutralipid MCT, Lipofundin) on functional properties of phagocytes.

The final concentrations of PLE were 0.05–0.0005%. The production of reactive oxygen species by leukocytes isolated from rat whole blood was analysed using chemiluminescence (CL) methods.

Spontaneous CL response of control rat leukocytes was fast with the maximum within the first 5 min after the start of the measurement. According to our previous studies, this peak is of extracellular origin. All emulsions except Omegaven inhibited the extracellularly induced CL depending on the type and the concentration of PLE applied. On the other hand, the rise of spontaneous CL activity in later time intervals was observed in the presence of PLE. This effect was the most significantly expressed in ClinicOleic, Deltalipid and Omegaven. The effect of PLE on the CL activity of leukocytes stimulated with opsonized zymosan was dependent on the type of PLE used. While ClinicOleic, Nutralipid P, Intralipid and Omegaven significantly inhibited CL response, the effect of Nutralipid MCT and Lipofundin was much milder.

It can be summarized that in vitro effects of PLE depended on the nature of PLE. The more important effect was observed in PLE composed mainly of LCT from soybean or fish oil. The activation of resting phagocytes and the inhibition of respiratory burst of activated phagocytes induced with these PLE have to be kept in mind when PLE are used parenterally.

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P2-14

Blood micromolar concentrations of kaempferol afford protection against ischemia/reperfusion-induced damage in rat brain

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The slow time course of neurodegeneration after brain ischemia/reperfusion opens a realistic time window for the application of protective therapies to prevent spreading of brain damage.

In this work, we studied the ability of micromolar concentrations of this flavonoid in the blood to protect against brain damage induced by transient focal cerebral ischemia in rats.

Intravenous injections of kaempferol, at a dose of 10–15 micromoles/L of blood 30 minutes before the induction of a 60 minutes ischemia-episode and just after reperfusion, led to $>90\%$ and 70–80% (TTC, H-E, TUNEL) decrease of brain damage in the temporal-frontal areas of neocortex and striatum, respectively, but only 40–50% decrease of brain damage was observed in the hippocampus and vicinal caudal areas of the striatum. This treatment with kaempferol also produced a similar reduction of metalloproteinase activation and loss of anti-laminin staining in cortical and striatum infarct areas. Kaempferol-treatment efficiently protected against nitrosative-oxidative stress after ischemia/reperfusion, as shown by nearly complete protection against the increase of protein nitrotyrosines, and also afforded strong protection against the increase of apoptotic cell death (TUNEL) and biochemical markers of apoptosis, such as caspase-9 activity and poly-(ADP-ribose) polymerase degradation.

On these grounds, a potential new therapeutic role of kaempferol to acute treatment of ischemic stroke is suggested.

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P2-15

High glucose acutely enhances the respiratory burst of peripheral blood monocytes but not of differentiated cells from the monocyte line U937

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The pathobiochemical mechanisms behind the 2- to 4-fold higher risk of atherosclerosis in diabetes mellitus are related to excessive elevations of blood glucose, particularly during the postprandial phase. In this project we focussed on monocyte/macrophage function by investigating the impact of high glucose on the respiratory burst (RB) of peripheral blood monocytes (PBMC) compared to the monocytic cell line U937. Differentiation (to dU937) by culture in retinoic acid and 1,25-dihydroxy vitamin D3 resulted in a time-dependent increase in the RB. After 72-hours the mRNA expression of the NADPH oxidase subunits p22 and gp91 increased by 8- and 6-fold respectively ($p < 0.001$). At normoglycemic D-glucose concentrations (5.55 mmol/L), the lucigenin-enhanced response of dU937 to opsonized zymosan (OZ) was similar to PBMC but was lower and longer lasting with luminol. In contrast, the response to phorbol ester (PMA) was substantially lower in dU937 cells than in PBMC. The absence of D-glucose (or its substitution by L-glucose) during the RB resulted in a 15% and 50% decrease for dU937 after PMA and OZ respectively ($p < 0.05$ and 0.005). This decrease was more pronounced in PBMC (75 and 90% respectively, $p < 0.005$). High D-glucose (33.3 mmol/L) caused a significant increase in lucigenin-enhanced chemiluminescence in PBMC (by 32% and 12% after PMA and OZ respectively, $p < 0.05$) but did not affect the RB of dU937 cells.

Our results demonstrate an absolute requirement of D-glucose during the respiratory burst of both dU937 and PBMC. The lower response of dU937 to PMA and their resistance to high glucose contrasts with PBMC and suggests a lower contribution of glucose-induced activation of PKC.

P2-16

Effects of a diet supplementation with vitamins E and C on variegate porphyria-associated proatherogenic lipid profile

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Variegate porphyria (VP) is the result of a decreased protoporphyrinogen oxidase (PPOX) activity, the penultimate enzyme of heme biosynthesis. Heme precursors accumulated in porphyria patients produce free radicals leading to oxidative stress. Lipid peroxidation is a key mechanism for the development of atherosclerosis and high density lipoproteins (HDL) are involved in oxidative stress protection mainly through its associated enzyme paraoxonase-1 (PON-1).

Our aim was to analyse the effects of VP and a diet supplementation with vitamins E and C on serum lipid profile and PON-1 activity. 12 women affected by VP and 12 control healthy women participated in a double-blinded cross-over study.

Each participant drunk, for six months, 500 ml/day of an almond-based beverage enriched with vitamin E (10 mg/ml) and vitamin C (30 mg/ml) or a not enriched beverage. After this period blood samples were obtained. Triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol levels were determined in serum. PON-1 and arylesterase activities and apolipoprotein-J (ApoJ) content were measured in plasma. VP patients had higher triglyceride and lower HDL-cholesterol levels than controls. Diet supplementation induced higher LDL-cholesterol and ApoJ content and lower PON-1 activity.

In conclusion, women affected by variegate porphyria present an enhanced proatherogenic lipid profile when compared to control healthy women and diet supplementation with vitamins E and C did not seem to improve this lipid profile.

P2-17

First trimester increase in oxidative stress and risk of foetal growth restriction

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Foetal growth restriction (FGR) is associated with high perinatal morbidity and mortality and an increased risk for the development of diseases in adulthood. It is well established that normal pregnancy is associated with a first trimester, physiological increase in the production of reactive oxygen species, however, further increased oxidative stress can have pathological effects. The objective of this study was to investigate the role of urinary 8-oxodG, as a non-invasive marker of oxidative stress, in late first and second trimester of pregnancy.

A longitudinal case-control, cohort study of low risk pregnant women recruited at the University Hospitals of Leicester NHS Trust and the University of Leicester, UK. Spot urine samples were collected at recruitment ($\sim 12 \pm 2$ weeks gestation) and again in the late second trimester (28 ± 2 weeks gestation). Cases ($n = 55$; ages 18–40 years) included all women giving birth to a growth restricted fetus defined as birth weight < 10th centile on the customised centile charts, Perinatal Institute, Birmingham, UK. The controls ($n = 55$; ages 18–40 years) were women with appropriately grown fetuses. Urinary 8-oxodG was quantified by mass spectrometry after solid phase extraction. Urinary creatinine was used to normalise for variations in urine concentration.

In our cohort of 110 subjects, the urinary 8-oxodG levels of 8-oxodG were significantly increased in the cases compared to the controls, at both 12 and 28 weeks gestation ($P = 0.0007$, $P < 0.0002$, respectively). Levels were decreased significantly between week 12 and 28 respectively ($P = 0.04$ and $P = 0.02$ for both controls and FGR cases respectively).

This study indicates the potential for urinary 8-oxodG to be used as a biomarker to identify pregnancies at risk of developing FGR.

P2-18

High fat diet-induced neuropathy of prediabetes and obesity: effects of low fat diet and 12/15-lipoxygenase gene deficiency

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Subjects with dietary obesity and prediabetes have increased risk for developing both nerve conduction slowing and small sensory fiber neuropathy. Animal model of this type of neuropathy has not been described.

This study evaluated neuropathic changes and their amenability to dietary interventions in mice fed high-fat diet, a model of prediabetes and alimentary obesity. Female C57Bl6/J mice were fed normal or high fat diets for 16 weeks.High fat diet fed mice developed obesity, increased plasma FFA and insulin concentrations, and impaired glucose tolerance. They had motor and sensory nerve conduction deficits, tactile allodynia and thermal hypoalgesia, in the absence of intraepidermal nerve fiber loss or axonal atrophy. Despite the absence of overt hyperglycemia, the mice displayed augmented sorbitol pathway activity in the peripheral nerve, as well as 4-hydroxynonenal adduct, nitrotyrosine and poly(ADP-ribose) accumulation and 12/15-lipoxygenase overexpression in peripheral nerve and dorsal root ganglion neurons.

A 6-week feeding with low fat diet after 16 weeks on high fat diet alleviated tactile allodynia and essentially corrected thermal hypoalgesia and sensory nerve conduction deficit without affecting motor nerve conduction slowing. 12/15-lipoxygenase gene deficiency alleviated motor and sensory nerve conduction deficits, but not manifestations of sensory neuropathy.

In conclusion, similarly to human subjects with prediabetes and obesity, high fat diet fed mice develop peripheral nerve functional but not structural, abnormalities, and, therefore, are a suitable model for evaluating dietary and pharmacological approaches to halt progression and reverse diabetic neuropathy at the earliest stage of the disease.

P2-19

Re-routing of metabolic pathways is a regulated response to oxidative stress

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Eukaryotic cells have evolved various response mechanisms to counteract the deleterious consequences of oxidative stress. Among these processes, metabolic alterations play an important role.

We observed that yeast with reduced activity of the key glycolytic enzyme triosephosphate isomerase exhibit increased resistance to oxidants. This phenotype is conserved in Caenorhabditis elegans and the underlying mechanism based on a redirection of the metabolic flux from glycolysis to the pentose phosphate pathway, resulting in altered equilibrium of the cytoplasmic NADP(H) pool. The inactivation of another key glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), provokes a similar redirection of the metabolic flux. Remarkably, GAPDH was known for a long time to be inactivated in response to various oxidants.

Hence, the naturally occurring inactivation of GAPDH functions as a metabolic switch for rerouting the carbohydrate flux to counteract oxidative stress. Thus, altering the homoeostasis of catabolic pathways is an active cellular response to balance the redox state of eukaryotic cells to counteract oxidative stress conditions.

P2-20

Lymphocytes from porphyria variegate patients are more susceptible to suffer DNA damage induced by H_2O_2 treatment

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The porphyrias are a group of uncommon metabolic diseases caused by enzyme deficiencies within heme biosynthesis that lead to neurotoxic or phototoxic heme precursor accumulation. Variegate porphyria results from a hereditary deficiency of protoporphyrinogen oxidase (PPOX) that is transmitted in an autosomal dominant fashion.

Twelve patients and twelve control women participated in the study. The study protocol was approved by the institutional bioethics committee. Blood samples were taken following an overnight fast and lymphocytes were purified from these blood samples. The activity of antioxidant enzymes and the PPOX gene expression were assessed in lymphocytes.

PPOX gene expression was significantly lower in variegate porphyria patiens than in controls. The lymphocyte carbonyl index, MDA levels, and DNA damage assessed by the comet assay, as oxidative damage markers, were also measured. In basal conditions, lymphocytes from affected women presented lower activities of catalase and GPx compared to control women ($p < 0.05$). However, markers of oxidative damage – MDA, protein carbonyl index, and DNA oxidation-presented similar values in both groups. When lymphocytes were treated with H_2O_2, the degree of DNA damage expressed as% DNA in tail and tail moment were significantly increased in women affected by porphyria variegate compared to controls ($p < 0.05$).

In conclusion, lymphocytes of porphyria variegate women are more susceptible to suffer from oxidative when they are submitted to a stressful situation such as H_2O_2 treatment.

P2-21

Superiority of flavonol 2,3-dehydrosilybin than its parental silybin in inhibiting DNA topoisomerase I

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Antioxidant silybin is the major compound found in silymarin extracts which have been used to treat liver diseases for several decades. Silybin is now being tested in clinical trials to treat prostate and breast cancers. We have previously reported that the oxidized form of silybin so-called 2,3-dehydrosilybin (DHS) exhibits more potent antioxidant activities than silybin by three folds in in vitro and in vivo systems. Compared with silybin, DHS was more cytotoxic and capable of inhibiting releases of metalloproteinase-2,-9 by five folds [BBA 1780(5):837, 2008].

In addition, we have shown that DHS (but not silybin) was capable of sensitizing TNF-alpha-induced apoptosis [Chemotherapy 54(1):23, 2008]. This implies that DHS could inhibit enzymes that regulate transcription such as topoisomerases (topo). Certain flavones and flavonols are potent and selective inhibitors of topo I. As a flavonol, DHS can directly interact with DNA topo I.Here we utilized model FIB and EPI cell lines exhibiting different levels of malignancies [Oncogene 22(38):6045, 2003]. After 24 h treatment of more transformed FIB cells, 30 μM DHS induced apoptosis measured as increases of mitochondrial membrane potential disruption and DNA fragmentation. We demonstrated that treatment of FIB cells with 30 μM DHS for 24 h produced significant decreases of extractable topo I activity while treatment with 30 μM silybin did not have any effects. Less transformed EPI cells were more resistant against 30 μM DHS-induced topo I inhibition. This indicates DHS selectivity towards cancer phenotype. Inhibitory effects of 10–50 μM DHS of specific topo I activity were also found in cell-free assays using purified topo I, while 50–100 μM silybin did not have any effects. Silybin at 100 μM however could inhibit topo I activity prepared from nuclear extracts.

Being superior to silybin, DHS induced apoptotic DNA fragmentation which was associated with topo I inhibition. Taken together, DHS may serve as a chemotherapeutic agent by targeting mitochondria and DNA topo I and with its enhanced sensitization potency and selectivity towards cancer phenotype.

P2-22

1,4-Dihydropyridine derivatives as antioxidants and therapeutics. A review

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1,4-Dihydropyridine (1.4-DHP) antioxidant activity was firstly detected in 1969. They are good synergists of a-tocopherol and BHT. Membranotropic properties of 1,4-DHP were determined by means of ESR and fluorescent techniques. 1,4-DHP are antimutagenics and prevent side effects of X-ray theraphy. Some of them possess neuroprotective and hepatoprotective properties, and potentiate anticancer drugs. The main favourable property of many 1,4-DHP is their low toxicity.

P2-23

The effect of quercetin on menadione toxicity in rat primary mixed glial cells

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Neurons and glial cells of the brain are highly susceptible to reactive oxygen species that play a key role in various neurodegenerative diseases. Menadione, a synthetic derivative of vitamin K, induces reactive oxygen generation via redox cycling. Quercetin, one of the most ubiquitous bioflavonoids in food of plant origin, has strong antioxidant activities on different cell types, however recent studies demonstrated that it has also prooxidant and cytotoxicity potentials.

The glial cells, obtained from 1–3 day old rat brain, were divided in two main groups. The first group of the cells were pretreated with either 10, 25, 100 or 250 μM quercetin for 1 hr then, washed out and menadione doses (10, 25, 50, 75 or 100 μM) were added for 6 hr in vitro. The second groups of the cells were treated with respective doses of quercetin combined simultaneously with the same menadione doses for 6 hr. The cells were washed and incubated for additional 24 hr for recovery period and cell viability was measured by using MTT colorimetric assay. All statistical analyses were performed by one way analysis of variance, followed by Tukey's multiple comparison test.

The pretreatment of the cells with respective quercetin doses for 1 hr could not eliminate menadione-induced toxicity. Although 10 and 25 μM quercetin combined with 10 or 25 μM menadione could not change menadione toxicity, 100 and 250 μM quercetin together with 10 or 25 μM menadione for 6 hr increased further the menadione toxicity. Since high dose of menadione is toxic to the cells, the combined treatment of all the quercetin doses with 50, 75 or 100 μM of menadione could not change menadione-induced toxicity.

P2-24

Fasting offers rapid and robust protection against ischemia reperfusion injury in mice

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Dietary restriction, or reduced food intake without malnutrition, is synonymous with extended longevity and increased stress resistance in model organisms. While the kinetics of onset and loss of benefits are rapid in lower organisms, they are unknown in mammals.

Using a mouse model of surgical ischemia reperfusion injury to the kidney, we found profound benefits of short-term dietary restriction, including four weeks of 30% reduced food intake as well as three days of water-only fasting, against organ dysfunction and death. Significant protection occurred within one day, increased for up to three days of water-only fasting, lasted beyond the fasting period, extended to another organ, the liver. Protection was associated with upregulation of oxidative stress protection genes, less cell death and reduced inflammation vs. ad libitum fed controls.

We suggest that mechanisms previously implicated in longevity extension by dietary restriction can respond rapidly to brief periods of fasting in mammals. We point out the potential clinical implications of preconditioning against oxidative stress associated with major surgery by preoperative nutritional care, including fasting.

P2-25

Toona sinensis roem extracts alleviated apoptosis induced by hydrogen peroxide in HepG2 cells

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Toona sinensis Roem (TS) is a traditional Chinese health food reported to exhibit many beneficial physiological functions. Hydrogen peroxide (H_2O_2) induced powerful oxidative stress in cells and led to apoptosis. In this study we focused on the anti-apoptotic effects of extracts from TS (TSL-2P and TSL-6). HepG2 cells (human liver carcinoma cell) were pre-treated with TSL-2P, TSL-6 and quercetin (a flavonoid, as a positive control) and then treated with H_2O_2. Cell viability was detected by MTT assay.

Results showed that cell viability was significantly reduced by H_2O_2 and alleviated by TSL-2P, TSL-6 and quercetin. Reactive oxygen species (ROS) production and apoptotic cells measured by flow cytometry were significant higher in H_2O_2 group than that in TS or quercetin groups. Activated Caspase-3 was significant higher in H_2O_2 group than that in TS or quercetin groups by western blot. Cell death induced by H_2O_2 was decreased by PD98059, an inhibitor of extracellular signal-regulatedkinase (ERK), which was similar to that by TSL-6. Protein expression of ERK was significant higher in H_2O_2 group than that in TS or quercetin groups by western blot.

We suggested that TSL-6 alleviated apoptosis induced by H_2O_2 via ERK pathway.

P2-26

Signs of oxidative stress persist in the gastric mucosa even after successful eradication of *Helicobacter pylori*

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Oxidative stress plays an important role in the pathogenesis of *Helicobacter pylori* associated peptic ulcer. Its intensity can be assessed by measurement of 4-hydroxy-2,3-trans-nonenal (4-HNE), an important secondary product of omega-6 fatty acids peroxidation.

The aim of present study was to determine the content and distribution of 4-HNE in the gastric mucosa of patients with *H. pylori* associated peptic ulcer disease (DPU) and evaluate their changes after successful eradication.

29 patients with H. pylori associated DPU (age $M \pm m$, 32.1 ± 1.7 yy.) and 20 healthy adults age $M \pm m$, 29.7 ± 1.4 yy.) were enrolled into the study. HNE was determined in the gastric mucosa (GM) biopsy samples taken before and 4–5 weeks after successful eradication consisting of 7-days triple-regimen treatment. The representative paraffin blocks were used for immunohistochemical staining with monoclonal antibodies for detection of HNE-modified proteins.

A content of 4-HNE in the body and antrum of the stomach of the DPU patients was increased comparing to healthy, including H. pylori positive, volunteers. Moreover, in 26.9% of patients 4-HNE was detectable in the glandular nuclei, while none of the controls had such findings. Eradication markedly reduced inflammation in the GM. However, it did not eliminate of 4-HNE from the glandular nuclei, while in the glandular cytoplasm its content was even increased.

Thus, despite the elimination of inflammatory changes, successful eradication of *H. pylori* does not relieve oxidative stress in the GM. Management of the DPU should include remedies aimed at correction of oxidative stress.

P2-27

Neuroprotective effects of polyphenols from diet: analysis of their antioxidant activities and intracellular targets

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Brain aging is associated with the accumulation of oxidative-induced damages, likely due to the imbalance between antioxidant defenses and intracellular generation of reactive oxygen species (ROS). A large body of evidence indicates that oxidative stress is also elevated in some neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's diseases (PD). Moreover, there is now substantial evidence indicating that oxidative damages to the brain is an early event in the pathogenesis of AD. Thus antioxidants have been studied for their effectiveness in reducing these deleterious effects and neuronal death in many in vitro and in vivo studies.

Many epidemiological studies have documented the influence of dietary habits and polyphenolic compounds with antioxidant activities on the incidence of neurodegenerative disorders such as AD and PD.

The aim of our study was to analyze the antioxidant activity of some polyphenolic compounds present in diet. This activity was compared to their neuroprotective property. Furthermore, some intracellular targets contributing to the control of the intracellular redox potential were analyzed.

Our results showed that the neuroprotective effect of some phenolic compounds against the amyloid-ß peptide (a peptide involved in the pathophysiology of AD) and MPP^+ (methyl-phenyl-pyridimium) (a neurotoxin used to mimic neurodegeneration as observed in PD) is not limited to their antioxidant activities but also involved the regulation of some redox-sensitive transcription factors. These data will be presented and discussed.

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POSTER-SESSION 3 — SELENIUM AND SEPSIS

P3-1
Effect of green tea and catechins on hepatic glutathione metabolism and oxidative stress in aged rats

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Green tea (GT) consumption is increasing worldwide due to its described health-promoting effects. This beverage contains polyphenolic compounds which are antioxidant in nature and have been extensively studied in vitro. However, its actions in vivo, and most particularly in long-term studies, are still scarce.
Our aim was to evaluate the influence of 6 months GT or GT extract (GTE) ingestion upon hepatic glutathione metabolism in aged rats.
For this, three groups of 6 rats each (12 months of age) were used: (1) controls, given free access to tap water (C); [2] GT-treated animals, given an infusion, as the only available fluid source, of GT prepared with 3 tea bags (1.3 g/bag, Lipton®) per liter of boiling water for 5 min; [3] GTE-treated rats, with access to an aqueous solution of 200 mg/L of catechins extracted from GT. Glutathione status [oxidized (GSSG), reduced (GSH) and total glutathione (GSX)] and glutathione-related enzyme activities [glutathione-S-transferase (GST), glutathione reductase (GR) and selenium-dependent glutathione peroxidase (Se-GPX)] were quantified in liver homogenates.
GT treatment increased GSSG and GSX but GTE decreased GSX and GSH. GR activity was equally enhanced by GT and GTE. Se-GPX and GST were inversely modulated by GT and GTE. GT decreased GST and GTE induced the opposite along with a decrease of Se-GPX. To better understand the full meaning of these results, other oxidative stress and hepatic function markers were also studied. Protein carbonyls were decreased after both treatments (more intensely with GTE) and no changes in DNA oxidation were observed. Total alkaline phosphatase (ALP) activity increased in GTE-treated animals in parallel with an increased mRNA expression of both tissue-nonspecific ALP and intestinal ALP.
GT seems to ameliorate glutathione status by increasing its availability. Although GTE treatment decreased glutathione availability and increased ALP activity we can not conclude about liver toxicity as shown by the absence of protein or DNA oxidative damage.
Support: FCT (POCTI, Feder, Programa Comunitário de Apoio, Unit 121/94, SFRH/BD/19497/2004 and SFRH/BPD/40110/2007).

P3-2
Influence of selenium on the accumulation of antioxidants in barley plants

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At present more attention is paid to this qualitative parameter of the vegetable food as the content in it of antioxidants. Antioxidants promote the scavenging of excess quantities of the free radicals and other reactive oxygen species in the cells and thus prevent the development of oxidative stress in the organism and its premature aging. The aims of this study were to investigate the influence of selenium on the accumulation of low-molecular antioxidants (carotenoids, anthocyan, rutin, glutathione, riboflavin, ascorbic acid) in barley plants. For analyses were used 10 days after sowing barley plants (Hordeum of 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. In the plants were determined the content of some low-molecular antioxidants (carotenoids, anthocyan, rutin, glutathione, riboflavin, ascorbic acid), and also malondialdehyde – product of the peroxide oxidation of lipids, proline and content of chlorophylls a and b.
The biochemical functions of the trace element selenium in the plants are not clear. In the present study the possibilities of increasing antioxidant status of vegetable food through the addition of selenium were investigated. The findings show the positive effect of selenium on the accumulation of 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.
One of the special features of selenium is its dual nature. Se can have antioxidant as well as prooxidant properties. Many authors note that an increase in the content of antioxidants can be related with the development of oxidative stress in the cells of plants. Therefore one of the reasons for the increase in the content of antioxidants under the addition of selenium could be the protection reaction of organism against the negative action of selenium. However, in our studies by the addition of selenium to nutrient solution was established the decrease of the content of malondialdehyde-the product of the peroxide oxidation of lipids-to 7%, proline-to 6%, and the content of chlorophylls a and b changed not significantly.
A reduction of the content of anthocyan (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 anthocyan is increased under the influence on the plants of a whole series of stress factors. The relationship of the accumulation of anthocyan with the development of oxidative stress in the plants can be demonstrated through the positive correlation dependences between the level of anthocyan pigments and the content of malondialdehyde ($r = 0.762$), and also between the level of anthocyan and the pool of free proline ($r = 0.842$). Thus, the results of investigating the influence of selenium on the accumulation of low-molecular antioxidants in the barley plants show possibility of induction in the plants the accumulation of some antioxidants (glutathione, ascorbic acid, rutin) through the selenium addition to nutrient solution.

P3-3

Activation of the glutathione peroxidase 2 (GPx2) via the Wnt/ß-catenin pathway

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Background and aims: GPx2, the gastrointestinal glutathione peroxidase, is a selenoprotein highly expressed in the proliferative area of the intestinal crypt-to-villus axis and in Paneth cells. Additionally, GPx2 is up-regulated during the development of gastrointestinal adenocarcinomas with the highest expression in early stages of malignancy. Both normal proliferation and differentiation of intestinal epithelial cells and carcinogenesis are regulated by the Wnt/ß-catenin pathway. The study was focused on the relationship between GPx2 and the Wnt/ß-catenin pathway and on the question whether GPx2 might be a target of the ß-catenin/TCF complex which transfers Wnt signals.
Results: The GPx2 promoter contains five putative ß-catenin/TCF binding sites. Reportergene assays showed that the promoter was active in two cell lines in which ß-catenin was constitutively active, HepG2 and SW480, but not in BHK-21 cells in which the Wnt pathway is silent. Cotransfection of wildtype ß-catenin and a constitutively active form of ß-catenin (S33Y ß-catenin) in combination with wildtype TCF4 activated the GPx2 promoter. Transfection of a dominant-negative TCF4 resulted in inhibition of promoter activity. Overexpression of wild-type APC in SW480 cells which harbour an APC gene with a defect in the ß-catenin binding site also inhibited GPx2 promoter activity. Truncation of the promoter identified one ß-catenin/TCF binding site that was sufficient for its activation. Mutation of this site reduced the response to ß-catenin/TCF by 50%. The effects of S33Y ß-catenin and dnTCF4 on the promotor activity can also be seen on the expression level of endogenous GPx2. In addition, the stable transfection of Wnt 3a in NIH-3T3 cells results, beside an increased basal GPx2 promoter activity, in an enhanced endogenous GPx2 RNA expression.
Conclusion: GPx2 is a target of the ß-catenin/TCF complex, as demonstrated in the different cell line models used. These findings suggest a function of GPx2 in the maintenance of normal renewal of the intestinal epithelium. Whether its up-regulation during carcinogenesis supports tumor growth or can rather be considered as counteracting is actually under investigation.

Reference

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P3-4

Glutathione S-transferase expression in upper urinary tract transitional cell carcinoma

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We featured a systematic functional investigation of different glutathione S-transferase (GST) classes in transitional cell carcinoma (TCC) of upper urinary tract (UUT) and the surrounding normal uroepithelium by array of different substrates and by using Western blot.
Tumor samples and surrounding normal urothelium were obtained from 20 patients with UUT TCC who underwent surgery. Determination of cytosolic GST activity was performed spectrophotometrically by using the following substrates with differential specificities: 1-chloro-2, 4-dinitrobenzene (CDNB) for overall GST activity; 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) for GSTA and GSTO; 1,2-dichloro-4-nitro-benzene (DCNB) for GSTM; 4-vinyl pyridine (VP) for GSTP1; and 1,2-epoxy-3-(p-nitrophenoxy)propane (EPNP) for GSTT1.
The results obtained at protein level have shown expression of GSTP1 in all samples of UUT TCC and corresponding normal urothelium. GSTP1 expression at protein level correlated significantly with activity towards VP. GSTM protein was detected in 9 of 20 samples, but expression did not correlate with DCNBactivity. Activity towards EPNP, specific GSTT1 substrate was found in all tumor and non-tumor specimens. No GSTA protein was detected, although 12 of 20 samples have shown activity towards NBD-Cl, which is also GSTO substrate. TCC tumors had the same GST expression profile as normal tissue, but they exibited significant upregulation of all GST enzymes tested.
We conclude that in UUT TCC and adjacent normal uroepithelium GSTM, GSTP1 and GSTT1 are expressed. Although GST isoenzyme pattern in TCC is similar to that of corresponding normal uroepithelium, during cancer progression there is a clear tendency towards an increase in all the GST subtypes expressed. It might be speculated that upregulation of GST in UUT TCC plays a role in the chemoresistance of these tumors.

P3-5

Activities of GSH-replenishing enzymes inversely correlate with cleaved caspase 3 index in transitional cell carcinoma of urinary bladder

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The increased ability of cancer cells to generate glutathione (GSH) could have a pathogenic role, leading to higher proliferation rate and drug resistance. In previous study, we showed that the redox balance in transitional cell carcinoma (TCC) of urinary bladder was in favour of a reduced state. However, relationship between activity of enzymes involved in replenishment of GSH, as well as antioxidant enzymes and apoptosis has not been investigated as yet.

We examined spectrophotometrically the activities of enzymes involved in GSH synthesis (gamma-glutamylcysteine synthetase, gamma-GCS), GSH regeneration (glutathione reductase, GR) and antioxidant protection (glutathione peroxidase, GPX; superoxide dismutase, SOD) in the cytosolic fraction of tumors and surrounding normal tissue of 20 TCC patients. For each tumor specimen, the corresponding paraffin embedded tissue section was also tested for cleaved caspase 3 labelling index by immunocytochemisty.

The results obtained have shown a significant increase in the activities of both GSH-replenishing and antioxidant enzymes in tumors when compared with normal uroepithelial tissue. Mean gamma-GCS and GR activities in tumors were about 4- and 2- fold higher, respectively than in corresponding normal tissue. Significant negative correlation was found between cleaved caspase 3 labelling index and gamma-GCS as well as GR activity in tumor specimens ($r = -0.474$, $p < 0.05$; $r = -0.542$, $p < 0.05$, respectively). No correlation was found between cleaved caspase 3 labelling index and activities of antioxidant enzymes GPX and SOD ($r = -0.248$, $p < 0.05$; $r = -0.060$, $p > 0.05$, respectively).

Based on inverse correlation between cleaved caspase 3 and gamma-GCS or GR activities in TCC of urinary bladder we conclude that up-regulated GSH replenishing pathways may account for decreased activity of executive apoptotic pathways.

P3-6

Enhanced GSTP1 expression in transitional cell carcinoma of urinary bladder is associated with down-regulated apoptosis

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Glutathione S-transferase P1 (GSTP1) might provide an important link between the activity of regulatory stress kinases and activities of apoptotic pathways. Recently, we have shown that expression of GSTP1 in transitional cell carcinoma (TCC) of urinary bladder is enhanced. In this study we aimed to establish whether relationship between GSTP1 expression and executive (procaspase 3, cleaved caspase 3) or regulatory (Bcl-2) apoptotic molecules in TCC exist. Samples were obtained from 84 TCC patients, who underwent transurethral resection, partial or radical cystectomy. Expression of GSTP1, procaspase-3 (CPP32) and Bcl-2 as well as labeling index of cleaved caspase-3 were determined by immunocytochemistry. Levels of expression were correlated with tumor stage and grade.

GSTP1 protein expression was demonstrated in all tumor samples examined. According to GSTP1 status all tumors were divided into three groups with low, moderate or high GSTP1 status. Expression of CPP32 and cleaved caspase 3 was positive in 80% of TCC patients. Levels of both CPP32 and cleaved caspase 3 differed significantly between groups with various GSTP1 expression ($p < 0.05$), with lowest CPP32 expression and cleaved caspase 3 index in tumors with high GSTP1 status. Moreover, significant negative correlation was found between GSTP1 level and cleaved caspase 3 labeling index ($r = -0.237$, $p = 0.030$). The positive rate of Bcl-2 protein expression was 48%. Most of the Bcl-2 positive patients exibited at the same time high GSTP1 positivity ($p = 0.078$). Significant association with tumor grade and stage was found for GSTP1 and all apoptotic parameters except for CPP32 according to tumor grade.

Based on results obtained we conclude that enhanced GSTP1 expression in TCC of urinary bladder is associated with down-regulated apoptosis. Molecular interplay between GSTP1 and members of apoptotic cascade, might at least partially, play role in the development of invasive characteristics of TCC.

P3-7

Control of selenoprotein P expression through interaction of the coactivator PGC-1a with FoxO1a and HNF-4a transcription factors

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Selenoprotein P (SeP), the major selenoprotein in blood plasma, acts as extracellular antioxidant and selenium carrier protein. SeP is synthesized and secreted mainly by the liver, although moderate to low expression of SeP is found in most organs, including the brain. In hepatoma cells, SeP is transcriptionally regulated by the forkhead box protein FoxO1a.

We demonstrate that overexpression of peroxisomal proliferator activated receptor-g coactivator 1a (PGC-1a) in hepatoma cells stimulated SeP promoter activity and enhanced the effect of FoxO1a. Adjacent to the FoxO-responsive element DBE2 in human SeP promoter, we identified a novel binding site for hepatocyte nuclear factor HNF-4a. Point mutations in both binding sites decreased basal activity and responsiveness of SeP promoter to PGC-1a. Moreover, the PGC-1a-inducing glucocorticoid dexamethasone strongly stimulated SeP transcription and secretion in cultivated rat hepatocytes, whereas insulin suppressed the stimulation of PGC-1a and SeP caused by dexamethasone treatment. In a brain-derived neuroblastoma cell line with low SeP expression, SeP transcription was stimulated by PGC-1a together with FoxO1a, and overexpression of HNF-4a potentiated this effect. In conclusion, high-level expression of selenoprotein P in liver is ensured by joint action of the coactivator PGC-1a and the transcription factors FoxO1a and HNF-4a.

As SeP is essential for adequate selenium supply of tissues throughout the body, our study establishes PGC-1a as a key regulator of selenium homoeostasis and potentially selenium-involved antioxidant protection in addition to its impact on oxidative energy metabolism.

P3-8

Tissue specific response of γ-glutamylcysteine synthetase on glutathione synthesis inhibition using buthionine sulfoximine

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Glutathione (GSH) homeostasis in the cells, essential for most pathophysiological processes, is maintained by both, intracellular and interorgan regulatory mechanisms. Cellular GSH level depends on γ-glutamylcysteine synthetase (γ-GCS)-determined rate-limiting step in GSH biosynthesis accompanied with GSH recycling by glutathione reductase (GR). This study was designed to examine the effects of in vivo GSH depletion by L-buthionine-[S,R]-sulfoximine (10 mM BSO), a specific γ-GCS inhibitor, on glutathione biosynthesis in rat liver, heart, kidney, brain, brown adipose tissue and intestine.

Adult Mill Hill hybrid hooded rats were divided into two groups: control and BSO-treated. Animals from BSO-treated group were sacrificed on day 1, 3, 7 and 21.

In the liver, significant depletion of GSH level from day 1 to day 21 of BSO treatment was associated with down-regulation of γ-GCS protein level. Similarly, there is a positive correlation between GSH and γ-GCS protein contents in the heart i.e. decrease of GSH level and γ-GCS protein expression at day 7 and 21 was observed. On the contrary, BSO treatment for 21 days induced marked increase in GSH concentration but didn't affect γ-GCS protein level in the kidney. Also, in contrast to significant depletion of GSH at day 21, there were no changes in γ-GCS protein content in the brain. In brown adipose tissue as well as in intestine, decrease in GSH level from 1 day of BSO treatment was accompanied with up-regulation of γ-GCS. In contrast to γ-GCS expression, GR activity was not affected with BSO in all examined tissues.

In conclusion, the response of γ-GCS translational level on GSH depletion with BSO is tissue-specific and it could be related to the tissue metabolic profile and its role in interorgan GSH metabolism. Also, it seems likely that modulation of GSH synthesis may not influence GR activity. These results suggest complex molecular mechanisms underlying GSH biosynthesis regulation and needed further investigation.

POSTER-SESSION 4 — OXIDANTS AND SIGNALLING

P4-1
Menadione attenuates gap junctional intercellular communication in rat liver epithelial cells: on the role of E-cadherin and beta-catenin

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Gap junctions are clusters of transmembrane channels connecting the cytoplasmic compartments of adjacent cells, allowing for the direct exchange of signalling molecules between cells. These channels consist of two connexin (Cx) hexamer hemichannels provided by each of the connected cells. Gap junctional intercellular communication (GJIC) has been hypothesized to play a critical role in the regulation of carcinogenesis and cellular proliferation/differentiation. We have previously demonstrated that the redox cycler and alkylating agent menadione (2-methyl-1,4-naphthoquinone) potently diminishes GJIC in rat liver epithelial cells by causing EGF-receptor-dependent activation of the mitogen-activated protein kinases ERK1 and ERK2 that in turn phosphorylate connexin43, the major connexin in this cell line. We here demonstrate that menadione also affects adherens junction integrity and that depletion of adherens junction proteins (E-cadherin and beta-catenin) strongly attenuates GJIC.

Exposure of rat liver epithelial cells to menadione for 30 min caused a 50–75% decrease in GJIC, which was accompanied by phosphorylation of Cx43. In addition, exposure to menadione lowered total amounts of the adherens junction proteins E-cadherin and beta-catenin as shown by immunoblotting and immunocytochemistry. Similarly, changes in the pattern of both beta-catenin and E-cadherin were observed in immunocytochemical studies, suggesting that menadione may affect GJIC by interfering with cell-adhesion. In line with reports on tyrosine phosphorylation of E-cadherin and beta-catenin induced by oxidative stress, general tyrosine phosphorylation of cellular proteins was strongly enhanced in cells exposed to menadione, with particularly prominent phosphorylation in the cell membrane. To further investigate the role of beta-catenin in the regulation of GJIC, rat liver epithelial cells were treated with specific siRNA.

After depletion of β-catenin GJIC was analyzed and the number of communicating cells found to be decreased to 40% of control. The loss of beta-catenin further caused a reduction of Cx43 and E-cadherin levels in regions of intercellular contact and a partial internalization of Cx43, as demonstrated by immunocytochemistry. In summary, it is hypothesized that menadione affects the integrity of adherens junctions, with consequences on gap junctional intercellular communication.

P4-2
PI3K/AKT dependent inactivation of Foxo3a mediates nitric oxide induced PGC-1a downregulation

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Nitric oxide (NO) production via endothelial nitric oxide synthase (eNOS) is associated with reduced levels of reactive oxygen species (ROS) in healthy non-proliferating vascular endothelium, but with elevated ROS levels in damaged or proliferating endothelium. While low endothelial ROS levels prevent the development of atherosclerosis, elevated ROS levels are necessary for angiogenesis. The transcriptional coactivator peroxisome-proliferator-activated receptor γ-coactivator-1 α (PGC-1α) positively modulates several genes involved in ROS detoxification. NO can regulate PGC-1α expression both positively and negatively *via* protein kinase G (PKG). We report that NO/PKG dependent downregulation of PGC-1α and the ROS detoxification system in endothelial cells is mediated by activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase Akt signaling pathway and subsequent inactivation of the FoxO transcription factor Foxo3a. Inactivation of either PI3K or Akt results in loss of NO dependent PGC-1α downregulation. Our results demonstrate that Foxo3a is a direct transcriptional regulator of PGC-1α and that overexpression of constitutively active Foxo3a cancels NO dependent PGC-1α downregulation. A direct role of Foxo3a inhibition in mediating NO activity is supported by the identification of a critical NO response element in the PGC-1α promoter as a functional FoxO binding site. We propose that inactivation of Foxo3a by NO is necessary for the reduction of cellular ROS detoxification capacity required for angiogenesis.

P4-3

Delayed ageing through damage protection by the Arf/p53 pathway. Role of oxidative stress

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The tumour-suppressor pathway formed by the alternative reading frame protein of the Cdkn2a locus (Arf) and by p53 (also called Trp53) plays a central part in the detection and elimination of cellular damage, and this constitutes the basis of its potent cancer protection activity. Similar to cancer, ageing also results from the accumulation of damage and, therefore, we have reasoned that Arf/p53 could have anti-ageing activity by alleviating the load of age-associated damage.

Here we show that genetically manipulated mice with increased, but otherwise normally regulated, levels of Arf and p53 present strong cancer resistance and have decreased levels of ageing-associated damage. They show lower lipid peroxidation and higher content of glutathione than the wild type animals. Moreover, the Arf/p53 animals live longer than their counterparts.

These observations extend the protective role of Arf/p53 to ageing, revealing a previously unknown anti-ageing mechanism and providing a rationale for the co-evolution of cancer resistance and longevity.

P4-4

Resveratrol suppresses peroxynitrite-triggered mitochondrial apoptotic pathway in endothelial cells by up-regulating intracellular levels of Bcl-2

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Peroxynitrite, the product of the diffusion-controlled reaction of nitric oxide with superoxide radical, is a strong oxidant and nitrating agent which represents a relevant mediator of LDL oxidative modifications and of endothelial dysfunction, recognized as important steps in the atherogenic process. On the other hand, resveratrol (3,4′,5-trihydroxystilbene) is a phytochemical believed to be partly responsible for the cardioprotective effects of red wine due to its numerous biological activities.

Therefore, the aim of this work was to study the biochemical pathways underlying peroxynitrite-mediated apoptosis in vascular endothelial cells and potential mechanisms responsible for resveratrol cytoprotective action.

Our data indicate that peroxynitrite triggers apoptosis in primary cultures of bovine aortic endothelial cells (BAEC) through caspases-8, -9 and -3 activation, implying both mitochondrial and death receptor apoptotic pathways. Resveratrol is able to prevent peroxynitrite-induced caspases-3 and -9 activation, but not caspase-8 activation. Moreover, peroxynitrite increases intracellular levels of Bax without affecting those of Bcl-2, increasing consequently the Bax/Bcl-2 ratio. This ratio decreases when cells are pre-incubated with low micromolar concentrations of resveratrol, mainly due to resveratrol ability per se to increase intracellular levels of Bcl-2 but not Bax levels.

These results propose an additional mechanism whereby resveratrol may exert its cardioprotective effects and suggest a key role for Bcl-2 in the resveratrol anti-apoptotic action, especially in disrupting peroxynitrite-triggered mitochondrial pathway. Paula Brito is a recipient of the grant SFRH/BD/7986/2001 and Núria Simões is a recipient of a research fellowship from FCT.

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P4-5

Methionine Sulfoxide Reductases A and B2 protect MOLT-4 cells against H_2O_2 and Zinc-mediated cell death and protein damage

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Oxidation of proteins by reactive oxygen species (ROS) has been implicated in aging and in several pathologies related to oxidative stress. Methionine is among the amino acids the most susceptible to oxidation and can be catalytically reduced within proteins by Methionine Sulfoxide Reductases A (MsrA) and B (MsrB). The Msr system, which represents one of the very few repair systems for oxidized proteins, plays a major role in the maintenance of protein homeostasis during aging and has also been involved in cellular defenses against oxidative stress, by scavenging ROS.

To evaluate the role of MsrB2, a mitochondrial MsrB member, in resistance to oxidative stress, we have overexpressed MsrB2 in the mitochondria of T-lymphoblastic human leukemia MOLT-4 cell line.

We showed that MsrB2 overexpressing cells are more resistant to H_2O_2 cytotoxity by delaying apoptosis and protecting against necrosis. Moreover, we demonstrated that the mechanisms by which MsrB2 protects against oxidative stress include: maintenance of a lower level of intracellular ROS, prevention of oxidized protein accumulation and protection of the proteasome against oxidative stress-induced inactivation.

Interestingly, Zinc has a pro-antioxydant effect by stimulating the Msr activity in lymphoblastic cells in both in vivo and in vitro situations but, in contrast, zinc treatment of MOLT-4 cells caused increased cell death, protein damage and ROS production.

Our results indicate that overexpression of MsrA or zinc-dependent MsrB2 enzymes counteracts the pro-oxidant effects of zinc which results in cellular protection against oxidative damage, especially protein oxidative damage and cell death.

Our results emphasize that upon oxidative stress the overexpression of MsrA or MsrB2 leads to the preservation of mitochondrial integrity by decreasing the intracellular ROS build-up through their scavenging role, hence contributing to cell survival and protein maintenance.

P4-6

Mutual interactions among collagen, platelets and professional phagocytes

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The hypothesis that collagen, platelets and professional phagocytes cooperate to modulate the oxidative burst of phagocytes and the extent of oxidative stress was tested.

Rat and calf type I collagen samples were subjected to the oxidative modification by incubation of collagen solutions (1 mg/mL in 10 mM HCl, pH adjusted to 5.0–5.5) with various oxidants: hydrogen peroxide (100 mM), hydroxyl radical (100 mM $FeSO_4$–2 mM H_2O_2) peroxyl radical (obtained by the thermal decomposition of 200 mM ABAP), and sodium hypochlorite (5% NaOCl). After the oxidation treatment all samples were subjected to an extensive dialysis against 10 mM HCl. Collagen sample dissolved in 50 mM acetic acid and dialyzed against 10 mM HCl only was designated as non-modified (control) sample. The denaturation temperatures of collagen samples were measured microcalorimetrically and spectrophotometrically. The procedures used led to the modification of collagen samples characterised by the decrease in denaturation temperature. Two different methods were used to evaluate the capacity of collagen samples to scavenge peroxyl radicals: TRAP (total peroxyl radical-trapping antioxidative parameter) and ORAC (oxygen radical absorbance capacity). Both methods revealed that even non-modified collagen has a very low capacity to scavenge peroxyl radicals. This capacity was further diminished in oxidatively modified collagen samples. The effect of collagen samples on platelet aggregation was measured in isolated platelets obtained after differential centrifugation. The non-modified collagen sample showed an aggregation behaviour comparable with that of thrombin. All oxidatively modified collagen samples, independently of the oxidation treatment applied, lost totally their platelet-aggregating activity. A production of reactive oxygen species by phagocytes in the whole blood was evaluated by luminol-enhanced chemiluminescence. Spontaneous chemiluminescence and chemiluminescence of phagocytes activated with one of the activators-opsonized zymosan particles, calcium ionophore A23187, phorbol-12-myristate-13-acetate or N-formyl-Met-Leu-Phe was determined.

Non-modified collagen itself has a capacity to induce an oxidative burst of phagocytes in whole blood. Oxidatively modified collagen samples exerted even stronger capacity to induce the oxidative burst of phagocytes the changes being independent on the type of oxidative treatment. In the case of activated phagocytes the effects of individual collagen samples differed according to the stimulus used. It can be concluded that reactive oxygen species produced by activated professional phagocytes are able to modify collagen, a major constituent of the extracellular matrix. On the other hand, both non-modified and oxidatively modified collagens can modulate the activity of professional phagocytes either directly or indirectly via mediators released from activated platelets.

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P4-7

Ochratoxin A induced oxidative stress in human caucasian colon adenocarcinoma (CaCo2) cells

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Introduction: Ochratoxin A (OTA) is a food-borne mycotoxin with multiple effects on gut epithelial cell lines. It seems that induction of oxidative stress plays an important role in the toxicity of OTA and generates apoptotic cell death. In this study, OTA induced oxidative stress and the modification of Bcl2/Bax gene expression were investigated in CaCo2 cell line.

Methods: The MTT test was used to assess cell viability after CaCo2 cells were treated with 10, 30, 50 and 80 μM of OTA up to 24 hours. The levels of total reduced glutathione, total superoxide anion concentration and lipid peroxidation, as well as glutathione reductase and glucose-6-phosphate dehydrogenase specific activities on cells treated with 50μM OTA for 24 hours were assayed. The gene expression of Bcl2 and Bax was analyzed by quantitative real time PCR after 6, 12 and 24 hours of exposure to OTA.

Results: After 24 hours of incubation, viability of CaCo2 was significantly decreased after exposure to 50μM OTA. The malondialdehyde concentration was significantly increased, whereas the reduced glutathione level was significantly decreased. This could be due to the increased level of superoxide anion and the decreased level of glucose-6-phosphate dehydrogenase specific activity, as well as unmodified glutathione reductase one compared to control. Our work showed that Bax mRNA level is overexpressed in CaCo2 cells compared to Bcl2 mRNA one after 4 hours of exposure to OTA.

Conclusion: According to our results, all events might represent important factors in the chain of cellular events leading to OTA induced apoptosis in CaCo2 cells.

P4-8

Peeping into the interplay of aging and caloric restriction at mitochondria

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Research on aging has flourished after the findings on common biology with dreadful diseases like cancer, Alzheimer's and schizophrenia. Dietary restriction appears as a promising experimental approach to enhance healthy lifespan. We used combined approach of blue-native PAGE and fluorescence difference gel electrophoresis (fluorescence DIGE) to monitor the quantitative changes in rat liver mitochondrial proteome with respect to age and caloric restriction. Proteins involved in energy transduction and detoxification pathways, besides those in anti-oxidant and cell death mechanisms were found changing in response to aging and/or diet.

The experimental set-up of BN-DIGE facilitated evaluation of abundance changes in membrane and soluble proteins across protein-protein interactions. It was fascinating to observe association of nucleoside-diphosphate kinase (DNA repair and transcriptional regulation of genes involved in DNA damage response) and glyceraldehyde-3-phosphate dehydrogenase (pro-apoptotic mitochondrial membrane permeabilization) with respiratory chain complex proteins, which highlighted a functional coupling between the energy transduction processes in mitochondrial membranes and oxidative stress induced cell death. Besides the transient or long-term effects of caloric restriction and the well appreciated events of age-retardation, we also observed aging-analogous effects.

The present work illuminates a network of interlinked pathways converged at mitochondria, which effectively responses to aging and caloric restriction. We hereby put forth a novel approach to screen the potential candidates directly involved in such complex physiological phenomena.

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P4-9

Role of nerve growth factor in modulating the survival of myogenic cells exposed to free radicals

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It is well known the capacity of Nerve growth factor (NGF) to rescue neuronal cells from injury by oxidative radical stress [Enokido and Hatanaka, 1990 [1]; Jackson et al., 1990 [2]; Jackson et al., 1990 [3]] by increasing both the level of cellular GSH and the activity of the antioxidant enzymes (Pan and Perez-Polo, 1993 [4]; Sampath, 1994). Utilising L6C5 myoblasts as in vitro model for skeletal muscle cells, our study focused on the role of NGF in the molecular mechanisms aimed to protect skeletal muscle satellite cells from the apoptotic cell death triggered by free radicals.

L6C5 myoblasts, in growing or differentiation medium, were exposed to different concentrations of exogenous NGF (20 ng/ml) in standard or oxidative stress conditions (H_2O_2 50–300 μM). Cells were then collected at different time during differentiation to be analyzed for cell growth and survival, NFkB activation, apoptosis induction, expression of specific myogenic markers and fusion rate.

We demonstrated that NGF is able to sustain L6C5 myoblast survival in FBS-free medium and that can improve their resistance towards oxidative stress, but without modifying the binding activity of NFkB or L6C5 susceptibility to apoptosis. Indeed, the inhibition of p75NTR, the only NGF receptor expressed in L6C5 line, did not cause a significant increase of apoptotic death, suggesting that the transduction pathway activated by p75NTR is not a main pro-apoptotic process in L6C5 myoblasts. However, although L6C5 cells don't express TrkA receptor, our cellular model is sensitive to treatments with a specific TRK receptor's inhibitor (AG879), which causes a significant decrease in cell survival, either in standard or oxidative stress conditions, together with a reduction in Akt phosphorylation and apoptotic proteolysis of poli (ADP ribose) polymerase enzyme (PARP). During L6C5 differentiation in vitro, we found that NGF enhances the activity of NFkB during the first phases of this process (2 days in DM), leading to decrease of L6C5 cell susceptibility to apoptosis induced by cytotoxic doses of H_2O_2. At the same time, NGF determines an over-expression of myogenin, an increase in the fusion rate of myoblasts into myotubes, with a consequent hypertrophy of multinucleated fibers.

In conclusion, our results demonstrate that NGF is able to sustain the survival of skeletal myoblasts in critical conditions, including oxidative stress, trough a cross talk between the transduction pathways activated by p75NTR and TRKs receptors. In addition, during differentiation, NGF is able to increase NFkB binding activity and the cell resistance to apoptosis induced by oxidative stress.

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P4-10

Alpha-Tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II, a new anti-cancer drug target

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Alpha-tocopheryl succinate (alpha-TOS) is a selective inducer of apoptosis in cancer cells, which involves the accumulation of reactive oxygen species (ROS). The molecular target of alpha-TOS has not been identified.

Here we show that alpha-TOS inhibits succinate dehydrogenase (SDH) activity of complex II (CII) by interacting with the proximal and distal ubiquinone (UbQ) binding site (Qp and Qd, respectively). This is based on biochemical analyses and molecular modelling, revealing similar or stronger interaction energy of alpha-TOS compared to that of UbQ for the Qp and Qd sites, respectively.

CybL-mutant cells with dysfunctional CII failed to accumulate ROS and undergo apoptosis in the presence of alpha-TOS. Similar resistance was observed when CybL was knocked down with siRNA. Reconstitution of functional CII rendered CybL-mutant cells susceptible to alpha-TOS.

We propose that alpha-TOS displaces UbQ in CII causing electrons generated by SDH to recombine with molecular oxygen to yield ROS. Our data highlight CII, a known tumour suppressor, as a novel target for cancer therapy.

P4-11

Heavy metal stress-induced modulation of FoxO signaling

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Exposure of human and rat hepatoma cells to stressful stimuli such as redox-active and thiol-reactive metal ions (Cu^{2+} and Zn^{2+}) was emonstrated previously to result in a phosphoinositide 3-kinase (PI3K)-dependent activation of the Ser/Thr kinase Akt, followed by a PI3K/Akt-dependent phosphorylation, inactivation and nuclear exclusion of transcription factors of the FoxO family as well as an impairment of FoxO-related expression of target genes [1, 2]. In mammalian cells, known FoxO targets include genes coding for proteins which are important for the cellular antioxidant defense as well as regulation of cell cycle or apoptosis. Although exposure to copper and zinc resulted in a phosphorylation of Akt, treatment with Mn^{2+}, Fe^{2+}, Co^{2+} and Pb^{2+} did not. Exposure of human hepatoma cells to Ni^{2+} ions, however, resulted in a PI3K dependent activation of Akt and phosphorylation of the established Akt substrate, glycogen synthase kinase-3. Furthermore, copper and nickel ions caused the phosphorylation of FoxO1a and FoxO3a. However, and in contrast to insulin and Cu^{2+}, Ni^{2+}-induced Akt activation and FoxO phosphorylation did not result in significant FoxO inactivation and nuclear exclusion. In line with this finding, no significant modulation of the activity of a FoxO-responsive promoter construct was observed. In summary, while several heavy metal ions are capable of eliciting a cellular stress response, only selected ions affect the PI3K/Akt/FoxO signaling cascade. The exact parameters defining the potency of heavy metal ions in modulating cellular signaling cascades remain to be fully established.

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P4-12

COX-2 expression is enhanced upon UVB irradiation in HaCaT keratinocytes: Regulation at the posttranscriptional level

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Cyclooxygenase-2 (COX-2) plays a crucial role in inflammatory processes. Ultraviolet (UV) radiation is known to stimulate COX-2 expression in skin cells. In HaCaT human keratinocytes such an elevated expression is observed only upon exposure to UVB (280–320 nm) but not UVA (320–400 nm) radiation, as demonstrated by RT-PCR as well as by Western blotting. The stability of COX-2 mRNA was dramatically increased by UVB irradiation. Moreover, p38MAPK was strongly activated.

Applying specific inhibitors of p38MAPK, both the stabilisation of COX-2 mRNA and the enhancement of COX-2 steady-state mRNA and protein levels caused by UVB exposure were prevented, suggesting p38MAPK-dependent mRNA stabilisation as a mechanism of induction of COX-2 expression by UVB. The mRNA binding protein hnRNP A0, a well-known downstream target of p38MAPK, was shown not to participate in COX-2 induction by means of siRNA-induced depletion.

However, HuR, a stress-responsive mRNA stabilising protein of the E LAV family, was found in a siRNA approach to be essential for UVB-induced COX-2 expression. Both the mode of p38 activation by UVB and the relation of p38 to HuR are currently being analysed.

P4-13

The supramolecular organisation of OXPHOS complexes in different rat brain regions changes during ageing

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Maintenance and regulation of cellular metabolism depend on activity of numerous reaction pathways which might get affected during ageing. Our studies focus on the mammalian mitochondrial membrane proteome, especially of the inner mitochondrial membrane with the oxidative phosphorylation (OXPHOS) complexes and other proteins possibly involved in life-span control and aging. Variations of the mitochondrial proteome during aging, with the emphasis on the abundance, composition, structure and activity of membrane proteins, are examined in various rat brain areas by native polyacrylamide gel electrophoresis (PAGE) techniques in combination with MALDI-TOF mass spectrometry. As analytical technique, blue-native PAGE separates not only individual proteins but also multi-subunit (membrane-) proteins, (membrane-) protein supercomplexes as well as interacting proteins in their native state. It reveals the occurrence and architecture of supramolecular assemblies of proteins. In contrast to common proteomic studies reflecting solely changes in the abundance of proteins, we examined in addition protein-protein interactions.

The mammalian brain is very complex. It is presumed that aging occurs differently in different brain areas. We studied the mitochondrial proteome of the largest region of the rat brain, the cortex, and two smaller regions, striatum and hippocampus, of two age groups, 4–6 and 28 months.

During aging, an increase in the amount of individual F_1-part of MF_oF_1 ATP synthase occurs in the cortex as well as in striatum and hippocampus. This indicates more damaged ATP-synthase or incomplete assembly in aged brain tissue. The age-related alterations in the oligomerisation of the MF_oF_1 ATP synthase and in the abundance of intact enzyme we observed in rat cortex might be a clue for understanding the link between respiration and longevity. Also, the abundance of OXPHOS supercomplexes, which are the natural assemblies of the respiratory complexes I, III, and IV into supramolecular stoichiometric entities, such as $I_1III_2IV_{0-3}$, differs between young and aged cortex tissue. Age-related changes in the supramolecular architecture of OXPHOS-complexes might explain alterations in ROS production during aging.

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P4-14

Nutritional modulation of sirtuin expression is mediated by redox changes

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Sirtuins are NAD-dependent deacetylases that modulate metabolism, the rate of ageing and other critical physiological parameters. Thus, metabolic regulation of these enzymes is of critical importance. The role of the NAD/NADH redox ratio in the regulation of sirtuins has been proposed.

To test this idea, researchers have measured NAD and NADH in several cellular models. However, in a pioneer study Krebs and co-workers showed that direct measurement of tissue content of NAD and NADH do not supply the required information: they fail to differentiate between free and bound nucleotides and they give no information on the distribution of the nucleotides between the various cell components which is known to be uneven?

This difficulty can be overcome by measuring the concentration of the oxidised and reduced metabolites of suitable NAD-linked dehydrogenases. We have used cell culture, invertebrates (Drosophila) and vertebrates (mice) models to study the regulation of sirtuins.

We have found that buffering the NAD/NADH pair with known concentrations of lactate and pyruvate regulates sirtuin expression in 3T3 fibroblasts in culture. Ethanol, which affects NAD/NADH ratio also up-regulates sirtuin expression in this model. In Drosophila, ethanol also up-regulates sirtuin expression and this results in an increased average life span of the flies.

NAD/NADH ratio also regulates sirtuin expression in vertebrates. Exercise causes a significant increase in lactate-pyruvate ratio and thus changes in NAD–NADH. This results in an increase in the expression of sirtuins. The importance of this study lies in the fact that sirtuin expression does not depend on levels of free NAD or NADH but rather on the ratio of these co-enzymes in tissues.

This ratio can be modulated by many physiological manipulations like exercise or moderate ethanol intake. We have observed that these metabolic changes result in significant increases in average life span of the animals.

The importance of these facts to understand the role of oxidation in longevity will be fully discussed.

P4-15

The novel gaseous vasorelaxant hydrogen sulfide inhibits angiotensin-converting enzyme (ACE) activity of endothelial cells

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Background: Beside NO (nitric monoxide) and CO (carbon monoxide), H_2S (hydrogen sulfide) was recently identified as the third gasotransmitter. A cardio- and vaso-protective action has been attributed to H_2S which is produced in micromolar (up to 100 μM) concentrations in the circulation. H_2S has been found to inhibit leucocyte-endothelial cell interactions, inhibit cellular apoptosis, could act as an antioxidant, could upregulate antioxidant defense and could protect cells from the cytotoxic action of hypochlorite (OCl^-) and peroxynitrite ($ONOO^-$). By directly acting on K^+ATP-channels of smooth muscle cells (SMC) H_2S possesses also vasorelaxing properties.
Study aims: As H_2S has the potential to react with metal ions (i.e. Cu, Fe, Zn) in metallo-proteins and ACE (Angiotensin-Converting-Enzyme), which is responsible for vasoconstriction, is a zinc (Zn^{++}) containing enzyme, we hypothesized that H_2S may interact with the Zn^{++} in the active center of ACE, modulating (inhibiting) its activity.
Methods: ACE activity was measured on the surface of human endothelial cells (HUVEC) monolayers in culture, ex-vivo in umbilical veins and in HUVEC protein extracts. Quantitative real-time PCR was used to study the effect of H_2S on ACE-mRNA expression in HUVEC.
Results: H_2S in a dose dependent manner inhibited the activity of ACE in HUVEC protein extracts, and only Zn^{++} but not Cd^{++}, Ca^{++} or Mg^{++} could counteract the inhibitory effect. Cell surface ACE activity was inhibited by H_2S on HUVEC monolayers and in ex-vivo umbilical veins. No influence of H_2S on ACE-mRNA expression was observed.
Conclusion: H_2S exhibits direct inhibitory action on ACE activity in HUVEC obviously by interfering with the Zn^{++} in the active center of the enzyme. Thus, beside the known influence of H_2S on SMC K^+ATP-channels, the observed direct ACE inhibitory effect may also add to the vasorelaxant, cardio- and vaso-protective effect of H_2S by reducing angiotensin II production and inhibiting bradykinin degradation.

P4-16

Flavonoids as inhibitors of bace activity and beta-amyloid peptide aggregation

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Proteolytic processing of the beta-amyloid precursor protein (APP) by beta-secretase (BACE) and gamma-secretase, and generation and accumulation of the beta-amyloid peptide is a main causal factor of Alzheimer disease (AD). Consequently, inhibition of BACE activity and inhibition of beta-amyloid peptide aggregation are attractive therapeutic approaches for the treatment of AD.
The purpose of the present study was to investigate the anti-amyloidogenic activity of several flavonoids to modulate BACE enzyme activity in a cell-free assay, and to suppress beta-amyloid peptide aggregation under in vitro conditions (electron microscopy analysis and Thioflavin T fluorescence assay).
We report that rutin, naringin and hesperidin at 10 μM had significant inhibitory effect on the suppression of beta-amyloid peptide aggregation and hidrosmin, rutin and naringin significantly inhibited BACE activity ($p < 0.05$).
Our results could contribute to the development of new BACE inhibitors and antiamyloidogenic compounds. These flavonoids and other structurally related compounds could be key molecules for the development of therapeutics for AD. Further studies with these flavonoids, in cellular and animal models, are essential to reveal which one of them exhibit anti-amyloidogenic and fibril formation effects *in vivo*.

P4-17

Effect of leukocyte apheresis on oxidative stress and fibrosis in inflammatory bowel diseases

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Inflammatory bowel diseases (IBD) are characterized by recurrent inflammatory injury and repair with consequent gross structural damage of the intestine, that involves the cross-talk of different cell types through the production of large variety of pro-inflammatory and pro-fibrogenic cytokines, such as TNF-alpha, IL-1beta, IL-6, IL-8 and TGFbeta1. In particular, TGF-beta1 is fundamental in maintaining the intestinal epithelial cell homeostasis, but its dysregulation has been observed in several chronic human diseases, including ulcerative colitis, Crohn?s disease and colon carcinoma. Besides cytokine production, another consistent feature of chronic inflammation, is an overproduction of different reactive oxygen species by activated leukocytes which overwhelms antioxidant tissue defenses.

The current IBD therapy, which mainly consists in a long-term administration of anti-inflammatory drugs, does not prevent intestinal fibrosis, stricture formation and consequent surgical intervention. Recently, leukocyte removal by apheresis has been widely accepted as therapeutical management of diseases that are mediated by humoral and/or cellular immunity (multiple sclerosis, rheumatoid arthritis, etc.), but few reports are presently available about the use of this technique in IBD.

The purpose of this study is to evaluate the effect of leukocyte apheresis on oxidative stress and fibrosis in patients IBD disease of moderate-to-serius activity which are resistant or intolerant to the commonly used steroid treatment.

Patients have been subjected to five cycles of leukocyte apheresis with an interval of one week between treatments. Up to now, we recruited 11 patients (2 Ulcerative Colitis, 9 Crohn?s Disease). Blood samples were taken at apheresis time-points we consider the most significant to evaluate oxidative stress (in terms of aldehyde-protein adducts in plasma) and profibrogenic activity (TGF-beta1 serum levels): before apheresis (T0), soon after the last cycle of apheresis (T1), 3 months (T2) and 6 months (T3) after the end of therapy. All patients will be constantly monitored for their overall health status.

Beneficial effects of leukocyte apheresis was observed in the 70% of the patients recruited, first of all their life-style was improved and the surgical intervention was delayed. In response to apheresis, the modifications of blood parameters of oxidative stress and fibrosis were highly correlated to patient's clinical status.

Selective leukocyte apheresis can remove activated neutrophils and monocytes/macro-phages from the peripheral blood, by this way reducing the major sources of inflammatory/fibrogenic cytokines and ROS, and favouring disease remission.

Thus, leukocyte apheresis may represent a promising new adjuvant or even an alternative therapy for all IBD, particularly in those patients who are refractory/intolerant to conventional pharmacological therapy with steroids.

P4-18

Nitric oxide modulates molecular basis of interscapular brown adipose tissue thermogenesis

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Brown adipose tissue (BAT) is highly specialized organ for both cold-induced and diet-induced thermogenesis. Nitric oxide (NO) regulates numerous processes in BAT, but little is known about the effects of NO on molecular basis of thermogenesis. Thus, we examined here possible effects of NO on transcriptional and translational profiles of key regulatory molecules of interscapular BAT (IBAT) thermogenic programme – uncoupling protein 1 (UCP1), peroxisome proliferator-activated receptor α (PPARα) and PPARα-coactivator 1 (PGC-1α), as well as on mitochondriogenesis and tissue hyperplasia during cold-acclimation.

Adult Mill Hill hybrid hooded rat males were divided into two main groups: control, kept at room temperature ($22 \pm 1^{\circ}$C) for 45 days and the group maintained at cold ($4 \pm 1\ ^{\circ}$C) during 1, 3, 7, 12, 21 and 45 days. Cold-acclimated group was divided into three subgroups: untreated, L-arginine-treated and NG-nitro-L-arginine methyl ester (L-NAME)-treated.

Results show time-coordinated transcriptional and translational activation of UCP1, PGC-1α and PPARα during cold-acclimation that was reflected by increased mitochondriogenesis, IBAT mass and protein content. These changes correlate with endothelial NO synthase (eNOS) transcriptional and translational activation suggesting NO involvement in shown molecular cascade activation.

L-arginine treatment, related to untreated-group, accelerated and prolonged cold-induced transcriptional and translational activation of UCP1, PGC-1α and PPARα and additionally increased mitochondriogenesis, tissue mass and protein content. In contrast, L-NAME attenuated UCP1, PGC-1α and PPARα transcriptional and translational activation and reduced IBAT hyperplasia.

Besides, L-arginine prolonged period of eNOS transcriptional and translational activation as well as translational activation of inducible NOS (iNOS), while L-NAME down-regulated eNOS transcription and decreased eNOS protein content from day 7 to 45 and iNOS on day 1 and 12 of cold-acclimation.

Presented data clearly indicate that NO positively affects molecular basis, as well as morphological aspects of IBAT remodeling. Namely, results suggest NO as important regulator of IBAT thermogenic programme, operating at all levels of organization, from genes transcription to the level of the tissue structural organization.

P4-19

Protection of insulin-producing INS-1 cells from H_2O_2-induced oxidative stress by phenolic extracts from Olea europaea L.

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Olea europea L. is the frequently used herb in Mediterranean countries for the management of diabetes. It contains generally phenolic antioxidant substances that may protect the function of insulin releasing pancreatic cells from free radical-induced damage.

Thus, we investigated the effects of the extracts from leaf (OLeaf) and fruit (OFruit) of Olea europaea L. var. europaea Zhukovsky and oleuropein as a standard phenolic compound on intracellular redox status and insulin secretion in insulinoma cell line (INS-1). We assessed the effects of these materials on reactive oxygen species (ROS) level (2,7-dichlorodihydro-fluorescein diacetate fluorescence assay, DCF-DA), cellular viability (MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay), activity of antioxidant enzymes (cholorimetric assay kit) including superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and insulin release (ELISA).

Incubation of INS-1 cells with H_2O_2 (35 μM for 45 min) resulted in a decrease in viability of cells (51 ± 9% of control), inhibition of glucose-stimulated insulin release (44 ± 6% of control), and an increase of intracellular ROS production (308 ± 57% of control). A 24-hour pre-incubation with OLeaf (0.1 mg/mL), OFruit (0.1 mg/mL) or standard oleuropein (0.1 mmol/L) showed a significant decrease ($P < 0.001$) in ROS production in oxidized cells. Although any protective efficacy was shown with OLeaf, the pre-treatment of the cells with OFruit resulted in the stimulation of insulin release by 229 ± 30% of control cells. Oleuropein led to almost complete recovery of insulin release (93 ± 1.0% of control) diminished by exposure H_2O_2 stress. H_2O_2 incubation also accompanied by a significant decreased in GPx and SOD activities (28.0 ± 9.2 and 59.0 ± 7.0 nmol/min/mL for GPx and SOD, respectively) compared to control cells (79.0 ± 14 and 71.7 ± 8.3 nmol/min/mL for GPx and SOD, respectively). However no such decrease was detected for CAT activity (6.1 ± 2.4 and 5.1 ± 3.3 U/mL for oxidized and control cells, respectively). The activity of GPx was remarkably elevated in cells pre-treated with OFruit compared to controls and oxidized cells (125 ± 11 nmol/min/mL). The pre-incubation with this fraction provided also a protection of SOD activity (75.9 ± 8.5 nmol/min/mL). OLeaf exerted a partial protection of GPx by 54.4 ± 11.5 nmol/min/mL together with over activation of CAT (74.0 ± 9.0 U/mL). However, oleuropein caused stimulatory effect on only CAT (43.4 ± 3.8 U/mL).

The results may justify the ethnomedical use of the Olea europea L. in the management of diabetes and suggest that the phenolic constituents of extraction fractions of leafs and/or fruits of this plant mostly mediate its protective actions on INS-1 cells by amelioration of redox status and insulin release impaired by H_2O_2-induced oxidative stress.

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P4-20

The molecular basis of thalidomide resistance in murine embryonic cells

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Thalidomide (Contergan), a potent treatment for multiple myeloma and leprosy, is also a teratogen, which induces embryopathies, such as eye defects (microphthalmia) or limb truncations (amelia, phocomelia) in humans [1]. In contrast to humans and chickens, mice are insensitive to the teratogenic properties of thalidomide. It has previously been shown in the chicken model system that thalidomide induces limb truncations via the formation of reactive oxygen species (ROS), suppression of Wnt and Akt survival pathways and induction of apoptosis during early embryonic limb development [2, 3].

Here, we describe the role of ROS and of antioxidant defense in thalidomide-sensitivity. According to the species specificity of thalidomide teratogenicity the drug induces apoptosis in embryonic fibroblasts of humans (HEFs) and chickens (CEFs) but not in those of mice (MEFs). MnTBAP, a superoxide dismutase (SOD) mimetic, but not cell- permeant catalase, completely abrogated thalidomide-induced cell death in HEFs and CEFs, suggesting that superoxide formation is involved in thalidomide toxicity. We sensitised MEFs for thalidomide by pre-treatment with the naphthoquinone derivative juglone or by depletion of cellular glutathione by application of diethyl maleate (DEM).

The addition of thalidomide to such pre-treated MEFs induces MEF apoptosis to an extent comparable to that found with thalidomide-treated CEFs and HEFs. In line with either higher levels of ROS generated or with glutathione depletion being an efficient means of sensitizing MEFs for thalidomide, the glutathione levels found in unstimulated cultured MEFs were higher than in thalidomide-sensitive CEFs. From these data, we conclude that thalidomide resistance depends on the capacity of the intracellular antioxidant defence.

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P4-21

The role of a yeast NAD(P)H-oxidase in aging and apoptosis

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One of the most widely accepted theories of aging posits that cellular damage by ROS (reactive oxygen species) is a prominent cause of aging. Superoxide is the primary oxygen radical which gives rise to a large number of ROS and other toxic and mutagenic molecules which can oxidatively modify proteins, DNA, and lipids. It is the well established that superoxide radicals mainly originate from a leakage of the mitochondrial electron transport chain. In a previous publication we showed that the dominant activated allele of the yeast RAS gene, RAS2ala18, val19, led to redox imbalance in exponential-phase cells. The mutant cells produce superoxide as shown by ESR spectroscopy and they do so even in the absence of a complete mitochondrial electron transport chain, pointing to the existence of a possible non-mitochondrial source for ROS generation. A very prominent source of superoxide in eukaryotic cells are the NAD(P)H-oxidases (NOX). These enzymes were originally found in phagocytes as a first line of defense in the human immune system. However, several isoenzymes exist which are expressed in other cell types of the human body. Their physiological function is speculated to be production of superoxide (or ROS) as a growth signal. The NOX enzymes and their homologs display a heme containing subunit of the flavocytochrome b, that consists of gp91phox, p22phox, and p67phox. NADPH oxidase enzymes were previously unknown in yeast.

Here we present experimental evidence that the yeast genome codes for a bona fide NADPH oxidase we named NOX1. It is a possible source for the non-mitochondrially generated superoxide in the yeast S. cerevisiae and is involved in the aging process and in cell cycle control at the G1/S boundary.

P4-22

Lack of involvement of mitochondrial ros during endoplasmic reticulum stress induced mitochondrial permeability transition and apoptotic cell death

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The precise role of the "mitochondrial permeability transition" (MPT) in apoptotic and necrotic cell death pathways are controversial.

Here we show that the MPT regulates the release of cytochrome c for apoptosis during endoplasmic reticulum (ER) stress by remodeling the cristae junction (CJ). CEM cells, HCT116 colon cancer cells and murine embryo fibroblast (MEF) cells were treated with the ER-stressor thapsigargin (THG) which led to cyclophilin-D (Cyp-D)-dependent mitochondrial release of the profusion GTPase Optic Atrophy 1 (OPA1), which controls CJ integrity, and cytochrome c leading to apoptosis.

Targeted RNAi knockdown of Bax blocked OPA1 and cytochrome c release after THG treatment, but did not prevent the MPT showing that Bax was essential for the release of cytochrome c by MPT. In isolated mitochondria, MPT led to OPA1 and cytochrome c release independently of voltage dependent anion channel (VDAC) and the outer membrane indicating that the MPT is an inner membrane phenomenon. Lastly, we show that the MPT was not regulated by mitochondria-derived reactive oxidant species (ROS) but was regulated by the mitochondrial electron transport chain (ETC) since (a) cell death was not blocked by antioxidants and (b) MPT and cell death did not occur in cells lacking mtDNA.

Our results show that the MPT regulates CJ remodeling for cytochrome c-dependent apoptosis induced by ER stress and that the mitochondrial electron transport is indispensable for this process whereas mitochondrial ROS are not.

P4-23

Flavonoids restore klotho gene expression in the hippocampus of rat with oxidative stress

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Decreasing ROS has been proposed to be helpful in delaying the aging process including loss of memory and learning ability. Human klotho (KL) gene was reported to play an important role in anti-aging. However, the regulation between KL gene and anti-oxidative reagents is still unknown.

In this study, Sprague Dawley (SD) rats i.p. injected with H_2O_2 showed a decreased expression of KL gene in hippocampus region compared to that in the healthy control. On the contrary, RT-PCR and Western analysis demonstrated that KL expression in the hippocampus of SD rats with oxidative stress was restored after quercetin or gallic acid treatment.

These results reveal that flavonoid exert protective effects on anti-aging through klotho gene pathway.

POSTER-SESSION 5 — HUMAN APPLICATIONS

P5-1
The serum concentration of homocysteine and reactive oxygen species in pacientes with renal transplant or submited to hemodialysis

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Background: Homocysteine is a non-protein sulfur containing aminoacid that is synthesized from methionine. Increased total homocysteine is very common in renal patients. Hyperhomocysteinemia is an additional factor that increases the risk of vascular diseases in general and in renal patients in particular. The aim of the paper was to point out the associations between the level of homocysteine and the oxidative stress in patients submited to hemodialysis and renal transplant.
Material and method: The study included 30 patients submited to hemodialysis from the Clinical Hospital Oradea-Department of Nephrology, and 25 patientes with renal transplant during five months after the surgery. Before and after the hemodialysis the concentration of homocysteine (total serum homocysteine was measured using the enzymatic homocysteine assay, cat no. FHER100, on Hitachi 912 instrument) of MDA (with tiobarbituric acid) and carbonylated proteins (guanidin method) were assessed. In the same time the level of ceruloplasmin, the main antioxidant factor in the plasma was measured (Ravin method). We also estimated the level of creatinine and urea during those months. The same investigation we made on the patient with renal transplant. The concentrations of cholesterol and triglicerides were also evaluated. All resultes were compared with a control group.
Results: Before and after the hemodialysis the homocysteine concentration was significantly elevated in the blood of the studied patients. The patients before hemodialysis had an increased level of MDA in comparison with the control group. Hemodialysis changed in little measure the level of MDA ($p = 1$). The carbonylated proteins had also a high concentration in patientes in comparison with the control group. This difference is very significant ($p < 0{,}001$). After hemodialysis no remarkable difference was noticed ($p > 0{,}1$). The concentration of ceruloplasmin in the serum of renal patients before hemodialysis was lower in comparison with the control group ($p < 0{,}05$). After hemodialysis the values were similar to those before the intervention ($p > 0{,}1$). We also assessed creatinine, urea and total protein (Table 1).
The patients with renal transplant have an increased level of MDA in comparison with the control group ($p < 0{,}001$). The concentration of ceruloplasmin in the serum of this patients is low comparing to the control group ($p > 0{,}1$). The level of serum homocysteine is increased in patients with renal transplant. We also assessed cholesterol, triglyceride which are considered cardiovascular disease risk factor (Table 2).
Conclusions: Concentrations of homocysteine in renal patients after hemodialysis are high. A high level of homocysteine is associated with oxidative stress in those patients. The level of MDA in patients with renal transplant is increased in comparison with the control group and the concentration of ceruloplasmin is reduced which means that an oxidative stress is present. The patients with renal transplantation had an hiperhomocysteinemia.

P5-2
Investigation of oxidative changes in human blood plasma after single bout of exercise in healthy and untrained subjects

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Physical exercise was used as a model of a physiological modulator of free radical production to examine the effects of exercise-induced oxidative modifications on the biochemical properties of the erythrocyte membrane and blood plasma. Studying exercise-induced oxidative modifications to tissues and molecules upon physiological conditions provide an opportunity to improve our understanding of the relevance of oxidative stress to the human body.
Venous blood was taken before, immediately after and 1h after an exhaustive incremental cycling test (30 W/min ramp) performed by 15 healthy untrained male volunteers: age (22 ± 2 y); height (187 ± 7.84 cm), weight (87 ± 15.6 kg), BMI (25 ± 4.5). Assays of plasma peroxides, carbonyl groups and -SH groups were chosen to examine plasma components damage (such as proteins and lipids). The reducing potential of plasma was measured by reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH). Statistically significant increase of plasma -SH groups has been observed. However, single bout of exercise did not induce significant changes in plasma carbonyl groups but the level of peroxides significantly decreased 1 hour after exhaustion. An increase in the reducing potential of plasma measured by DPPH was observed 1h post-exercise.
Results indicate that single bout of exercise in healthy, untrained subjects on normal and balanced diet (without antioxidant supplementation, non-smokers, non-drinkers, not exercising regularly) did not induce excessive ROS production. Therefore, it can be recognized as a factor, which induces defensive plasma antioxidant system, such as peroxidases and low molecular weight antioxidants. It has been evidenced by the increase of reducing potential of the plasma, peroxides decrease and elevation of -SH groups.
Grants: This study was supported by the research grant: N 404 117 33 from the Polish Ministry of Science and Higher Education.

P5-1. Table 1. The values of biochemistry parameters in renal patients.

Parameters	Normal values	May	June	July	August	September
Creatinina (mg%)	0,6–1,1 mg%	8,52 ± 2,64	9,14 ± 2,1	9,36 ± 1,87	8,81 ± 2,07	8,47 ± 2,25
Uree (mg%)	15–45 mg%	158,73 ± 36,42	152,15 ± 35,21	165,38 ± 57,78	145,68 ± 28,97	146,87 ± 36,90
ac uric (mg%)	3,5–7,2 mg%	6,77 ± 1,11	6,87 ± 1,03	7 ± 0,69	6,9 ± 0,83	6,6 ± 0,91
Total proteins (mg%)	6,6–8,7 mg%	6,83 ± 0,52	6,81 ± 0,37	6,6 ± 0,62	6,69 ± 0,58	6,53 ± 0,54

P5-1. Table 2. The Values of cholesterol and trigliceride in pacients with renal transplant.

Parameters	Normal value	March	April	May	June
Cholesterol (mg%)	200–239 mg%	185 ± 36,99	159 ± 46,48	152,85 ± 43,62	260,27 ± 36,35
Triglyceride (mg%)	150–199 mg%	160,57 ± 86,92	184,3 ± 74,99	260,89 ± 96,44	223,05 ± 97,14

P5-3

The study of correlations between aerobic metabolism and heart rate variability in duodenal peptic ulcer patients: an effect of amaranth oil

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Introduction: The recent studies showed that the heart rate variability (HRV), a highly sensitive and widely available noninvasive physiological examination, is closely related with condition of metabolism and can reveal manifestations of oxidative stress in organism. Therefore, the study of correlations between aerobic metabolism parameters and HRV in duodenal peptic ulcer (DPU) patients, before and after standard as well as modified (with Amaranth oil (AmO) supplementation) treatments, appears to be interesting and important, and became the aim of the study.
Methods: 75 DPU patients underwent HRV examination and biochemical studies (catalase and superoxide dismutase (SOD) activities, TBARS, hydroperoxides, middle mass molecules, oxidatively modified proteins and LDL-cholesterol levels) in blood serum. Correlation coefficients (r) and its significance (p) were calculated in groups: before treatment [1], after standard treatment [2] and standard treatment accompanied by AmO supplementation [3].
Results: The DPU patients (group 1) were characterized by decreased HRV parameters, as well as, manifestations of oxidative stress (by biochemical indices), revealed a number of significant correlations. Among them the most valuable were positive correlations of many HRV parameters with SOD-activity. This means that inhibited SOD activity could be responsible for the depression of parasympathetic tone and overall HRV decrease. Standard treatment scheme strengthened the tendencies, observed before treatment. In addition, manifestations of oxidative stress persisted and, by some parameters, even increased. Supplementation with AmO caused mild prooxidant effect and shifted correlations of HRV from positive with SOD-activity (groups 1 and 2) to negative with TBARS levels (group 3). Thus, higher HRV parameters were observed in patients with better TBARS utilisation.
Conclusions: The study revealed existence of correlations between HRV and aerobic metabolism parameters. Their strength and character depend on the functional and metabolic condition of the DPU patients. AmO which has mild prooxidant influence provided modulation of correlations between studied parameters, which supported increase of HRV and stress resistance.

P5-4

European standards committee on urinary (DNA) lesion analysis (ESCULA): towards consensus for the measurement of urinary 7,8-dihydro-8-oxo-2′-deoxyguanosine

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Urine provides a non-invasive, potentially high throughput route to assess oxidative stress via analysis of selected biomarkers, applicable to large scale epidemiological studies. There is growing evidence that, rather than simply being a non-invasive marker of whole body oxidative stress, measurement of urinary DNA oxidation products, such as 7,8-dihydro-8-oxo-2?-deoxyguanosine (8-oxodG), may also reflect DNA repair activity. Multiple techniques have been used to analyse urinary 8-oxodG, primarily chromatography (e.g. GC/MS following prior HPLC pre-purification; LC-MS/MS, or LC-EC) or immunoassay (ELISA). A discrepancy in basal urinary 8-oxodG levels has been noted when comparing chromatographic techniques with ELISA, although all techniques have been shown to discriminate between diseased and healthy subjects, and possess good within-technique agreement. ELISA has received significant use, and is clearly amenable to the greatest number of laboratories, but this discrepancy compared to chromatographic measurements continues to raise questions regarding the utility of ELISA. Understanding the basis of this discrepancy will aid our understanding of the significance of urinary lesions. In addition to inter-laboratory validation exercises, through the distribution of standard materials, enabling provision of robust methods for widespread dissemination and application, ESCULA aims to: determine Reference ranges; address individual variability, sample collection and correction factor issues; achieve a better understanding of the sources of DNA lesions in urine; examine how levels of other urinary lesions compare to urinary 8-oxodG levels.
ESCULA, supported financially via ECNIS (Environmental Cancer Risk, Nutrition and Individual Susceptibility, a network of excellence operating within the European Union 6th Framework Program) currently has >20 participating laboratories with a geographical spread outside of its initial scope (13 in Europe, 5 in the Americas, 7 in Asia). Completion of a second round of inter-laboratory validation is imminent, conducted using aqueous standards, urine with exogenously added 8-oxodG and a series of unadulterated urine samples. Complimentary, ECNIS-supported work is also ongoing to determine the roles of diet, cell death and DNA repair to the production of urinary 8-oxodG.
Further details of both these projects can be found at http://www.escula.org

P5-5

The level of oxidatives stress markers and the effect of vitamine C on the carios proces

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Introduction: Reaserches on the saliva have shown its importance in the diagnose of varios disease in medical practice. Saliva acts as a cleansing solution, a lubrificant, an ions reservoire and buffer due to its content. To this properties it can be also a first line of defence against free radical mediated oxidative stress. Vitamin C is an important water-soluble vitamin for humans and a powerful reducing agent. Vitamin C also protects plasma lipids against lipid peroxidation. It has been shown to scavange superoxid anion,hydrogen peroxide, singlet oxygen. The aim of our study was to demonstrate the evolution of the salivary oxidative stress in persons with dental caries and if the administration of vitamin C improves the antioxidant capacity and if there are measured modifications of transaminases, phosphatase, LDH or C-reactiv protein (CRP) in saliva.

Material and method: The studied persons were divided into 2 groups (each group consisting of 16 persons), one group with a single tooth with a carious process non- smokers, and the otherone with multiple caries. The first group took 200 mg vitamin C 3 time a day, for 3 weeks. For proving the salivary and serum oxidative stress we established the level of MDA utilizing method with tiobarbituric acid and ceruloplasmin with Ravin method. GOT and GPT, alkaline and acid phosphatase, LDH and CRP were determined out of saliva on analyzer Hitachi 912, Roche Diagnostic, Switzerland, using reactive Greiner Diagnostic, Germany. The obtained results were compared to an experimental group of 15 volunteers with no dental lesions or restorations.

Resultes: Salivary MDA was significantly increased at the group with a single tooth with a carious process and with multiple caries.The same thing was observed in blood sample on those groups.The result could be an indirect prove that serum MDA do not pass in saliva through filtration. The resultes proved the strong antioxidant efect of vitamin C even in small doses. Salivary level of MDA were reduced. The tissues destruction from dental cavities and periodontitis is testified by the increase of salivary transaminses, and the presence of a inflammatory reaction is pointed out by the increased concentrations of LDH and CRP. The presence of bony reshaping caused by the studied dental lesions is reflected by the variations of alkaline and acid salivary phosphatase.

Conclusions: This findings suggest that there was a high level of MDA and lower salivary ceruloplasmin level caused by the carious proces-s.Our study indicates the protective effects of vitamin C in saliva for the prevention of oral inflamations. The level of GOT, GPT, LDH, CRP indirectly indicates the presence of an inflamatory process. Due to non-invasive and non-noxious ingathering saliva could be used for diagnosis purposes.

P5-6

Biological variation and critical difference of exercise related oxidative stress metabolites

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The concept of biological variation (BV) and critical difference (CD) is often neglected in the field of exercise free radical biochemistry. Reference data exist for a wide range of haematological analytes, however, there are currently no data for any exercise related oxidative stress metabolite.

We therefore studied the daily BV and CD of lipid-derived free radicals (PBN-adduct), lipid hydroperoxides (LOOH), malondialdehyde (MDA), ascorbic acid, retinol, lycopene, alpha-tocopherol, beta-carotene and alpha-carotene in 10 apparently healthy male volunteers (age 24 + 3 yrs, body mass 80 + 14 kg; stature 178 + 007 cm). Blood was drawn from an antecubital forearm vein once every hour, over an eight hour period (9.00am to 5.00pm) under standardised laboratory conditions. Blood was also obtained from 12 male volunteers (age 23 + 3 yrs, body mass 75 + 8 kg; stature 179 + 01 cm) at rest and following an acute bout of exhaustive aerobic exercise.

The BV of PBN-adduct, LOOH, MDA, ascorbic acid, retinol, lycopene, alpha-tocopherol, beta-carotene and alpha-carotene was 43.3, 8.8, 17.5, 3, 9.3, 38, 1, 9.1 and 38.4% respectively, and the CD 121, 27.5, 50.3, 8.5, 28.7, 106, 13, 28.2 and 107% respectively. Following exercise there was a comparatively greater concentration of PBN-adduct, LOOH and beta-carotene ($P < 0.05$ vs rest), representing a change of 115, 60.2 and 100% respectively. Ascorbic acid, alpha-tocopherol, retinol and lycopene all increased following exercise (9.3, 9.1, 9.6, 64.1% respectively) but no mathematical significance was observed ($P > 0.05$).

This data demonstrates that oxidative stress indices have a range of BV and CD values, and that exercise can cause a mathematical change in the absence of a clinical change and vice versa. We would emphasise that exercise biochemists who routinely measure oxidative stress take into consideration the biological variation and critical difference in order to determine whether exercise and intervention protocols and/or pathology contributes towards a real physiological/clinical phenomenon.

Keywords: Antioxidants, Free radicals, Biological variation, Critical difference, Exercise.

P5-7

Oxidative stress in the pathogenesis of Gaucher's disease: effect of substrate reduction therapy

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Background: Gaucher's disease (GD) is an inborn error of glycosphingolipid (GSL) metabolism caused by deficient activity of the lysosomal enzyme β-glucocerebrosidase that cleaves the ?-glucosidic linkage of glucosylceramide (GlcCer) yielding glucose and ceramide. The pathology of GD results primarily from the progressive lysosomal accumulation of GlcCer and other GSLs in the mononuclear phagocyte system with subsequent macrophage activation and tissue inflammation. Phenotypically, GD is considered a continuum of disease states ranging from the milder forms, displaying only peripheral symptoms, to the most severe forms with early neurological onset. In non-neuronopathic GD patients (type 1 variant, GD1) GlcCer accumulation is restricted to visceral tissues, predominantly liver, spleen and bone marrow, without central nervous system involvement. Substrate reduction therapy (SRT), based on the partial inhibition of GSL synthesis by N-butyl-deoxynojirimycin (Miglustat), is a treatment used to balance the reduced catabolic capacity of Gaucher's patients.

Aims: To delve further into the underlying pathophysiological mechanisms related to oxidative stress in GD and assess the effect of SRT on molecular oxidative damage in GD1 patients.

Patients and methods: Indicative parameters of lipid and protein oxidation together with the liposoluble antioxidant a-tocopherol were analysed before and during 24 months of oral miglustat treatment (Zavesca) in plasma of 27 patients with mild-to-moderate GD1.

Results: Compared with healthy controls, GD1 patients had greater oxidative damage to lipids and proteins demonstrated through the analysis of malondialdehyde (MDA), advanced oxidation protein products (AOPPs) and oxidized-LDL, all of which were significantly raised in plasma of GD1 patients prior to treatment and decreased progressively over the following two years of SRT. In plasma of untreated GD1 patients, MDA concentrations were on average 2-fold higher than in controls (1.3 vs. 0.6 nmol/mL), AOPPs 1.8-fold (140.7 vs. 77.9 nmol/mg prot) and ox-LDL 1.3-fold (63.3 vs. 47.8 U/L). During substrate depletion therapy, levels of oxidative stress biomarkers in plasma decreased significantly in all the GD1-treated patients. Plasma a-tocopherol concentrations were normal and did not differ in untreated, SRT-treated GD1 patients or healthy controls.

Conclusion: Our results support the hypothesis that the GD-associated accumulation of peroxidable lipids provokes oxidative damage to macromolecules that declines significantly in patients receiving SRT which diminishes GSL synthesis. Thus, oxidative stress appears to be a new pathomechanism of GD, possibly implicated in the phenotypic expression of the disease, and that is improved in SRT-treated GD1 patients. This study indicates that although GD is a classical lysosomal storage disorder, the pathology may in part be caused by extra-lysosomal processes that directly contribute to the complex pathophysiology of the disease.

P5-8

Possible mechanism of mechanotransduction induced by external stress like shockwave application. participation of reaktive oxygen species and nitric oxide

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It was supposed, that during shockwave application reactive oxygen and nitrogen species play an important role.

Reactive nitrogen species (RNS) like the NO-radikal were measured by an ozone induced chemiluminescence-assay. Reactive oxygen species (ROS) were measured by ultra weak chemiluminescence.

It was shown, that NO was produced during shockwave application by measuring the NO signal through the skin. On the other hand reactive oxygen species, which we cannot discriminate now, were induced by shockwave application and were identified by ultra weak chemiluminescence.

We have shown under other circumstances, that an extrinsic stress like UV-light, ozone or cigarette smoke can induce the generation of reactive oxygen and nitrogen species. We believe that the application of shockwaves are nothing else than another kind of extrinsic stress and can induce via mechanotransduction the production of RNS and ROS. The generation of reactive oxygen and nitrogen species may play a role in the mechanotransduction pathway, a hypothesis of a possible mechanism will be discussed.

P5-9

Influence of moderate daily exercise on formation of reactive oxygen species in healthy volunteers

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The balance in reactive oxygen species (ROS) formation is the key for different physiological and pathological conditions such as exercise, cell growth, aging and cancer. The appropriate detection of reactive oxygen species in human still requires further development of applications and techniques. The aim of our study was to analyze the correlation of in vivo formation of ROS with changes of hemodynamic parameters during a moderate increase in difficulty of a treadmill exercise and to quantify the formation of ROS before and after a training period of 8 weeks. The measurements were taken during the day over a period of 8 weeks of moderate daily exercise.

Methods: In 2 groups of healthy human volunteers in the age of 49 ± 5 years with minimal previous exercise history and regularly exercised volunteers in the age of 23 ± 3 years we observed the changes of cardiac hemodynamic parameters, ex-vivo formation of ROS (BenchTop EPR spectrometer E-SCAN, Noxygen GmbH) measured before and after 2 cycles of moderate increase in difficulty of treadmill exercise, before and after a daily training for 8 weeks. Additionally, plasma reduced SH-groups concentration, total cholesterol were analysed using EPR spectroscopy and using photometrical assay, respectively.

Results: Moderate exercise induced EPR detectable enhancement in formation of ex-vivo ROS parallel to changes of hemodynamic parameters. Moderate daily exercise over 8 weeks in 49 ± 5 years old human volunteers lead to significant decrease in formation of ROS down to the level of 22 ± 3 years old human volunteers and to the adaptation of exercise dependent changes of hemodynamic parameters such systolic blood pressure and heart rate.

We observed a significant decrease in day time dependent and exercise dependent formation of ROS, total cholesterol level and increase in total SH-group concentration.

Conclusion: We demonstrated a suitable approach for detection of ROS in human volunteers reflecting the adaptation to moderate daily exercise as well as changes in cellular/metabolic adaptation (reduced SH-group/total cholesterol concentration) which are involved in improving the resistance to oxidative stress. Release of ROS during exercise can be used as an index for of resistance to oxidative stress.

P5-10

Inflammatory disease activity and antioxidative potential in juvenile idiopathic arthritis

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Oxidative stress is probably involved in the pathophysiology of juvenile idiopathic arthritis. This disease is characterized by chronic joint inflammation. In adult patients with rheumatoid arthritis an increase of radical damage and a decrease of antioxidative defence in synovial fluid or blood is seen. Fewer studies discuss the relations of antioxidative potential and inflammatory activity in paediatric patients.

Patients with juvenile idiopathic arthritis ($n = 100$) seen consecutively in a paediatric rheumatology unit were stratified according to inflammatory disease activity (erythrocyte sedimentation rate) in groups with low (65%), medium (25%) or high activity (10%; $t = 0$ months). A follow-up of these patients was performed after 3, 6 and 12 months. The effectiveness of antioxidative defence in the blood of these patients (total antioxidant capacity, antioxidant concentrations, lipid peroxidation) was correlated with inflammatory disease activity and compared to a control group.

The concentrations of SH-groups were decreased compared to control in the medium inflammatory activity group ($t = 0$). After three months there was a shift in the distribution of patients according to inflammatory disease activity (low: 75%; medium: 21%; high: 4%); simultaneously there was an increase in total antioxidant capacity in plasma. After 6 and 12 months the plasma concentrations of vitamin C and sulfhydryl-groups were increased; simultaneously the malondialdehyde concentrations were decreased. After 12 months the inflammatory disease activity measured as erythrocyte sedimentation rate was significantly decreased compared to $t = 0$ months.

During a one-year observation period of patients with juvenile idiopathic arthritis the inflammatory disease activity decreased. At the beginning of the study the antioxidant defence was decreased compared to the control group. The decrease of inflammatory disease activity was paralleled by a time dependent increase of hydrophilic antioxidants and a simultaneous decrease of lipid peroxidation.

These findings illustrate the role of free radicals in the end pathway of inflammatory disease activity in juvenile idiopathic arthritis.

P5-11

Oxidative stress measurements in pancreatitis patients

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Introduction: Pancreatitis is inflammation of the pancreas, an organ that produces several enzymes to aid in the digestion of food, as well as the hormone insulin, which controls the level of sugar (glucose) in the blood. Pancreatitis may be either acute (sudden and severe) or chronic. Both types of pancreatitis can cause bleeding and tissue death in or around the pancreas.

In this study oxidative stress parameters have been measured to investigated whether patients suffer from a disturbed redox or antioxidant status.

Methods: Consecutive patients admitted with a diagnosis of acute pancreatitis were divided into mild ($n = 22$) and severe ($n = 17$) and a control group ($n = 28$). General serum parameters (albumin, ALT, AST, total cholesterol, HDL- and LDL-cholesterol, triglycerides, uric acid, creatinine, HS-CRP, γ-GT, glucose, have been measured with an autoanalyzer (LX-20 Pro, Beckman). Oxidative stress markers in serum were measured including reactive oxygen metabolites (ROM) and ferric reducing antioxidant power (FRAP). In erythrocytes glutathione status (GSH_{tot}, GSH, GSSG), GPX, SOD, Hb, HbA1c and fatty acid patterns have been measured.

Results and discussion: The general clinical chemical parameters in patients with both mild and severe pancreatitis showed all a large deviation from those of the control group, with also a substantial difference between mild and severe patients. All enzymes and CRP showed dramatically increased values indicating general tissue damage and increased inflammation. Also the lipid and albumin values showed a substantial deviation.

In contrast the parameters in erythrocytes (glutathione, GPX, SOD, Hb, fatty acid patterns) showed no statistically significant differences between all groups. The serum oxidative stress parameters ROM and FRAP showed a complete unexpected behaviour. Both pancreatitis groups showed a statistically significant decreased level of oxygen metabolites and an increased total antioxidant capacity compared with the control group.

The present results showed a completely disturbed status of the general physiology of the pancreatitis patients, but without any sign of increased oxidative stress. On the contrary they showed a lower oxidative stress in a number of serum parameters. Whether this is caused by lower albumin concentrations or lower iron status has to be investigated in detail.

P5-12

Alpha-lipoic acid attenuates exercise-induced oxidative stress and induces thioredoxin reductase activity

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The benefits of antioxidant supplementations have been often questioned. Alpha-lipoic acid (LA) is a natural, potent thiol antioxidant that can regenerate major physiological antioxidants of the lipid and aqueous phases. On the other hand, thiol antioxidants like LA and its dithiol form DHLA, have been suggested to function also as pro-oxidant depending on the type of stress and/or physiological circumstances. Thioredoxin is another thiol group antioxidant which plays an essential role in cell function by limiting oxidative stress directly via its antioxidant effects and indirectly by protein/protein interactions with key signalling molecules.

The aim of this study was to examine the effects of LA on the exercise-induced oxidative stress and antioxidant responses. Six standardbred trotters were examined at rest and at different time points of recovery after 75 minutes of aerobic treadmill exercise. They were supplemented orally with LA (25 mg kg-1 day-1) for five weeks without any additional vitamins.

Using electro paramagnetic resonance (EPR) assay, we showed that strenuous aerobic exercise increases significantly free radical formation in gluteus medius muscle of control animals but not in LA-supplemented horses. LA-supplementation decreased the amount of post-exercise lipid hydroperoxides in plasma and exercise-induced increase in malondialdehyde in plasma and muscle. There was no increase in muscle protein carbonyl levels; a marker of protein oxidation, or thioredoxin due to exercise or LA-supplementation; however, the activity of thioredoxin reductase was significantly increased in LA-supplemented horses.

According to our results, LA appears to decrease exercise-induced oxidative stress at lipid phase and enhance TRX defence.

P5-13

Measurment of multiple oxidized lipid products from a single plasma sample from healthy volunteers and clinical patients

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Many oxidized lipid products have been associated with diseases such as atherosclerosis, stroke, thalassemia and diabetes mellitus. These products include F2-isoprostanes (F2-IsoPs), hydroxyeicosatetraenoic acid products (HETEs) and oxysterols or cholesterol oxidation products (COPs). Each product is often measured individually in separate blood samples. Here we present a method capable of measuring F2-IsoPs, HETEs, COPs and arachidonate using a single plasma or serum sample. As examples for the application of the method, clinical patient data from stroke, dengue and diabetic mellitus patients are presented.

A volume of plasma or serum sample (1 ml) is spiked with heavy isotopic standards and hydrolysed in alkali with organic solvent. It is then purified using anionic exchange solid phase extraction (SPE). After washing the SPE column, hexane and hexane/ethyl acetate portions are collected and combined for COPs measurement. Afterwards, the column is loaded with hexane/ethanol/acetic acid and fractions collected for total F2-IsoPs, total HETEs and arachidonate measurement. All compounds in the eluates are measured by gas chromatography-mass spectrometry. The robustness of the method was tested by the efficiency of SPE and reproducibility for all compounds measured.

The method showed high and reliable efficiency of SPE and reproducibility of oxidized lipid products and were measurable in plasma of healthy volunteers and clinical patients. For example, levels of total F2-IsoPs 0.45 ± 0.26 ng/ml ($n = 157$), total HETEs 34.1 ± 16.4 ng/ml ($n = 21$) total arachidonate 68.4 ± 24.5 ug/ml ($n = 33$), and COPs 7-ketocholesterol 12.3 ± 6.6 ng/ml, 7b-hydroxycholesterol 6.3 ± 3.5 ng/ml, 7a-hydroxycholesterol 15.1 ± 7.1 ng/ml, 24-hydroxycholesterol 41.4 ± 18.2 ng/ml and 27-hydroxycholesterol 29.1 ± 16.8ng/ml ($n = 26$) were recorded in healthy volunteers. The method was also applicable in stroke patients where total F2-IsoPs 0.98 ± 0.38 ng/ml ($n = 21$), total HETEs 33.9 ± 22.1 ng/ml ($n = 15$), total arachidonate 90.4 ± 30.7 ug/ml ($n = 21$), and COPs 7-ketocholesterol 27.9 ± 17.6 ng/ml, 7b-hydroxycholesterol 31.5 ± 18.9 ng/ml, 7a-hydroxycholesterol 5.5 ± 1.6 ng/ml, 24-hydroxycholesterol 124.6 ± 102.4 ng/ml and 27-hydroxycholesterol 37.6 ± 19.0 ng/ml ($n = 21$) were recorded. Our investigation presented indicates that the method developed is applicable for the measurement of lipid oxidation products in human clinical settings or in small animals where plasma samples are limited.

P5-14

Pure dietary flavonoids, quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy human volunteers

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Background: Dietary flavonoids may have beneficial cardiovascular effects by improving endothelial function.
Objective: Pure dietary flavonoids may benefit endothelial function by modulating nitric oxide and endothelin-1 production.
Design: A randomised, placebo controlled, cross-over trial in 12 healthy men was conducted to compare the acute effects on nitric oxide, endothelin-1 and oxidative stress after oral administration of 200 mg of quercetin, (-)-epicatechin and epigallocatechin gallate. Nitric oxide production was assessed by measuring plasma S-nitrosothiols, plasma/urinary nitrite and nitrate levels, while their effects on oxidative stress by measuring plasma/ urinary F2-isoprostanes. Plasma and urinary levels of quercetin, (-)-epicatechin and epigallocatechin gallate were measured to establish the absorption of these flavonoids.
Results: Relative to water (control), quercetin and (-)-epicatechin resulted in a significant increase in plasma S-nitrosothiols and nitrite and urinary nitrate concentrations ($p < 0.001$), but not plasma nitrate or urinary nitrite. Epigallocatechin gallate did not alter any of the measures of nitric oxide production. Only treatment with quercetin resulted in a significant reduction in urinary endothelin-1 concentration ($p < 0.05$). All three treatments did not significantly change plasma or urinary F2-isoprostane concentrations. Significant increases in the circulating levels of the three flavonoids were observed ($p < 0.05$) after each treatment, however, plasma levels of EGCG were very low (approx 0.1 μM) even after supplementation.
Conclusions: We showed that dietary flavonoids, such as quercetin and (-)-epicatechin augment nitric oxide status and thereby may improve endothelial function. We have also showed that flavonoids such as quercetin, (-)-epicatechin and epigallocatechin gallate and have no acute effect on systemic oxidative stress assessed using F2-isoprostanes.

P5-15

Regular light exercise does not induce oxidative stress in healthy children

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Aim: The purpose of this research was to study the oxidative/antioxidative status in healthy children which perform regular light exercise and which do not perform exercise.
Methods: We evaluated 37 healthy children (age 10–11) which were divided in 2 groups: 16 children which perform regular light exercise and 21 which do not perform any exercise. Blood samples were taken and malondialdehyde, carbonyl protein and ceruplasmin levels in serum assessed. The results were also compared with a group of 25 healthy adults, blood donors in Transfusion Center.
Results: There are no differences between the 3 serum parameters in the 2 groups of healthy children. The children compared to the adults have significant reduced carbonylated protein concentrations ($p < 0.02$), and also elevated levels of ceruplasmin.

P5-16

Oxidant/antioxidant balance in chronic hepatitis C

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Aims: Hepatitis C virus produces a persistent infection. Tissue damage is mainly caused by immunopathological mechanisms, but studies demonstrate the important role of ROS (reactive species of oxygen) also. The purpose of our study was to evaluate the oxidant/antioxidant balance in patients with chronic hepatitis C.
Methods: The antioxidant response of the organism was tested by assessing the levels of SOD (superoxide dismutase) and GSH-Px (glutathione peroxidase) in the red blood cells using Randox Laboratories Ltd. reagents (Cat. No. SD 125, Cat. No. SR 505) on Cobas Mira Plus analyser. Serum levels of ceruloplasmin (CER) and two markers of oxidative stress: malondialdehide (MDA) and carbonyl proteins (CP) were also measured. Our research was performed on 54 patients with chronic hepatitis C (CHC). We compared them to a group of 39 blood donors.
Results: MDA and CP levels were considerably increased in CHC patients ($p < 0{,}001$; $p < 0{,}01$). CER levels were decreased ($p < 0{,}01$). The activity of the two intraerythrocyte enzymes (SOD, and GSH-Px) were also decreased ($p < 0{,}001$; $p < 0{,}05$).
Conclusion: In CHC patients exists a marked imbalance between reactive species of oxygen (ROS) and antioxidants.

P5-17

Vitamin D deficiency and oxidative stress in patients with oral squamous cell carcinoma and healthy subjects

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Background: Recent evidence suggests that the most advantageous serum levels for 25(OH)D3 appeared to be at least 75 nmol/l and for cancer prevention, desirable 25(OH)D3 levels are between 90–120 nmol/l. In this study, we aimed to determine the relationship between oxidant-antioxidant balance and vitamin D status in cancer patients and healthy individuals.

Material and methods: Circulating levels of 25(OH)D3, thiobarbituric acid reactive substances (TBARS), erythrocyte reduced glutathione (GSH), catalase and superoxide dismutase (SOD) were measured in 55 patients with stage III–IV squamous cell carcinoma and 38 controls.

Results: Patients with cancer had non-significantly lower circulating levels of 25(OH)D3 levels compared to controls (32.2 ± 9.3 vs. 34.5 ± 15.3 nmol/L). Circulating levels of 25(OH)D3 were found lower than 40 nmol/L in 75% of patients from cancer group but also in 65% of healthy subjects. The vitamin D intake was very low in both groups (60 ± 25 IU/d in cancer group and 66 ± 38 IU/d in control group). Serum TBARS levels were found significantly increased (7.4 ± 2.1 vs. 5.1 ± 2.3 nmol/ml, $p < 0.0001$) and erythrocyte GSH (9.4 ± 2.4 vs. 10.1 ± 2.6 μmol/ml, $p < 0.01$), catalase (1.0 ± 0.5 vs. 1.7 ± 0.1 U/ml, $p < 0.01$), SOD (3.2 ± 0.7 vs.3.9 ± 0.7 U/ml, $p < 0.001$) were found significantly decreased in patients with cancer as compared to controls.

Conclusion: Results of our study suggest that the levels of vitamin D in population are very low and the majority of patients with cancer have values below 40 nmol/l. Lipid peroxidation was significantly increased and antioxidative systems were significantly decreased in cancer patients as compared to control group.

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P5-18

The balance of oxidants and antioxidants factors in pregnancy and in umbilical cord blood

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Background: The rendox status is necessary for the normal function of cell. The excesiv intracellular production of reactive oxygen species (ROS) as superoxid anion, hydrogen peroxide, singlet oxygen, nitric oxide, are able to induce noxious effects. It is well knowed that human placenta exercise a lot of influences upon the maternal homeostasis. An explanation is the rich content in mitochondria of the placenta. When placenta is fully developed consumes about 1% of the basal metabolism rate of the pregnant women. Mitochondria generates ROS as undiserabile waste products derivate from the oxidative metabolism. Pregnancy is a physiological condition which is characterized by an incressed oxygen consumption and in different organs and also in the fetoplacentar unit. The aim of our the study was to point out the markers of oxidative stress during different ages of pregnancy and in the umbilical cord blood during delivery.

Materials and methods: The 60 studied pregnant women treated at the Obstetric Gynecology Clinical Hospital Oradea, Romania were examined at the 30^{th} week, at the 38^{th} week of pregnancy and in the 4^{th} day after delivery. The markers of oxidative stress assessed by us were MDA (method with tiobarbituric acid), carbonylated proteins (CP) (a guanidine hydroclorid method), and like the most important antioxidant plasmatic factor the level of circulating ceruloplasmin (the Ravin method). We also determined the same parameters from umbilical cord blood during delivery. All resultes were compared with a control group. Supplimentary biochemistry determination such as glycemia, uric acid ASAT, ALAT and bilirubin were also done.

Results: The level of MDA, CP were increased in the at 30^{th} week and the 38^{th} week of pregnancy compared with the control group ($p <$ 0,001). The ceruloplasmin concentration was highest in the 30^{th} week and in the 4^{th} day after delivery($p < 0{,}001$). The resultes in the umbilical cord blood revealed a high level of MDA, CP and a low concentration of the ceruloplasmin in comparison with Reference. The biochemistry parameters were between normal ranges.

Conclusions: Lipid peroxidation was increased in pregnant women during delivery, but in umbilical cord blood was highest. This is perhaps due to umbilical cord compression in the period of delivery or is due to the reperfusion of the pulmonary tissue. An other explanation can be that in the period of delivery the labor effort and the increase oxygenation during intensive breathing determine production of a high level of ROS. In our study the normal level of glycemia in pregnant women demonstrated that ROS are not induced by nonenzymatic glycation. The plasma antioxidant potential was able to counteract the oxidative stress in normal pregnancy.

P5-19

Low plasma levels of beta-carotene in obese men with advanced coronary artery disease

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Coronary heart disease (CHD) is the leading cause of morbidity and mortality in developed countries with west-east stratification. Differences in classical risk factors do not account for the variability in the incidence found in different populations. Considerable evidence indicates that oxidants are involved in the development and clinical expression of CHD and that antioxidants may contribute to disease resistance. An important lipid-soluble antioxidant dietary component is alpha-tocopherol, major chain-breaking antioxidant in body tissues, and beta-carotene. Significant regional differences in plasma levels of these compounds were found. The aim of this study has been to determine plasma concentrations of beta-carotene and alpha-tocopherol, the level of systemic inflammation and oxidative stress in patients with advanced coronary artery disease. Oxidative stress parameters were determined in group of patients with advanced coronary artery disease (at least 50% stenosis of the left main coronary artery or 70% stenosis of the epicardial coronary artery according to coronarographic examination; S, $n = 91$) and control group of examined patients with normal coronary arteries (normal coronarographic status; C, $n = 49$).

Plasma levels of beta-carotene, alpha-tocopherol and malondialdehyde were determined by HPLC, free radical concentration by direct method based on chlorophyllin acceptance of electrons. Plasma levels of high sensitive C-reactive protein, Interleukine-6 (IL-6), glucose, total cholesterol, triglycerides, HDL-ch, LDL-ch and fibrinogen were followed by standard procedures. In patients with coronary stenosis (S) lower levels of beta-carotene (BC) and alpha-tocopherol (AT) coincided with higher level of inflammation and in lesser extent of free radicals (FR). BC: S: 0.10 ± 0.11 μmol/l vs C: 0.14 ± 0.08 μmol/l ($p < 0.05$); AT: S: 22.59 ± 7.16 μmol/l vs C: 23.75 ± 5.95 ($p > 0.05$); IL-6: S: 5.75 ± 3.55 ng/l vs C: 3.92 ± 2.76 ng/l ($p < 0.05$); MDA: S: 3.02 ± 0.51 μmol/l vs C: 2.85 ± 0.39 μmol/l ($p > 0.05$). In S group significantly ($p < 0.05$) higher levels of fibrinogen, glucose and lower levels of HDL-ch were found. Levels both of beta-carotene and alpha-tocopherol have been markedly below recommended plasma concentrations (beta-carotene 0.4 μmol/l, alpha-tocopherol 30 μmol/l). Low levels of beta-carotene, higher systemic inflammation and in lesser extent oxidative stress have been associated with advanced coronary atherosclerosis.

According to statistical analysis obese people with coronary stenosis had the lowest beta-carotene plasma levels, men having significantly lower beta-carotene plasma levels than women. Our study has revealed very low levels of beta-carotene especially in obese men with advanced CHD in our region. This work was supported by Ministry of Education, Youth and Sports CR grant COST 926 OC 124 and grant 0021627502.

P5-20

Redoxomics of plasma proteins in uremic patients

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A large body of evidence suggests that end stage renal disease (ESRD) patients suffer of chronic inflammation and oxidative stress. These conditions are particularly exacerbated in the course of maintenance hemodialysis (HD) therapy and may cause post translational modifications (PTMs) of plasma proteins, which are potential uremic toxins involved in phagocytic cell activation and apoptosis. Proteomics techniques, often described with the name of "redoxomics", have been preliminarily used to investigate such large (or proteinaceous) solutes. These techniques are essentially based on the immunorecognition of the most abundant PTMs followed by mass spectrometry (MS) analysis to confirm protein identity. The immunologic approach remains a preferential tool to investigate PTMs in clinical studies since current MS methods do not allow to directly identify and quantify PTMs in plasma proteins.

Redoxomics was used in this study to investigate the presence of 4 different oxidative stress-related PTMs in plasma and dialysis ultrafiltrate samples pooled from HD patients treated with standard high flux and protein-leaking dialysers. The PTMs investigated by immunoblotting were 3′-Nitro-Tyrosine (3′-N-Tyr), 2,4-DNPH-derivatizable protein carbonyls (PC), and the advanced glyc(oxid)ation and lipoxidation end products carxymethyl-lysine (CML) and 4-hydroxy-2-nonenal (4-HNE), respectively. In some experiments, the 3′-N-Tyr containing proteins of plasma samples were enriched by immunoaffinity, and were investigated for the coexistence of the other types of PTMs. Therefore, the cytotoxic power of uremic plasma was assessed in THP-1 human monocytic cells. This effect was thus correlated with the extent of immuno-detectable PTMs accumulation in such samples.

Uremic plasma was characterized by the presence of proteins that contained PTMs to a higher extent than in healthy control plasma. The dialysers with higher protein leakage (nominal cut-off > 70 KDa) provided a significant removal of immuno-detectable PTMs in the ultrafiltrate, which represents a proof of the in vivo efficacy to remove such potential large toxins. In these samples albumin was the main target of nitration and carbonylation; however, these PTMs were present also on other proteins with apparent MW of 160 KDa and 70 KDa, respectively. On the contrary, 4HNE and CML accumulate preferentially on proteins with a lower apparent MW (between 25 and 40 KDa). Albumin and these other main proteins are recognized simultaneously by all the four antibodies investigated in this study.

The incubation of uremic plasma (10% vol/vol), but not of healthy control plasma, exerted a significant cytotoxic effect on THP-1 cells (lower viability and proliferation rate). This effect correlated with the extent of immunodetectable PTMs in these samples.

Therefore, this study demonstrates that several oxidative stress-related changes are encountered in plasma proteins of uremic patients and are partially removed by protein-leaking dialysers. Since these large solutes might have a role as cytotoxic agents, further laboratory and clinical investigation is required to ascertain their role in ESRD.

P5-21

The effects of obturation with composite materials incorrect coronary reconstruction on the salivary oxidative balance

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Introduction: Involvement of the reactive species of the oxygen in oral pathology has been the theme of several reasearch during the last years. The detailed knowledge of mechanisms and effects of the reactive species of the oxygen creates new possibilities of treatment and prevention in oral pathology. It has been noticed that restaurative dental materials and the adhesives, through the damaging processes release cytotoxic products that may cause apoptosis or necrosis. Involvment of ROS is well konwn, especially of the anion super-oxyde and of the hydroxi-radical in apoptosis. We have intended to prove the existance of the concordance between the level of the salivary ROS and electromicroscopical changes at the level of the marginal periodont, damaged subsequent inappropriate stomatological intervention.
Material and method: There have been studied 65 individuals; students from the specialisation dental medecine at Medicine and Pharmacy Faculty Oradea. They are all aged between 20–30 years, non-smokers. Out of them, 25 had improper obturations with composite materials and 25 had inappropriate dentures. At the same time, a group of 15 individuals with no restauration has been studied. The oxidativ stress markers were determined with TBA method and ceruloplasmin determined with Ravin method. Also it was mesured the concentration of uric acid with enzymatic-colorimetric uricase PAP using reactive Greiner Diagnostic, Germany, on analyzer Hitachi 912. Together with the testing of the oxidative stress for the two groups of subjects, it have been determined acid, alkaline phosphatase. In order to determine the total acid phosphatase it has been used the colorimetric method using reactive Randox Laboratories Ltd, Great Britain, cat No AC 1011 on analyzer Hitachi 912, Roche Diagnostic, Switzerland. In order to determine the alkaline phosphatase we have used the Kinetic optical clorimetric method DGKC, using reactive Greiner Diagnostic, Germany, cat No 105203, on analyzer Hitachi 912, Roche Diagnostic, Switzerland. Ingathering of the pathological and normal tissues have been made from individuals aged between 20 and 30 years, in order to be studied at the Transmission Electonic Microscope (TEM), type Jeol JEM 1010 from The Centre of Electronic Microscopy of Babes Bolyai University in Cluj-Napoca, Romania.
Results: The research made in cases of individuals with incorrect obturations from composite materials have shown an increase, even if not significant, of the MDA to the affected ones ($p < 0,2$). Individuals with inappropriate dentures had also an obvious increase of the MDA, but an insignificant one ($p < 0,2$). In cases of individuals with incorrect obturations from composite materials the ceruloplasmine has much decreased values compared to those of the control group. ($p < 0,001$). Compared to the control group, the ceruloplasmine of the individuals with incorrect dentures is also significantly low ($p < 0,001$). It has also been measured the uric acid from the saliva, which also fulfills an antioxydant role. In cases of individuals with improper obturations with composite materials the value of the uric acid is situated between the limits of the control group ($p > 0,1$). As for those with incorrect dentures, the concentration of the uric acid does not differ from the one of the control group ($p > 0,1$). The values of the alkaline phosphatase in cases of individuals with incorrect obturations from composite materials have decreased statistically insignificanly ($p > 0,1$). In cases of individuals with improper dentures there have been obtained the same insignificant values ($p > 0,1$). Individuals with incorrect obturations using composite materials had a decreased enzymatic activity of the acid phosphatase as compared to the control group ($p < 0,5$). The values of the acid phosphatase in cases of individuals with improper dentures have been slightly decreased ($p > 0,1$).

Conclussions:

1. Individuals with obturations made using composite materials and incorrect dentures are associated with an increase salivary ROS.
2. The concentration of the ceruloplasmine decreases. This demonstrates a reduced antioxydant ability against the oxydative stress.
3. Insignificant changes of the alcaline phosphase in cases of patients with incorrect obturations an inappropriate dentures demonstrate that these proceedings are not able to activate the osteoblasts in the dental cement.
4. After incorrect obturations and inappropriate dentures it has been noticed a decrease of the salivary acid phosphatase (compared to the control group), maybe due to the inhibitory role on the osteoclasts.
5. The presence of vascular lessions in the electromicroscopical field, of leucocytes, macrophages, mastocytes, desmosoms, fibroblasts and altered collagen fybres represent microscopic signs of the inflamation.

P5-22

Ozone therapy: a clinical study on the pain management

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Ozone therapy is widely used in many countries since many years. Oxidative preconditioning is the essential bases of ozone therapy. Recently, the increasing widespread of this complementary therapy has been accomplished by an increased number of basic and clinic papers published on international journals. Many of the basic mechanisms of the ozone action are now well outlined. In addition, the modulation of interleukins productions and of some biochemical pathways related to inflammation and pain, indicate the rationale of its use in many pathological conditions related to pain.
We show data collected on patients treated in the last three years for disorders related to pain either in sport traumatism (232 subjects) or derived for inflammatory disorders (770 subjects). The evolution of patients was follow using the Overall Patient Satisfaction Scale. The maximal score (8–10), corresponding qualitatively to "very good" was reached in 80% of patients. A rapid and sustaining success was reached in pubalgy to a score 8–10 as soon as the therapy was started. In patients with pain from inflammatory disorders a progressive evolution in time was reached to a score 8–10. However, the maximal score in joint inflammatory diseases was 6–8 (qualitatively "good").
No side effects were recorded at short and long-term follow-up. In our experience, O_2/O_3 treatment of pain and inflammatory diseases has revolutionized the approach to radiculopathy and articular disease pains manage.

P5-23

Effects of an ironman triathlon on the DNA as detected by the scge and cbmn (CYT) assay

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Background: Regular moderate physical activity is related to various health benefits including decreased risk of cardiovascular diseases and diabetes. However, the enhanced formation of reactive oxygen species during acute and strenuous exercise can induce damage to lipids, proteins and nucleic acids. Currently there are no data available dealing with the influence of an Ironman triathlon or a similar load comparable duration on genomic stability. Thus, the major aim of the present study was to investigate the effect of an Ironman triathlon race (3.8 km swim, 180 km cycle, 42 km run), as a prototype of ultra-endurance exercise, on genomic stability and a possible DNA damage.
Materials and methods: The cytokinesis-block micronucleus cytome (CBMN Cyt) assay is a biomarker for assessing genomic instability, cytotasis and cytotoxicity. Within this study the number of micronuclei (Mni), nucleoplasmic bridges (NPB) and nuclear buds (Nbuds) in once-divided binucleated (BN) lymphocytes were measured in a subgroup of 20 triathlets (age 32 ± 6 years; VO_2 peak 60.8 ± 8.8 ml/kg/min; height 182.8 ± 6.2 cm; weight 76.7 ± 8.1 kg) (total group $n = 42$) 2 days (2 d) before, within 20 min after the race, 5 days (5 d) and 19 days (19 d) post race. The single cell gel electrophoresis (SCGE) assay is a sensitive method for detecting DNA single-strand breaks, alkali labile sites and DNA cross-linking and was applied for a subgroup of 28 triathlets (age 33 ± 6 years; VO_2 peak 58.9 ± 8.5 ml/kg/min; height 181.3 ± 6.4 cm; weight 75.1 ± 7.7 kg) (total group $n = 42$) 2 d before, within 20 min after the race as well as 1 day (1 d), 5 d and 19 d post race.
Results: The number of BN cells with MNi decreased significantly ($p < 0.05$) after the race, remained at a low level until 5 d post exercise and declined further to 19 d post race ($p < 0.01$). The frequency of NPBs and Nbuds did not change significantly immediately after the triathlon, but the number of NBP declined significantly from 2 d pre race to 19 d post exercise ($p < 0.05$). The number of Nbuds increased after the triathlon reaching a maximum 5 d post race ($p < 0.01$) and then decreased significantly 19 d after the race to basic levels ($p < 0.01$).
The DNA migration (tail moment) decreased significantly ($p < 0.05$) after the race, then increased significantly ($p < 0.05$) reaching a maximum 1 d post race and declined significantly below basic levels 19 d post exercise.
Conclusion: The present study demonstrates that an Ironman triathlon race does not cause DNA damage as detected by the CBMN Cyt assay, but leads to increased DNA migration 1 d post exercise as shown in the SCGE assay; however, this marker decreased to basic levels 5 d post race. Thus, it seems that adaptive mechanisms including the upregulation of repair mechanisms and increased capacity of the endogenous antioxidative systems may prevent severe oxidative stress and long lasting DNA damage after strenuous exercise.

P5-24

Oxidative stress in patients with spinocerebellar ataxia type 2

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Spinocerebellar ataxia type 2 is an autosomal dominantly inherited neurodegenerative disease characterized by ataxia, dysarthria and slow saccades. Recent evidences suggest that increased oxidative damage as well as deficits in antioxidants defence systems could be related to the pathogenesis of some hereditary ataxias.
The aim of this study was to investigate some of redox status biomarkers to evaluate its relation with the pathological events of the disease. Samples from 33 patients and 22 control subjects, from Holguin province, were used to determined plasmatic levels of malondialdehyde and enzymatic activities such as Cu/Zn superoxide dismutase and catalase by spectrophotometric methods. Besides, DNA damage in peripheral blood cells was evaluated using Comet assay.
Our results evidenced that oxidative damage is higher in spinocerebellar ataxia type 2 Cuban patients in comparison with the control group. In addition, we found an increase of antioxidant activity of evaluated enzymes in affected subjects. We concluded that redox status is affected in these patients.

P5-25

Oxidative stress biomarkers in patients diagnosed with probable alzheimer disease

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Increasing evidence suggests that oxidative stress is associated with several neurodegenerative diseases, including Alzheimer's disease (AD). Neuronal lost in this pathology result from a complex interaction among oxidative damage, excitotoxic stimuli, dysfunction of proteins and genetic factors.

To evaluate oxidative damage levels and antioxidant activity in subjects diagnosed as probable Alzheimer patients. Levels of malondialdehyde (MDA), advanced oxidation protein products (AOPP), glutathione reduced (GSH) and activity of the antioxidant enzyme Cu/Zn Superoxide Dismutase (SOD) were measured in plasma of 25 AD patients and 30 healthy aged-matched controls.

The level of MDA was significantly higher in plasma of patients compared to control individuals. SOD activity was significantly lower in AD patients compared with the control group. There were no significant differences in GSH and AOPP levels between groups.

Results presented here shows that oxidative status is actually affected in AD patients. Our findings underline the role of free radical in the pathogenesis of this neurodegenerative disease.

P5-26

Involvement of R192A PON-1 gene polymorphism in the response of postmenopausal women to oral hormone replacement therapy

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Menopause is a normal period during woman ageing, characterized by ovarian dysfunction and decreased circulating estrogens. This condition is associated with the risk of osteoporosis and cardiovascular disease. Ageing and degenerative diseases are associated with the accumulation of oxidation products formed during the normal metabolism. In order to prevent the adverse effects of the lack of estrogens, hormone replacement therapy (HRT) is often used, although its effects on the cardiovascular system are not well defined. We have proposed that allele variations of genes involved in the redox balance contribute to the genetic component that originates the interindividual differences in response to HRT.

Regarding this hypothesis, the aim of this work was a) to determine the effects of HRT on oxidative stress- and cardiovascular risk biomarkers of postmeopausal women, and b) establish gene markers determinant in the response to the therapy. Paraoxonase-1 (PON1) possesses high density lipoprotein-associated arylesterase activity, protecting the body against oxidation. The enzyme eliminates lipid peroxides, preventing their accumulation in low density lipoproteins. We analysed by RFLP (restriction fragment length polymorphism) R192Q PON1 gene polymorphism. The G to A substitution in exon 6 of the PON1 gene originates a change in arginine to glutamine (R192Q) in the final protein. This supposes an increased activity against lipid peroxides and is associated to a lower atherosclerosis risk. The study population consisted of 45–55 years old post-menopausal women ($n = 42$) (no menses for 12 or more months, with plasma FSH 40 U/l and estradiol < 50 pmol/l).

Women ($n = 21$) received combined HRT (continuous 50 μg/day transdermic estradiol, and 200 mg/day micronized progesterone orally the first 14 days of each month) during 6 months. A follow-up group with no treatment ($n = 21$) was also included in the study. Statistical analyses for paired data were done for comparisons between the initial and final stages.

HRT decreased levels of the soluble adhesion protein, ICAM-1 only in R allele carriers of the R192Q PON1 polymorphism. The beneficial effect of the low activity R allele of the PON1 gene on the cardiovascular risk factor was unexpected. However, carriers of this allele presented significantly higher ($p = 0{,}009$) sICAM-1 levels than the QQ genotype carriers when entering in the study. For this genotype, in contrast to at least one R allele carriers, THS did not reduce serum total antioxidant activity.

These results suggest a key role of the PON1 gene expression in response to HRT.

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P5-27

Involvement of a-463g MPO gene polymorphism in the response of postmenopausal women to oral hormone replacement therapy

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Menopause is a normal period during woman ageing, characterized by ovarian dysfunction and decreased circulating estrogens. This condition is associated with the risk of osteoporosis and cardiovascular disease. Ageing and degenerative diseases are associated with the accumulation of oxidation products formed during the normal metabolism. In order to prevent the adverse effects of the lack of estrogens, hormone replacement therapy (HRT) is often used, although its effects on the cardiovascular system are not well defined. We have proposed that allele variations of genes involved in the redox balance contribute to the genetic component that originates the interindividual differences in response to HRT.

Regarding this hypothesis, the aim of this work was to determine a) the effects of HRT on oxidative stress- and cardiovascular risk biomarkers of post-menopausal women, and b) specific genes, which are keys in the response to the therapy. The myeloperoxidase (MPO) enzyme generates free radicals; high levels of MPO have been associated with increased risk of atherocoronary disease.

We analysed by RFLP (restriction fragment length polymorphism) the A-463G polymorphism located in the promoter region of the MPO gene. The A allele is associated with lower MPO expression. The study population consisted of 45–55 years old post-menopausal women ($n=42$) (no menses for 12 or more months, with plasma FSH 40 U/l and estradiol <50 pmol/l). Women ($n=21$) received combined HRT (continuous 50 μg/day transdermic estradiol, and 200 mg/day micronized progesterone orally the first 14 days of each month) during 6 months. A follow-up group with no treatment ($n=21$) was also included in the study. Statistical analyses for paired data were done for comparisons between the initial and final stages. HRT decreased the serum total antioxidant activity ($p=0.013$), expressed as Trolox equivalents, and the levels of the soluble adhesion protein, ICAM-1 ($p=0.013$), not modifying other cardiovascular risk markers (E-selectin, C reactive prot). The high MPO activity GG genotype of the A-463G MPO gene negatively affected the HRT response, since only carriers of this variant showed a reduced serum total antioxidant activity and did not undergo reduced circulating sICAM-1 levels.

We thank Drs. Miguel Angel Elorriaga and Fernando Rodríguez, who attended the outpatients in the health centres of Ortuella and Zalla, respectively. This work was supported by Gobierno Vasco and ERRASMIK/IRALMET.

P5-28

Association of blunt chest trauma severity and septic load with the increase of oxidative stress in patients

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Chest trauma represents for patients a heavy burden accompanied by the development of the oxidative stress. Patients suffer from the chest injury itself and furthermore from a subsequent reperfusion that can have similar important impact because of oxidative stress.

The aim of this study was to reveal the potential correlation between the severity of the chest trauma plus septic load and the increase of the oxidative stress. Oxidative stress parameters were determined in a group of patients with blunt chest trauma ($n=53$, age 16–76 years) divided into 4 subgroups according to Injury Severity Score (ISS1-S4). The blood samples were taken in the days 0–1–2–4–7–10th. Reactive oxygen and nitrogen species (RONS) concentration was tested by a direct method based on chlorophyllin acceptance of electrons (kit Free Radicals, Sevapharma, CR). Total antioxidant status was tested by the kit TAS (Randox, UK). Plasma levels of high sensitive C-reactive protein (hs-CRP) and prealbumin were followed by standard procedures. The extent of septic load was evaluated by the Inflammation and Septic Index (ISI; ratio hs-CRP/prealbumin).

In patient group ISS1 (light injury without complications) and group ISS2 (polytrauma with system reaction) only slight increase of RONS level and inflammation index were found. ISS1: RONS 7.18 ± 3.04 mmol/l; ISI 0.32 ± 0.42; ISS2: RONS: 6.68 ± 3.04 mmol/l; ISI 0.58 ± 0.65. In group ISS3 (very hard injury) increase of RONS and inflammation levels reached statistical significance. ISS3: RONS 10.07 ± 6.13 mmol/l; ISI 0.95 ± 0.69; $p<0.05$. In group ISS4 (necessity of mechanical ventilation) enhanced oxidative stress and inflammation response were found as well. ISS4: RONS 8.94 ± 3.35 mmol/l; ISI 1.31 ± 0.87; $p<0{,}05$.

Patients are endangered by the chest trauma itself. Furthermore the stress burden consisting of psychic, haemorrhagic and traumatic stress factors is accompanied by increased RONS formation. Elevation of RONS levels occurs after trauma as the part of the organism defense response to the acute state and reperfusion. Concurrently the septic load is enhanced. The increase of the reperfusion extent and the oxidative stress are in coincidence with the trauma severity. These results are in a good correlation with the significance of a septic load characterized by ISI, the dynamics of hs-CRP/prealbumin ratio.

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P5-29

Lipid peroxidation biomarkers as indicators of oxidative stress prevention

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In pathological conditions balance between oxidants and antioxidants is disturbed and ROS react with cell components. All diseases and metabolic disorders are accompanied by oxidative modifications of lipids. Determination of oxidative stress biomarkers, especially markers of lipid peroxidation, allows to confirm oxidative stress occurrence and its localization in tissues and biological fluids. Lipid hydroperoxides, low molecular aldehydes (malondialdehyde and 4-hydroxy-2-nonenal) and isoprostanes are determined most frequently. All mentioned lipid peroxidation biomarkers may be determined by high performance liquid chromatography in liver homogenates and biological fluids.

In our studies we established the sensitivity, repeatability, precision and limit of detection for measurement: LOOH level by HPLC method with spectrophotometric detection, MDA and 4-HNE level by HPLC method with fluorometric detection and 8-iso-PGF2a level by LC-MS, in serum and liver of rats which were chronically intoxicated with ethanol to develop oxidative stress. Deproteinization was needed for all tissues.

Determination of LOOH and 8-iso-PGF2a required Folch extraction. Moreover determination of aldehydes needed derivatization while for HNE and 8-iso-PGF2a determinations solid phase extraction was also used. Application of these methods allowed to receive limit of detection (LOD): 7 pmol/ml for LOOH and MDA, 15 pml/ml for 4-HNE and 18 fg/ml for 8-iso-PGF2a. Limit of quantification (LOQ) for LOOH, MDA, 4-HNE and 8-iso-PGF2a was 18 pmol/ml, 20 pmol/ml, 30 pmol/ml and 18 fg/ml with limit of linearity: 5–250 nmol/ml, 0.1–2.2 nmol/ml, 0.10–2.25 nmol/ml and 100–1200 pg/ml,respectively. The CV values were as follows: 5.1% for LOOH, 4-HNE and 8-iso-PGF2a and 4.6% for MDA analysis. Significant increase was observed in concentration of all examined lipid peroxidation biomarkers in rats chronically intoxicated by ethanol (for LOOH and MDA by about 70% while for HNE by 30%). However liver level of 8-iso-PGF2a was increased by about six times in comparison with control group. The preventive effect of black tea in the liver and serum caused smaller increase in examined biomarkers of oxidative stress (0.9–25%).

Above results univocally confirm antioxidant action of black tea against free radicals generated during ethanol metabolism.

P5-30

Effect of pyruvate on antioxidants and antioxidative enzymes under hypercholesterolemia

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Hypercholesterolemia (HC) induced formation of reactive oxygen species (ROS) which interferes with vascular tone, resulting in cellular damage and organ dysfunction. Pyruvate was used acting as an antioxidant with beneficial metabolic effects for studying glutathione, glutathione reductase (GR), glutathione-S-transferase (GST) activity and changes in ascorbic acid concentration in plasma and liver.

Methods: Hypercholesterolemia (HC) was achieved with a 0.5% cholesterol diet, with or without 20 mM pyruvate in drinking water for 8 weeks for male guinea pigs. Plasma cholesterol was estimated using a Sigma Diagnostic kit, ascorbic acid was quantified by HPLC with electrochemical detection. ROS formation and cellular thiols (reduced SH-groups) were detected by the BenchTop electron spin resonance spectrometer E-Scan (Noxygen GmbH). GR and GST activity in liver tissue, plasma lactate and pyruvate was analyzed by absorbance spectroscopy. Blood cells were counted using Sysmex cell counter.

Results: Cholesterol supplementation enhanced the plasma cholesterol concentration, additional pyruvate in turn diminished the increase in plasma cholesterol (basal: 0.6 ± 0.1 mmol; chol: 5.4 ± 0.4 mmol; pyr: 4.8 ± 0.1 mmol). ROS formation in whole blood of HC is significantly enhanced and decreased by coadministration of pyruvate. The lactate/pyruvate ratio in plasma (indicator of metabolism), decreased in the pyruvate group. Induction of oxidative stress under HC diminished significantly ascorbic acid concentrations in plasma and liver tissue, pyruvate reversed these effects. The diminished SH-group concentration in red blood cells as well as the liver GR and GST activities were improved by pyruvate supplementation.

Conclusions: Application of pyruvate substantially diminishes oxidative stress. This may result in maintaining of the relevant antioxidants concentration (reduced thiols, ascorbic acid) and in the improved activity of SH-groups reducing enzymes (glutathione reductase, glutathione-S-transferase).

P5-31

Sesame supplementation does not affect cardiovascular disease risk factors in overweight volunteers: a controlled crossover trial

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Background: Pre-clinical studies suggest that sesame and its lignans affect micronutrient metabolism, and induce beneficial changes in risk factors related to cardiovascular disease. However very few controlled intervention trials have investigated these potential bioactivities of sesame in humans.

Objective: We aimed to investigate the effects of sesame supplementation in humans on micronutrient metabolism, blood lipids, blood pressure, systemic inflammatory and oxidative stress biomarkers.

Design: Overweight men and women ($n = 33$) completed a randomized cross-over intervention trial. Participants consumed 25 g/day of sesame (~ 50 mg/d sesame lignan) and an iso-caloric placebo matched for macronutrient and tocopherol composition for 5 weeks each. Each intervention period was preceded by a 4 week washout period.

Results: Results are presented as the effect of sesame supplementation relative to placebo. gamma-tocopherol increased 17% ($P = 0.012$), and urinary excretion of its metabolite, gamma-CEHC, decreased 31% ($P < 0.001$). Serum alpha-tocopherol and excretion of its urinary metabolite remained unchanged. Urinary excretion of the mammalian lignans enterolactone and enterodiol, increased approximately 8-fold ($P < 0.001$). Blood lipids, and blood pressure assessed by ambulatory blood pressure monitoring, were not altered. In addition, markers of systemic inflammation (CRP, IL-6, TNF-alpha) and lipid peroxidation (F2-isoprostanes) were not affected.

Conclusions: 25 g/day sesame supplementation caused no beneficial changes to markers of cardiovascular disease risk in overweight men and women. However, it did significantly increase serum gamma-tocopherol and urinary mammalian lignans.

POSTER-SESSION 6 — PROTEIN OXIDATION AND PROTEOLYSIS

P6-1
In vitro oxidation study of bovine serum albumin: a proteomics approach

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Albumin is widely considered as an important antioxidant plasma protein. This is largely due to its large quantity and high turnover, as well as to the reactivity of its sulfhydryl groups with oxidant species, contributing to the protection of cellular and regulatory long-lived proteins.
In this work we have studied the structural modifications of albumin induced by oxidative stress. To do so, bovine serum albumin (BSA) was submitted to in vitro model oxidative stress through metal-catalyzed oxidation. The determination of the structural alterations was performed through a mass spectrometry based approach (MALDI-MS and MALDI-MS/MS), combined with previous off-line nano liquid chromatography of tryptic digests. A total of 106 different residues were identified as oxidatively modified, being the main affected residues lysines, cysteines, arginines, prolines, histidines and tyrosines.
Together with protein hydroxyl derivatives and oxygen additions, other modifications were identified such as deamidations (arginines), carbamylations (arginines and lysines), arginine oxidation to glutamic semialdehyde, cysteine oxidation to oxoalanine, histidine oxidation to aspargine and to aspartic acid, lysine oxidation to a-aminoadipic acid and to aminoadipic semialdehyde, proline oxidation to pyroglutamic acid and to pyrrolidinone, tryptofan oxidation to oxolactone, tyrosine oxidation to 2-aminotyrosine and to quinone. Three main regions were affected by the oxidative damage and were affected at different time points in the oxidative process.
Results indicated that oxidative damage appeared at first in surface-exposed regions near cysteine disulfide bridges, spreading after to more buried regions as albumin unfolded with the oxidation of cysteines residues implicated in disulfide bridges.

P6-2
In vitro properties of artificial lipofuscin

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A typical indication of aging is the intracellular accumulation of lipofuscin, a hydrophobic yellow-brown material that accumulates especially in the lysosomal compartment, where it can be neither degraded or exocytosed from the cell. It can only be diluted by cell division and subsequent growth. This way of lipofuscin removal is not possible for postmitotic cells including neurons and muscle cells.
For *in vitro* studies, an artificial model of lipofuscin that behaves like the native one would be a very useful tool for investigating its intracellular interactions. We developed a method generating artificial lipofuscin by irradiation of erythrocyte-lysates with UVA and UVB, according to the protocol of Nilsson and Brunk using isolated mitochondria. This artificial material has been investigated relating its properties and its abilities to accumulate iron. Its resistance against proteolytic degradation and its ability to reduce proteasomal activity was also proven.
The artificial material showed all characteristics found in native lipofuscin and offered an interesting model of proteasomal inhibition.

P6-3

Plasmatic levels of asymmetric dimethylarginine, age and AOPP in different methods of renal replacement threrapy

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Background: Asymmetric dimethylarginine (ADMA) is an important mediator of endothelial dysfunction and a marker of cardiovascular mortality in patients with end-stage renal disease (ESRD). Production and elimination of ADMA can be influenced by type of renal replacement therapy and by oxidative stress.
Methods: We studied levels of ADMA, advanced oxidation protein products (AOPP) and advanced glycation end-products (AGE) in 20 patients on hemodialysis (HD), 19 patients on peritoneal dialysis (PD) and 20 control subjects. Furthermore, we followed an immediate and long-lasting effect of low-flux HD and hemodiafiltration (HDF) on plasmatic values of ADMA, AOPP and AGE. Results are expressed as median [interquartile range] if not stated otherwise.
Results: Patients on HD showed higher ADMA values than controls (1.20 [0.90 to 1.39 μmol/L] and 0.89 [0.77 to 0.98], resp., $p < 0.01$), whereas PD patients didn't differ from controls (0.96 [0.88 to 1.28]). Decrease of ADMA in plasma after HD (95% CI = 0.34 to 0.79 μmol/L, $p < 0.001$) a HDF (95% CI = 0.38 to 0.64, $p < 0.001$) was comparable. AOPP and AGE in HD (62 [55.9 to 75.4]; 8.88 [7.65 to 9.8], resp.); and PD (69.7 [61.2 to 72.75]; 6.73 [6.38 to 7.88]) groups were higher ($p < 0.0001$ for both AOPP, AGE and HD, PD groups) than controls (36.7 [32.87 to 43.45]; 3.32 [3.06 to 3.77]) in HD and PD groups. The change of blood purification method from HD to HDF (and vice versa) for 8 weeks didn't influence measured parameters.
Conclusions: HD and HDF procedures temporarily decrease levels of ADMA, but have no long-term effect on ADMA, AOPP and AGE concentrations in plasma. PD patients have comparable ADMA concentrations with healthy controls.
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P6-4

N-chloroamino acids: factors implicated in protein oxidation *in vivo*?

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The increase in the amount of oxidatively modified proteins is a hallmark of ageing and age-related disorders. Hypochlorous acid/hypochlorite is one of physiological oxidants responsible for oxidative protein modifications. N-Chloroamino acids, *in vivo* products of hypochlorite reactions, may also contribute to this effect.
This study was aimed at characterization of oxidative modifications of erythrocyte membrane proteins by N-chloroamino acids and HOCl. N-Monochloroamino acids were synthesized by reaction of amino acids (Ala, Lys, Ser, Asp, Phe) with HOCl at a molar ratio of 5:1. Protein tryptophan, formylokynurenine and dityrosine residues were estimated fluorometrically, hydroperoxides with xylenol orange, thiol groups with the Ellman reagent and amine groups with fluorescamine. Acetylcholinesterase activity was estimated according to Ellman. SDS-PAGE of membrane proteins was performed under both reducing and non-reducing conditions.
All chlorocompounds employed oxidized protein thiol groups, induced formation of protein aggregates and hydroperoxides; all of them but AspCl induced tryptophan loss and decreased acetylcholinesterase activity.
Formylokynurenine was formed after treatment with AlaCl, PheCl and HOCl. HOCl only induced dityrosine formation. The loss of amine groups was evident after AlaCl, LysCl and SerCl treatment. Formation of protein aggregates was noticed under non-reducing conditions indicating formation of disulphide bonds.
These results indicate that chronic inflammation augmenting the formation of HOCl and N-chloroamino acids may contribute to oxidative modifications of erythrocyte membrane proteins which are an obvious target of their reactions in blood.

P6-5

Polyphenol-induced dissociation of various amyloid fibrils results in a methionine-independent formation of ROS

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Fibrilization of amyloid polypeptides is accompanied by formation of reactive oxygen species (ROS), which, in turn, is assumed to further promote amyloid-related pathologies. Different polyphenols, all of which are established antioxidants, cause dissociation of amyloid fibrils. We have investigated the dissociation of A? 42 fibrils by six different polyphenols, using electron microscopy and spectrofluorometric analysis. We also studied the antioxidative potency of these polyphenols and found that the capacity of the various polyphenols to induce defibrilization correlates with their antioxidative potency. Curcumin, which is the most potent antioxidant (and defibrillating agent) also causes dissociation of four other amyloids. In addition, we followed the production of ROS during dissociation, using electron spin resonance and a peroxide assay and found that most, if not all, the investigated dissociation processes are accompanied by ROS formation. Unlike dissociation, the production of ROS is independent of methionine residues. Both the mechanistic significance and the possible implications of the results require further experimentation.

P6-6

Oxidative modulation of sarcoplasmic reticulum from rat skeletal muscles in animals suffering from adjuvant arthritis

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Oxidative stress and redox imbalance contribute to the pathogenesis of chronic inflammatory diseases including rheumatoid arthritis (RA). Oxidative stress is tightly connected with imbalance of calcium homeostasis. Intracellular calcium level plays a role in signalling function and is modulated by calcium regulating proteins, including the Ca-ATPase from sarco/endoplasmic reticulum (SERCA).

In the present work we studied the mechanisms of SERCA modulation in vivo in rats suffering from adjuvant arthritis (AA). AA is an animal model of RA and was induced by intradermal administration of Mycobacterium butyricum (MB) to the base of the tail of Lewis rats. Injury of SERCA from skeletal muscles of hind paws of AA rats was analyzed on days 14, 21 and 28 after MB injection.

ATPase activity of SERCA from AA animals decreased 1.8-fold on day 21 after MB injection and was associated with a significant 6-fold increase of protein carbonyls in sarcoplasmic reticulum (SR). In contrast on day 28, an increase of SERCA ATPase activity (1.7-fold) was observed and protein carbonyl level reversed to control level. Concerning kinetic parameters, maximum reaction velocity (V_{max}) decrease and increase was observed on day 21 and day 28, respectively. Neither fragmentation nor aggregation of SERCA protein was observed in animals with AA in the time course from day 14–28.

The decrease of SERCA activity on day 21 of AA may be associated with increase of protein carbonyl formation and in fully developed AA on day 28 after MG injection, adaptive mechanisms might be responsible for increased SERCA activity.

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P6-7

Cytoskeleton dynamics modulates the regionalization of plasma membrane-bound cytochrome b5 reductase in cerebellar granule neurons in culture

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Previous studies of our laboratory have pointed out that the NADH oxidase activity and superoxide anion production of the plasma membrane of isolated rat brain terminals (synaptosomes) are several-fold higher than these activities in the plasma membrane of another mammalian cells. More recently we have shown that cytochrome b5 reductase accounts for most of the NADH oxidase activity and flavoproteins fluorescence of the plasma membrane. On the other hand, the dynamics of the actin cytoskeleton plays a major role in synaptic secretion and plasticity.

In this work we have studied the effects of drugs that alter the polymerization state of actin on the regionalization of the flavoprotein cytochrome b5 reductase in synaptic terminals in mature rat cerebellar granule neurones in culture. Depolymerization of actin microfilaments was assessed by fluorescence microscopy using phalloidin labelled with Alexa-546 and also by the decrease of fluorescence resonance energy transfer (FRET) between Alexa-488 and Alexa-546 dyes bound to myosin V antibody and to phalloidin, respectively. FRET between flavins and red fluorescent marker dyes has been monitored through green fluorescence quenching and red fluorescence intensity measurements with epifluorescence microscopy images acquired with a CCD camera.

FRET pairs were built up with anti-cytochrome b5 reductase, anti-caveolin 2 and anti- SNAP-25, labelled with secondary fluorescent antibodies tagged with Alexa-488 (donor) and Cy3 (acceptor).

The results pointed out that depolymerization of actin microfilaments elicit a large drop of the efficiency of FRET between donor/acceptor pairs tagged to caveolin 2 and cytochrome b5 reductase, and also to SNAP-25 and cytochrome b5 reductase. We conclude that actin depolymerization leads to an altered regionalization of cytochrome b5 reductase in the neuronal plasma membrane.

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