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Oral Presentation Abstracts

OP1
Molecular mechanisms of mitochondrial protection against oxidative damage in hibernators - the anti-aging effects of heterothermy

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Natural hypothermia, in addition to allowing energy saving in hostile conditions, has been associated with delayed aging and increased longevity. However, the molecular basis responsible for observed correlations between the use of daily torpor/hibernation and indices of rate of aging is hitherto unclear. Considering central role of mitochondria dysfunction in the ageing process, we examined several mechanisms that might be involved in mitochondrial protection against oxidative damage during euthermia-hypothermia (and vice versa) transition, in brown adipose tissue (BAT) of the European Ground Squirrel (*Spermophilus citellus*).

Results showed that in hibernation increased protein expression of Mn superoxide dismutase coincides with decreased content of ATP synthase and uncoupling protein 1. This suggests that BAT mitochondria during hibernation are protected from oxidative injuries by suppressed oxidative capacity, as well as by upregulated antioxidant defense. Also, the data indicate that such molecular pattern of changes is initiated already during prehibernating period. Namely, in this period we observed accumulations of hypoxia-inducible factor-1α (HIF-1α) and nuclear factor (erythroid 2-related)-like 2, which are probably responsible for suppressed oxidative metabolism, i.e. increased antioxidant capacity, respectively. Increased expression of the mitofusin 1 and detection of the megamitochondria in the prehibernating period indicate intensive mitofusion process in the BAT. This may be another mechanism of protection of mitochondrial content/function during euthermia-hypothermia transition.

The results of the study suggest mechanisms that might be associated with increased resistance of "hibernating" mitochondria to the oxidative damage. Also, the data showed that biochemistry responsible for redox balance within the cell involves integration of antioxidant response and transcription control of overall metabolism. Finally, the results go in favor of the previous reports that suggested HIF-1 as a negative modulator of aging.

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OP2
HSP90 impairment through oxidative cleavage leads to oxidized proteins accumulation

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Oxidative stress (OS), has been associated to a variety of phenomena as cancer progression, neurodegeneration, obesity and ageing itself. At the molecular level, OS leads to protein carbonylation, a non-enzymatic irreversible event and common feature of aged cells. Carbonylated proteins are dysfunctional and can accumulate in the form of insoluble protein aggregates that alter cellular functioning.

To cope with carbonylated proteins, cells employ the proteasome, the main non-lysosomal structure for carbonylated proteins turnover. However, if the degrading rate is inferior to carbonylated proteins formation rate, protein aggregates form.

In a previous study, in oxidative stress challenged cells we could verify that cytoplasmatic actin becomes heavily carbonylated and forms oxidized actin aggregates, which lead to proliferation impairment and proteasome activity diminishment, similar to senescence like states. Because under these oxidative conditions there is a proteostasis disturbance, such as oxidized proteins (especially actin) accumulation and proteasome activity impairment, Hsp90 involvement was studied. Hsp90, a molecular chaperone, assists oxidized proteins degradation and also protects the 20S proteasome from oxidative inactivation. We reasoned that the mechanism

by which protein aggregates form, is mainly due to Hsp90 lesser functionality, which we attribute to cleavage. In our study, we verified cleaved Hsp90 in protein aggregates and that its fragmentation occurred before actin insolubilization. Adding to this, when Hsp90 cleavage was prevented employing a drug, no protein aggregates were found. Furthermore, preliminary results show that under stress, cells overexpressing cleaved Hsp90 exhibited an increase, comparing to non-transfected or full Hsp90 overexpressing cells, in carbonylated proteins levels content.

We are convinced, that Hsp90 cleavage is an important event for oxidized protein accumulation and proteasome inactivation. However, further studies should follow to confirm our hypothesis.

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OP3

Impact of the transcription factor NRF2 in the modulation of autophagy on TAU and β-amyloid pathology in a combined mouse model of Alzheimer's disease

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NRF2 might orchestrate a defensive response against proteinopathies such as Alzheimer's disease (AD). Indeed, a bioinformatics analysis allowed us to identify several putative ARE-containing autophagy genes whose expression was next confirmed to be regulated by NRF2 in vitro. To explore the relevance of NRF2 in this context, we generated a new AD mouse model consisting on expression of human APPV717I and TAUP301L in the wild type background (biAT) and in Nrf2-knockout mice (triAT). NRF2-deficiency aggravated the long term potentiation defects and impaired spatial memory (Morris water maze). The levels of pro-inflammatory markers as well as astrogliosis and microgliosis were exacerbated in the biAT animals compared to age-matched triAT mice. Intracellular insoluble TAU aggregates were more evident in hippocampus of triAT mice. All mice developed Aβ plaques after 13-14 months, but Nrf2-/- mice developed fewer plaques and a larger number of intracellular APP-positive granules. Immuno-colocalization analyses showed that these aggregates were contained in autophagy vesicles (p62, NDP-52, etc), but triAT mice showed a lower colocalization of APP with these markers, suggesting alterations in the autophagy flux and secretion. These animals will be an excellent tool to study the relevance of NRF2 as a drug target in AD and other proteinopathies.

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OP4

Nitroglycerin-induced cardioprotection is endothelial nitric oxide synthase- dependent

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Purpose: We sought to evaluate the contribution of the endogenous NO pathway to the cardioprotective action of nitroglycerin (NTG).

Methods and Results: Anesthetized rabbits were subjected to 30-min myocardial ischemia (isc) and 3-h reperfusion (rep) and randomized into: Control group (no further intervention); PostC group (application of 8 cycles 30-sec isc/rep) and NTG treated group (2 μg/kg-1/min-1 IV bolus) for 65 min starting 10 min prior to rep. In additional groups, pharmacological inhibitors of NOS, nNOS, iNOS, PI3K, adenosine receptors and PKG were administrated with or without NTG. The infarcted (I) to risk (R) ratio was estimated. In a second experimental series tissue samples were collected from Control, PostC, NTG and NTG L-NAME groups in the 10th min of rep for determination of eNOS and Akt and of myocardial ROS-RNS by DHE staining, and nitrotyrosine and MDA levels evaluation. Inhibition of NOS or PI3K along with NTG eliminated the effect of NTG on %I/R ($37.9 \pm 2.0\%$, and $38.3 \pm 2.6\%$ respectively vs $23.0 \pm 3.2\%$, $p < 0.05$). Inhibition of adenosine and PKG did not affect the protection afforded ($16.0 \pm 1.8\%$ and $18.5 \pm 3.4\%$ respectively, p=NS vs NTG). Inhibition of nNOS increased NTG protection ($12.3 \pm 1.0\%$, $p < 0.05$) whilst 7-NI itself exerted protection ($27.05 \pm 1.6\%$ vs $48.05 \pm 2.0\%$ in Control, $p < 0.05$). Inhibition of iNOS did not affect the benefit of NTG on %I/R ($14.4 \pm 1.3\%$). eNOS and Akt phosphorylation was higher in PostC and NTG groups. Nitro-oxidative stress markers' levels were reduced in NtG treated animals. To further investigate the role of eNOS and mPTP function on NTG-mediated protection; wild type, eNOS KO and cyclophiline D (CypD) KO mice underwent isc-rep with NTG administration. NTG had no effect on %I/R in either eNOS KO or CypD KO. Conclusion: NTG induces protection in an eNOS and CypD dependent manner.

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OP5

Investigation of the crosstalk between proteasome function and nucleotide excision repair mechanism during ageing

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The proteasome is a fundamental enzyme complex that conducts the degradation of abnormal, damaged or unnecessary proteins of the cell and plays a fundamental role in the maintenance of cellular homeostasis. Nucleotide excision repair (NER) is one of the major DNA repair mechanisms in mammals. There are two modes of NER; repair of lesions over the entire genome (GG-NER) and repair of transcription-coupled lesions present in transcribed DNA (TC-NER). Many proteins participate in that pathway, including CSB which is essential for the recognition of the damage during TC-NER, XPC which is necessary for the recognition of the damage during GG-NER, XPA which facilitates repair complex assembly and ERCC1 which forms a complex with XPF and catalyzes the 5'-incision of the damaged DNA in both GG- and TC-NER. Impairment of proteasome as well as nucleotide excision repair has been associated with ageing.

Despite the central role of NER and proteasome-mediated proteolysis in the maintenance of cellular homeostasis, a possible crosstalk between DNA repair and proteasome function has never been investigated. We therefore sought to investigate the potential interplay between the proteasome and NER with emphasis in ageing. We have employed a group of well characterized animal models carrying defects in the NER pathway to assess the proteasome function. More specifically, we have used mouse embryonic fibroblasts and tissues derived from knock out mice for ERCC1, XPA and XPC NER factors and mutant animals for CSB NER factor and we have thoroughly investigated the proteasome status. Our results reveal a potential link between proteasome and NER.

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OP6

Sirtuins pathways and redox homeostasis: a pilot study on young and old monozygotic twins

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Sirtuins are NAD+ dependent deacetylases that play a key role in the regulation of many processes related to homeostasis, such as the regulation of metabolism, apoptosis, DNA repair and inflammatory response. Studies on humans reported a relation between the alteration of their expression and the incidence of various diseases such as metabolic, cardiovascular, cancer and neurodegenerative diseases. According with these findings the maintenance of their optimal level of activity is considered important to ensure a low risk of pathologies related with the aging process. Indeed, SIRT1 has been correlated with the redox homeostasis maintenance and with the levels of oxidative damage, such as those affecting telomeric sequences of DNA.

The aim of this study is to verify the correlation between sirtuins' expression, histone deacetylation and redox status in young and old monozygotic twins.

For this study we took advantage from blood samples of young (20-40 years old) and old (70-80 years old) monozygotic twins couple, where we analysed the expression of SIRT1 and SIRT2, Lysine acetylation proteins and Histone H4 acetylation, the protein oxidation through the detection of carbonyl groups have been analysed in PBMCs, plasma level of oxidized and reduced glutathione.

The putative increase of SIRT1 and SIRT2 levels that should also lead to an improvement in redox and inflammatory homeostasis, hormonal response to stress, and in the maintenance of telomeric DNA sequences could open an interesting view in this field. Particularly the twin model can help to better understand the interaction between genetic and epigenetic effects in the age-related mechanisms.

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OP7

Redox-directed interventions targeting skin photodamage

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Abstract not provided by speaker.

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OP8

The dark and the bright sides of the transcription factor Nrf2 in skin protection and disease (Nrf2 and epidermal barrier function)

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The epidermis is the outermost layer of the body. It serves as a barrier, which prevents excessive water loss and protects from environmental insults, such as UV light and air pollutants. However, these insults can damage the epidermis by formation of reactive oxygen species (ROS). A key player in the protection of cells against ROS damage is the transcription factor Nrf2, which regulates expression of various antioxidant enzymes, cytoprotective proteins and transporters. Therefore, Nrf2 is a promising pharmacological target for skin protection and cancer prevention.

We analyzed the consequences of pharmacological and genetic Nrf2 activation on keratinocyte protection, barrier formation and hair follicle regeneration. Nrf2 activation protected murine keratinocytes from UVB damage through enhancement of ROS detoxification. On the other hand, strong Nrf2 activation resulted in thickening and hyperkeratosis of the interfollicular epidermis and hair follicle infundibulum as well as in inflammation. As a consequence, Nrf2 transgenic mice have scaly skin and form cysts upon ageing. This resulted from upregulation of the cornified envelope proteins small proline-rich proteins (Sprr) 2d and 2h and of secretory leukocyte peptidase inhibitor (Slpi), which we identified as novel Nrf2 targets in keratinocytes. Since Sprrs are potent ROS scavengers and since Slpi has antimicrobial activities, their upregulation contributed to Nrf2's protective function. However, it also caused corneocyte fragility and impaired desquamation, and consequently alterations in the epidermal lipid barrier, inflammation and overexpression of mitogens that induced keratinocyte hyperproliferation.

Thus Nrf2 is a central regulator in protection of keratinocytes from oxidative damage but strong and prolonged Nrf2 activation leads to epidermal barrier disturbance, hyperkeratosis and cyst formation. This limits the therapeutic potential of Nrf2 activators for skin protection.

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OP9

The use of an N/TERT epidermal model for skin sensitizer identification

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Reconstructed human skin equivalents (HSEs) recapitulate most of the *in vivo* characteristics of human skin. For this reason they are widely used for research purposes, clinical applications and screening purposes. Since testing of chemical ingredients for cosmetic purposes is prohibited, there is an urgent need for *in vitro* models that can predict the safety of these constituents, including the identification of chemical allergens.

It is known that donor-to-donor variability of primary cell culture models result in inconsistencies that makes the interpretation of the data difficult. In order to minimize this donor variability, we believe that the use of an epidermal model generated with a cell line would be of added value for screening purposes. Moreover, fresh human skin needed for construction of primary human skin equivalents is often difficult to obtain and the use of a cell line can overcome this problem.

In human skin, keratinocytes are abundantly present and are key players in the initiation of allergic contact dermatitis. One of the pathways that has been shown to be induced by sensitizers is the Keap1-Nrf2-ARE pathway. In this study we compared the response of four keratinocyte-based models including (a) primary human KCs, (b) N/TERT monolayer cultures, (c) the Leiden Epidermal models (LEMs) and (d) the N/TERT epidermal models (NEMs). All keratinocyte-based models were subjected to chemical exposure of the sensitizer 2,4-dinitrochlorobenzene (DNCB) and irritant Sodium dodecyl sulphate (SDS) at nontoxic concentrations. Activation of the Keap1-Nrf2-ARE pathway was evaluated by measuring Nrf2 protein levels as well as nuclear translocation and activation of transcriptional targets of Nrf2. Results show that the Keap1-Nrf2-ARE pathway is activated by the sensitizer DNCB in monolayer keratinocytes and as well as the LEMs and NEMs and not by the irritant SDS. Collectively our data demonstrate that the N/TERT models respond similarly as primary KCs and could therefore serve as an alternative model for skin sensitizer identification, thereby overcoming the need for primary skin tissue.

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OP10

Phospholipid oxidation of the skin and its role in stress response, autophagy and senescence

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Oxidation of lipids and proteins is not only a manifestation of aged skin but also potentially causative for age-related aesthetic decline and pathologic damage. Extrinsic oxidative stress, like UV radiation promotes the accumulation of reactive lipid oxidation products.

To study which oxidation products are generated by UV other age promoting stressors and in replicative senescence of keratinocytes, fibroblasts and melanocytes, we performed lipidomic analysis of oxidized and non-oxidized phospholipids (PL) using a HPLC-tandem-MS method accompanied by transcriptomic and proteomic profiling. With that approach we could quantify several hundred oxidized lipid species in KC after acute physiological UVA stress, exposure to promoters of cellular senescence like Paraquat over replicative senescence and in aged skin.

Oxidized phospholipids activate the antioxidant response system in the various compartments of the skin, they regulate macroautophagy and they fine tune the innate immune system, and they are candidate molecules to be comprised in the senescence associated secretory phenotype of sencescent skin cells.

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OP11

The role of paraoxonase 2 (PON2) in modulating the oxidant-antioxidant balance of the peripheral blood mononuclear cells in newly diagnosed type 2 diabetic patients

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Background: Obesity is caused by chronic energy discrepancy between energy expenditure and intake. Monocytes by means of their respiratory burst (RB) are an important source of free radicals.

The aim: of the present study was to investigate the role of paraoxonase 2 (PON2) in the peripheral blood mononuclear cells (PBMNC) in newly diagnosed type 2 diabetic patients (NDT2D).

Methods: 367 NDT2D were compared regarding clinical, biochemical and oxidative stress parameters with 130 healthy subjects. All subjects were divided according to their body mass index (BMI) into overweight (BMI 25-29.9Kg/m2) and obese (BMI $\geq$ 30kg/m2). The capacity of the PBMNC to release pro-oxidants and to neutralize them was determined by measuring the RB and the antioxidant enzyme PON2. Serum levels of leptin and adiponectin were determined by ELISA. For frucosamine, glutathione peroxidase (GPx) and superoxide dismutase (SOD) photometric methods were used.

Results: PON2 activity in PBMNC was significantly lower in NDT2D ($p < 0.001$) and in obese in all groups while RB do not differ. They had decreased levels of adiponectin ($p < 0.05$), GPx ($p < 0.001$), SOD ($p < 0.001$) and increased insulin ($p < 0.05$), proinsulin ($p < 0.05$) and fructosamine ($p < 0.05$) levels. PON2 levels were inversely correlated to measures of adiposity (BMI and WC: r – 0.42, $p < 0.001$), of glucose control (fructosamine and HbA1c) and insulin resistance (HOMA-IR: r -0.21 p 0.01). In multivariate models, 39% of the PON2 variance was explained by diabetes and waist circumference.

Conclusions: The lower PON2 enzymatic activity in PBMNC is partly explained by abdominal obesity and insulin resistance. Up-regulation of monocyte PON2 activity may provide a compensatory protective

mechanism against oxidative stress damage in early (prehyperglycaemic) phase of T2D.

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OP12

Gut microbiome is rescued by dietary nitrate during dysbiosis: the impact on epithelial fence function and inflammatory pathways during antibiotic therapy

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Nitrate from green-leafy vegetables is sequentially reduced to nitrite in the oral cavity and to nitric oxide (ŸNO) in the stomach. Here, ŸNO increases mucosal blood flow, mucus thickness and prevents microbial infections. Gut microbiota is pivotal in the maintenance of local and systemic welfare as dysbiosis is associated with increased epithelial permeability and with the activation of inflammatory pathways. Herein, we investigated the impact of nitrate on gut microbiome and ensued mucosal effects.

Wistar rats were divided in 4 groups and the drinking water was supplemented with 1) antibiotic cocktail (neomycin, bacitracin, imipenem), 2) antibiotic cocktail nitrate, 3) nitrate, 4) tap water (control). Animals were weighted daily. After 7 days they were anesthetized and euthanized. Feces were collected before and after the treatment. Ceca were collected and weighted. The stomach and ascending colon were isolated and occludin, claudin-5, -15 as well as myeloperoxidase (MPO) and iNOS were analyzed. Bacterial DNA was analyzed by DGGE.

Antibiotics induced weight loss and cecamegalia ($p < 0.05$) in all animals but nitrate supplementation prevented such effects, likely through a more efficient harvesting of nutrients and preserved motility. The gastric expression of occludin and claudin-5 was decreased during dysbiosis, but both protein levels were recovered by nitrate ($p < 0.05$). Similarly, nitrate prevented MPO and iNOS overexpression under dysbiosis ($p < 0.05$) in the rat stomach. In the large intestine, nitrate increased claudin-5 expression under dysbiosis ($p < 0.01$) but had the opposite effect on occludin ($p < 0.001$). Rats treated with antibiotics and nitrate showed a microflora richness similar to control animals, which suggests that gastric generation of ŸNO recovers gut microbiota during dysbiosis. This data supports that dietary nitrate may rescue gastric epithelial integrity and gut microbiota during dysbiosis and therefore its consumption may be useful when antibiotics are prescribed to treat infections.

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OP13

Chemotherapy Resistance: The role of proteasomal degradation and heat shock response

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Proteasomal degradation is crucial to prevent the accumulation of cellular damage. The removal of the damage is a required process for healthy organisms to keep the integrity while in cancer cells this situation may induce drug resistance. Regarding chemotherapy for the cancer treatment, degradation mechanisms such as proteasomal system and autophagy have been focused recently and proteasomal inhibition in cancer cells have been shown to induce autophagy. This induced pathway may prevent the cancer cells from death or can cause autophagic cell death which is an important reason for chemotherapy resistance. There are many preclinic studies to improve the results and on the other hand heat shock proteins are accepted to be protective which may bring new approach. In our laboratory, several cancer cell lines have been tested from different aspects of proteasomal activity. HCT116 colon cancer cell line was used to test the role of HSP70 and proteasome inhibition on autophagic cell death. In this direction, heat shock treatment has been applied to the cells which is also an applied process for cancer patients. Cell viability, proteasome activity, degradation of long-lived proteins, and the expressions of HSP70, LC3, beclin1, caspase 9 and PARP have been analysed. Additionally, mouse hippocampal cell line is used to test the proteasomal activity in relation to heat shock proteins which highlighted another important point for the chemothrapy resistance with antioxidant gene expressions. Different breast cancer cell lines have also confirmed the role of proteasomal degradation in the failure of chemotherapy.

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OP14

Influence of curcumin on a pediatric hepatocellular carcinoma model in vitro and in vivo: significant reduction of alpha-fetoprotein and curcuminoid levels in mice

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Introduction: In children with hepatocellular carcinoma (pHCC) overall survival is poor. In adult HCC several antitumor properties are described in in vitro models for the use of curcumin (CUR).

Methods: Hepatoma cell lines (HuH6, HepT1, HepG2, HC-AFW1) were treated with CUR or, and Cisplatin (PDDT), cultures were either kept in the dark or exposed to blue light (PDT; 480 nm, 300W, 10 seconds), MTT-tests were performed. ROS production was measured. Reduction of cancer stem cells (CSC) was investigated with FACS analyses. Orthotopic growth of the pediatric hepatocellular carcinoma cell line HC-AWF1 in NSG mice was induced. By the increase serum alpha fetoprotein AFP >5 U/mL mice were treated with micellar CUR, PDDT, or CUR PDDT and compared to controls. Curcuminoid levels in serum and organ lysates as well as AFP serum levels were investigated.

Results: In all cell lines IC50 were significantly lower after blue light exposure than after CUR alone ($p < 0.001$). Blue light exposure resulted in significant ROS production in all cell lines. CUR alone reduced HEK-6D6 positive CSC not as effectively as CDDP alone or as curcumin with PDT. Serum CUR decreased from 3513.89 $\pm$ 2791.84 nmol/L two hours after administration to 769.74 $\pm$ 448.61 nmol/L after five hours. CUR concentrations significantly differed between organs ($p=0.000$), highest concentrations were observed in the lungs 11.33 $\pm$ 9.17 nmol/Kg, lowest in the brain 0.16 $\pm$ 0.24 nmol/Kg. The concentrations in the tumor tissue (2.57 $\pm$ 1.49 nmol/Kg) were higher than in the liver (1.77 $\pm$ 1.50 nmol/Kg). Combination therapy significantly reduced AFP concentrations compared to control group (week 3: 1.04 $\pm$ 0.67 vs. 2.73 $\pm$ 0.64, $p = 0.004$; week 4: 2.05 $\pm$ 1.01 vs. 3.35 $\pm$ 0.43, respectively, $p = 0.02$).

Conclusion: These data prove the potential of micellar curcumin as a complementary agent in pediatric oncology to enhance the overall survival of patients with pediatric liver tumors.

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OP15

Uncovering changes in the redox protein interactome of PTEN

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Phosphatase and tensin homolog (PTEN) is a redox-sensitive, dual-specificity protein phosphatase involved in regulating a number of cellular processes including metabolism, apoptosis, cell proliferation and survival. It acts as a tumor suppressor by negatively regulating the PI3K/Akt pathway. While direct evidence of a redox regulation of PTEN downstream signaling has been reported, the effect of cellular oxidative stress or direct PTEN oxidation on the PTEN interactome is still poorly defined. To investigate this, PTEN-GST fusion protein was prepared in its reduced form and an H2O2-oxidized form that was reversible by DTT treatment, and these were immobilized on a glutathione-sepharose-based support. The immobilized protein was incubated with cell lysate to capture interacting proteins. Captured proteins were eluted from the beads, analyzed by LC-MSMS and comparatively quantified using label-free methods. After subtraction of interactors that were also present in the resin and GST controls, 97 individual protein interactors were identified, including several that are novel. Fourteen interactors that varied significantly with the redox status of PTEN were identified, including thioredoxin and peroxiredoxin-1. Except for one interactor, their binding was higher for oxidized PTEN. Using western blotting, altered binding to PTEN was confirmed for 3 selected interactors (Prdx1, Trx, and Anxa2) and DDB1 was validated as a novel interactor with unaltered binding. Our results suggest that the redox status of PTEN causes a functional variation in the PTEN interactome which is important for the cellular function of PTEN. The resin capture method developed had distinct advantages in that the redox status of PTEN could be directly controlled and measured.

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OP16

Investigations on the oral bioavailability of *trans*-resveratrol and *trans*-ε-viniferin from native and micellar Vineatrol®30 grapevine-shoot extract in healthy volunteers

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Vineatrol®30 grapevine-shoot extract contains the stilbenes trans-resveratrol as well as considerable amounts of the resveratrol dimer trans-ε-viniferin. Resveratrol is a potent antioxidant, anti-inflammatory, and chemopreventive agent in vitro and in vivo, and is thought to be cardio- and neuroprotective. The bioavailability of these stilbene compounds in humans is low (ca. 1-2%). The aim of our project was therefore to enhance the oral bioavailability and thus potentially the biological activity of trans-resveratrol and trans-ε-viniferin from Vineatrol®30, and to assess its safety in humans. We performed a single-blind crossover trial with twelve healthy volunteers, with the two study arms separated by $\geq$1-week washout periods. All participants orally ingested in random order capsules with a single dose of 500 mg Vineatrol®30 as native powder or liquid micelles. Blood samples were collected before (0 h) and after (0.5, 1, 2, 4, 6, 8 and 24 h) Vineatrol®30 intake. Urine samples were collected before (0 h) and 1-6, 6-12 and 12-24 h after intervention. Liver and kidney function parameters were quantified in serum samples before as well as 4 and 24 h after intake of Vineatrol®30. Blood and urine samples are currently being analysed for total trans-resveratrol and trans-ε-viniferin by HPLC and serum samples for the activity of liver enzymes and further safety parameters. The pharmacokinetics of native and micellar stilbenes as well as the tolerability and safety of the native and micellar formulations will be presented.

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OP17

Natural antioxidants accelerate cachexia development in colon cancer

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Cancer cachexia is a multifactorial syndrome and a complex metabolic disorder, characterized by a continuous loss of muscle mass with or without depletion of adipose tissue. It manifests mainly in patients with advanced stages of colon cancer and accounts for more than 20% of mortality in total cancer patients. Muscle atrophy is one of the most relevant clinical events in cancer cachexia that negatively impact patient's quality of life. Currently, it is well established and recognized that cachexia-induced muscle atrophy is intimately linked to oxidative stress (OS), since oxidative damages were increased in the skeletal muscle of cachectic patients and were positively correlated with muscle proteolysis. Thus, supplementation with natural antioxidants could be a valuable strategy to prevent the deterioration of patient's quality of life and/or alleviate cachexia-related symptoms, like muscle atrophy. Herein, we tested the effectiveness of this strategy in a model of C26-tumor bearing mice. Five-week old Balb/c mice have received a subcutaneous injection of PBS or C26 cancer cells with or without daily supplementation with a cocktail of natural antioxidants. Venous blood and skeletal muscles were removed at 20-22 days after injection. We found that supplemented mice started to lose weight faster and died prematurely compared to no supplemented mice. Muscle atrophy occurred earlier in supplemented mice as evidenced by the decrease in fibers diameter, skeletal muscle weights and muscle endurance. These events were concomitant with an increase in systemic and muscular oxidative stress (e.g. carbonyls proteins, 4-HNE). Surprisingly, oxidative damage markers were decreased only in tumor of antioxidants-supplemented mice and were associated with a decrease in cell cycle inhibitors expression (e.g. p21), leading to tumor proliferation and progression. In line with recently published reports, our study support the evidence that antioxidants supplementation, if there is no need, could have deleterious consequences on health and well-being.

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OP18

Xanthohumol derivatives as mild mitochondrial uncouplers for treatment of metabolic syndrome

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Xanthohumol (XN) is a prenylated flavonoid found in hops, beer, and in dietary supplements. There is substantial evidence in the literature that XN improves glucose and lipid metabolism of mammalian cells in vitro, but relatively little is currently known about the potential benefits/risks of chronic exposure to XN *in vivo*. Even less is known about the underlying mechanisms of action. Using Zucker fa/fa rats and diet-induced obese C57BL/6J mice as models of obesity and metabolic syndrome, we treated animals with XN at three dose levels 6-12 weeks (n=12-16/dose group). The XN-treated animals had significantly lower plasma glucose levels and smaller body weight gain compared to the control groups, while food intake was not affected by treatment. Using an untargeted metabolomics approach, we found a dose-dependent decrease of hepatic triglyceride content and metabolic products of dysfunctional lipid metabolism (medium-chain acylcarnitines, dicarboxy fatty acids, hydroperoxy and hydroxy fatty acids). Taken together, the results indicate that XN improves β-oxidation of fatty acids. Untargeted metabolomics analyses in combination with oxygen consumption measurements of mouse skeletal C2C12 muscle cells treated with XN revealed that it increased basal oxygen consumption and induced an adaptive stress response. Collectively, these findings are consistent with XN acting as a mild mitochondrial uncoupler of oxidative phosphorylation. Because XN has undesired electrophilic and pro-estrogenic properties, we prepared two hydrogenated derivatives of XN that lack electrophilicity and pro-estrogenicity. The derivatives were tested in the diet-induced obese C57BL/6J mice and in the C2C12 cells. They showed comparable activity as the parent XN but at lower doses and concentrations. These data suggest that hydrogenated XN derivatives are promising candidates for further development to treat metabolic syndrome.

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OP19

Genetic predisposition of grapevine to polyphenols accumulation

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OP20

The evolution of vitamin C biosynthetic pathways in plants and algae

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Ascorbic acid (vitamin C) is present in high concentrations in leaves and, in some species, in fruit. In leaves it has a major role in photoprotection of photosynthesis both as scavenger of reactive oxygen species but also as a cofactor for violaxanthin de-epoxidase, an enzyme required for the synthesis of the photoprotective xanthophyll pigment zeaxanthin. Animals use an ER-localised gulonolactone oxidase (GULO) for ascorbate biosynthesis but this also generates hydrogen peroxide. The biosynthetic pathway in plants and photosynthetic protists is distinct from animals in using a mitochondrial galactonolactone dehydrogenase (GLDH) in the last step that does not generate hydrogen peroxide. Genome sequence analysis and biochemical data suggest that GULO has been lost repeatedly throughout eukaryote evolution (resulting in inability to synthesise ascorbate in primates and some other animal groups) and has been functionally replaced by GLDH in all photosynthetic eukaryotes. Green algae and plants derived from primary endosymbiosis with a cyanobacterium synthesise ascorbate by a pathway distinct from animals while photosynthetic protists with chloroplasts derived by secondary endosymbiosis appear to have a "hybrid" pathway combining an animal-like pathway with plant-like GLDH in the final step. It is proposed that unlike the animal pathway, the plant pathway and the "hybrid" pathway provide a high capacity for ascorbate biosynthesis without hydrogen peroxide formation that provides sufficient ascorbate for photoprotection of photosynthesis.

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OP21

Different ROS-scavenging properties of flavonoids determine their abilities to extend shelf life of tomato

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The shelf-life of tomato (*Solanum lycopersicum*) fruit is determined by the processes of over-ripening and susceptibility to pathogens. Post-harvest shelf life is one of the most important traits for commercially grown tomatoes. We compared the shelf life of tomato fruit that accumulate different flavonoids and found that delayed over-ripening is associated with increased total antioxidant capacity caused by the accumulation of flavonoids in the fruit. However, reduced susceptibility to *Botrytis cinerea*, a major post-harvest fungal pathogen of tomato, is conferred by specific flavonoids only. We demonstrate an association between flavonoid structure, selective scavenging ability for different free radicals and reduced susceptibility to B. *cinerea*. Our study provides mechanistic insight into how flavonoids influence shelf life of tomato, information which could be used to improve the shelf life of tomato, and potentially of other soft fruit.

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OP22

Glutathione transport into the nucleus

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The low molecular weight thiol antioxidant glutathione is present in the nucleus of plant cells, where it functions as an important redox buffer. The glutathione redox potential of the nuclei determined using redox-sensitive (ro-) green fluorescent protein was found to be the same at that of the cytosol (-295 /- 2.5 mV) in the first two layers of diving cells above the quiescent centre in roots over the first 48h period after germination. Using 3mM hydroxyurea to synchronise the cell cycle, together with a CYCB1:1GUS marker for the G2 phase of the cell cycle, we established that the cells accumulated at G2 between 16 and 18h after the start of incubation with the inhibitor. At this point the glutathione redox potential was the same in the nuclei and the cytosol, which were both highly reduced. However, the redox potential of the nuclei can become oxidised relative to the cytosol under different conditions. This talk will focus on the glutathione redox potential of the nuclei and on how it might be controlled.

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OP23

Mitochondrial ROS formation in diabetic cardiomyopathy

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OP24

Centenarians overexpress BCL-xL, which confers them a protection against apoptosis, oxidative stress and immunosenescence

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Centenarians not only have an extraordinary longevity, but also show a compression of morbidity. They preserve the capacity of maintaining homeostasis, and this is the reason for them to reach such a long life. We studied their mRNA expression profile and identified 1721 mRNAs differentially expressed by centenarians when compared with septuagenarians and young people. A sub-network analysis showed six common genes: interferon, T-cell receptor, tumor necrosis factor, SP1 transcription factor, transforming growth factor and IL-32.These six centenarian-specific genes are related to Bcl-xL, Fas, and Fas ligand all of them involved in the control of apoptosis. RT-PCR analysis confirmed that centenarians up-regulate Bcl-xL. This is in keeping with the fact that they have lower plasma cytochrome C levels than septuagenarians. Bcl-xL is a mitochondrial protein involved not only in the control of apoptosis, but also in mitochondrial damage protection, control of mitochondrial respiration and immune response. We found that mitochondrial membrane potential ($\Delta\Psi m$) as assessed by JC-1 cytometry was significantly higher in PBMCs obtained from centenarians versus septuagenarians as well as young people, suggesting that the functional state of mitochondria was maintained. Moreover, centenarians showed lower malondyaldehyde and protein carbonyl levels than septuagenians. When analyzing the immune system, we found that leukocyte chemotaxis and NK cell activity were significantly impaired in septuagenarians compared with young people whereas in centenarians these indicators of immunosenescence were similar to the picture noted in young people. In conclusion, centenarians, who constitute an example of successful ageing, overexpress Bcl-xL, which confers them a protection against apoptosis, oxidative stress and immunosenescence.

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OP25

The pivotal role of proteasome enhancement in ageing, longevity and proteostasis across species

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Ageing is a natural biological process, determined by both genetic and environmental/stochastic factors. It is characterized by a gradual decline of physiological function and the eventual failure of organismal homeostasis. The phenomenon is represented in vitro by replicative senescence. The proteasome is one of the major cellular proteolytic systems. It is responsible for the degradation of normal proteins as well as of damaged proteins (abnormal, misfolded and otherwise modified proteins) that tend to accumulate during ageing. Impaired proteasome function has been tightly correlated with ageing both *in vivo* and in vitro. We have shown that proteasome is down-regulated during replicative senescence of human fibroblasts. Its partial inhibition triggers a premature senescence phenotype that is p53-dependent. By contrast, its genetic activation through overexpression of proteasome subunits confers lifespan extension and maintenance of youthful morphological features for longer. We have also identified natural compounds that activate the transcription factor Nrf2, a key molecule involved in cellular protection against chemically-induced oxidative stress thus promoting proteasome activation. Proteasome induction results in lifespan extension and delayed establishment of senescence morphology, consistent with the effects of proteasomal genetic activation. More recently, we have revealed the results of proteasome activation on the lifespan of the *Caenorhabditis elegans* model. Our initial results confirm the beneficial effects of the proteasome genetic activation on the lifespan of the nematode. We also provide evidence that proteasome activation might be a potential strategy to minimize protein homeostasis deficiencies underlying aggregation-related diseases such as Alzheimer's or Huntington's. We are currently screening for compounds with proteasome activating properties that could be used in preventive or therapeutic approaches against Alzheimer's disease. In conclusion, our results show that there is a dynamic interconnection between the proteasome, the proteostatic mechanisms and the progression of ageing and age-related diseases.

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OP26

Oxidative Stress and the Decline of Adaptive Homeostasis in Aging

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Oxidative stress causes major perturbations of homeostasis. The classical view of homeostasis considers a single range of biological capacities, extending above and below a mean value. Thus, we may consider mean blood pressure for a 'normal' 20 year old female to be approximately 110/70, with a high end ranging up to 130/80 and a low end ranging down to 100/60. Over the past two decades, however, studies from this laboratory (and several others) have demonstrated that in responding to oxidative stress cells, simple organisms, and even mammals, can temporarily expand the homeostatic range by undergoing transient adaptation. Such adaptive responses to oxidative stress depend on altered gene expression and are orchestrated by signal transduction pathways, such as the Nrf2-Keap1 system. Such adaptive pathways allow cells and organisms to cope with transient changes in (internal or external) environments, and many forms of stress, including oxidative stress. Thus, in addition to the 'normal' range of homeostatic capabilities, there is an additional range of adaptive capacity that I propose should be called, 'Adaptive Homeostasis.' Importantly, several oxidative stress studies from this laboratory now show that Adaptive Homeostasis declines with age in cells, worms, flies, and rodents; in other words, a decline in Adaptive Homeostasis appears to be a 'normal' age-dependent phenomenon. Declining Adaptive Homeostatic capacities may make older organisms (and people?) more susceptible to multiple stresses, and to disease. On the other hand, declining Adaptive Homeostasis may be protective against cancer. While the full explanation for age-dependent declining Adaptive Homeostasis is still under study, our research indicates that diminishing Nrf2 responsiveness, and increasing levels of (competitive?) Nrf1, Bach1, and c-Myc may all play important roles.

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OP27

Resveratrol and Foxo1 modulate glucose metabolism

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The liver plays a central role in energy homeostasis and metabolism. Resveratrol, a potent antioxidant derived from grapes, exerts important effects on glucose and lipid metabolism, yet detailed mechanisms mediating these effects remain unknown. Among the direct redox modulating activities resveratrol acts on the activation of sirtuin 1 (SIRT1) and the forkhead transcription factor-1. This cascade appears to be important in liver and recent findings about the mechanisms by which resveratrol contributes to this regulation will be discussed.

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OP28

Laminin, a key component of tissue extracellular matrix, is a major target for peroxynitrous acid

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Basement membranes (BM) are specialized extracellular matrices underlying endothelial cells in the artery wall. Laminin, the most abundant BM glycoprotein, is a structural and biologically active component. Peroxynitrous acid (ONOOH), a potent oxidizing and nitrating agent, is formed in vivo at sites of inflammation from superoxide and nitric oxide radicals. Considerable data supports ONOOH formation in human atherosclerotic

lesions, and an involvement of this oxidant in atherosclerosis development and lesion rupture. These effects may be mediated, at least in part, via extracellular matrix damage.

In this study we demonstrate co-localization of 3-nitrotyrosine (a product of tyrosine damage by ONOOH) and laminin in human atherosclerotic lesions. ONOOH-induced damage to laminin was characterized with purified murine laminin-111, and murine BM extracts containing multiple matrix components. Exposure of laminin-111 to ONOOH resulted in dose-dependent loss of protein tyrosine and tryptophan residues, and formation of 3-nitrotyrosine, 6-nitrotryptophan and the cross-linked material di-tyrosine, as detected by amino acid analysis and Western blotting. This damage was modulated by bicarbonate, a known modifier of ONOOH reactions. These changes were accompanied by protein aggregation and fragmentation as detected by SDS-PAGE. Significant damage was detected with equimolar or greater concentrations of ONOOH. Endothelial cell adhesion to isolated laminin-111 was significantly decreased (~25%) compared to controls, on exposure to 10 μM or higher levels of ONOOH.

These data indicate that laminin is oxidized by equimolar or greater concentrations of ONOOH, with this resulting in structural and functional changes. These modifications, and resulting compromised cell-matrix interactions, may contribute to endothelial cell dysfunction, a weakening of the structure of atherosclerotic lesions, and an increased propensity to rupture.

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OP29

Redox regulation of FoxO activity: impact on micronutrient homeostasis

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OP30

More damage, longer life in *C. elegans*?

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Since its inception almost 60 years ago, the free radical theory of ageing (FRTA) has become the foremost damage accumulation theory of ageing. The FRTA posits that ageing is caused, at least in part, by mitochondrial reactive oxygen species (ROS) and the resulting oxidative damage. However, antioxidant intervention studies have often failed to extend lifespan in model organisms such as *C. elegans* and have also generally failed to show consistent beneficial effects in mammals and humans. In addition to the often questionable in vivo antioxidant efficacy of such compounds, one explanation for these largely disappointing results of antioxidants in modulating lifespan and healthspan may be a complex role of homeodynamic systems during perturbations. Both ROS production and ROS detoxification are part of a complex regulatory network affecting the expression of damage-repair and stress-response genes, but also directly impacting energy production, metabolism and life-history traits. Such complexity often leads to surprising (even counter intuitive) outcomes. This complexity also provides an exciting new area of development for interventions against age-dependent functional decline. Hormesis refers to the phenomenon whereby a stimulus or challenge that is detrimental or lethal at high dose exhibits beneficial effects at low dose. Hormetic interventions are one way of leveraging endogenous homeodynamic systems and responses instead of trying to control or overwrite them. Hormesis can even result in lifespan and healthspan benefits following exposure to low levels of stressors. However, this poses new questions. For example, does the success of hormetic interventions challenge damage accumulation theories or does, as often suggested, activation of endogenous stress response and damage repair pathways following hormetic insult result in a net decrease in damage? I will attempt to illuminate some of these questions based on data from our *C. elegans* mtDNA damage assay.

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OP31

Role of molecular chaperones in protein folding in health and disease

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OP32

The challenge of maintaining redox balance during protein folding

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OP33

Protein misfolding and ER stress in malignancy

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OP34

Endoplasmic reticulum stress during development and progression of diabetes mellitus

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Abstract not provided by speaker

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OP35

Mitochondrial oxidative stress in skeletal muscle and cardiac aging

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Mitochondrial dysfunction plays an important role in many chronic diseases, including aging. However the role of oxidative stress in this dysfunction remains controversial. We tested whether mitochondrial oxidative stress underlies not only mitochondrial deficits, but also declines in performance of skeletal and cardiac muscle in aged mice. Direct manipulation of mitochondrial H_2O_2 (mtH_2O_2) production by lifelong overexpression of mitochondrial targeted catalase reduced mitochondrial deficits in aged skeletal muscle and protected against age-related declines in cardiac function. To test whether manipulating mitochondrial oxidative stress late in life could reverse deficits in aged skeletal muscle and heart we used a novel mitochondrial targeted peptide, SS-31, that interacts with cardiolipin on the inner mitochondrial membrane. SS-31 reduces mtH_2O_2 production in dysfunctional aged mitochondria and improved glutathione redox status while not affecting young healthy mitochondria. Acute (1 hr) and 8 week treatment with SS-31 had similar effects on mitochondrial function and skeletal muscle performance. Both treatments reversed age-related declines in in vivo maximal mitochondrial ATP production and efficiency of oxidative phosphorylation (P/O) while increasing muscle fatigue resistance. Treatment for 8 weeks also reversed cardiac dysfunction, especially diastolic dysfunction. These improvements in skeletal muscle and cardiac function translated to improved treadmill endurance capacity in the aged mice. These results reveal a dynamic relationship between mitochondrial deficits and the redox environment of the cell that affects function in these tissues. To examine the mechanisms underlying this interaction we are examining the effect of manipulating mitochondrial oxidant production on the thiol redox proteome. Aging is associated with increased S-glutathionylation of the thiol proteome in aged skeletal muscle. Treatment with SS-31 partially reverses many of these age-related changes, including proteins involved in E-C coupling, muscle contraction, and protein quality control. These results demonstrate that mitochondrial oxidant production alters redox dependent post-translational modifications throughout the muscle thereby providing a potential mechanism linking mitochondrial oxidative stress to loss of muscle function with age. Furthermore, we demonstrate that reversing this mitochondrial oxidative stress can rapidly improve function highlighting the interaction between mitochondria and cellular redox environment as an attractive target to improve health in the elderly.

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OP36

Redox control of FOXO through disulfide-dependent heterodimerization

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The forkheadbox O (FOXO) family of transcription factors regulates a variety of cellular programs, including cell cycle arrest, reactive oxygen species (ROS) scavenging, and apoptosis, and are of key importance in the decision over cell fate. In animal model systems it has been shown that FOXO is involved in the regulation of long lifespan. FOXO activity is tightly controlled by the insulin signaling pathway and by a multitude of ROS-induced posttranslational modifications. Over the past years my lab has discovered that cysteines in FOXO transcription factors become oxidized in response to redox signaling and that this leads to the formation of highly specific intermolecular disulfide formation with a number of regulators of FOXO activity. We have shown that ROS-induced Lysine acetylation on FOXO depends on the formation of an intermolecular disulfide with the p300 or CBP acetyltransferases. Furthermore, nuclear shuttling of FOXO is triggered by a shift in the cellular redox state towards more oxidizing conditions and this depends on intermolecular disulfide formation between FOXO and nuclear import receptors. By comparing cysteines present in human FOXO3 and FOXO4 isoforms by a quantitative proteomics approach we have identified paralog-specific redox signaling. We speculate that intermolecular disulfide formation could be a more common phenomenon in redox signaling and that it could serve to stabilize protein-protein interactions that otherwise would be of low affinity. By this means specific signaling cascades can be reversibly activated for as long as the cellular redox state remains more oxidizing.

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OP37

Redox proteomic approaches to understanding age-related changes in redox signalling

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Redox modifications of regulatory amino acids offers a dynamic and versatile means to rapidly alter the activity or structure of proteins in response to external and internal stimuli. These stimuli often refer to endogenously generated reactive oxygen or reactive nitrogen species (ROS/RNS), which play a role in normal cellular functioning including glycolysis, oxidative phosphorylation, biogenesis, autophagy, transcription, translation. The activities of redox sensitive proteins are often determined by the redox state of specific cysteine (Cys) residues located in catalytic sites or involved in co-factor binding. Disruption or dysregulation of many of these pathways by excessive or diminished levels of ROS/RNS signalling has been suggested to play a crucial role in ageing and many metabolic diseases. Skeletal muscle provides an ideal model to study age related changes in redox signalling, as muscle generates ROS/RNS during contractions and ageing is associated with a loss of skeletal muscle mass and function, with particular muscle types more susceptible than others. In this presentation, I will describe techniques that we have applied to study the effects of age on the redox proteome of a number of different skeletal muscles (Soleus,

Gastrocnemius, and Vastus lateralis). Combining global label free proteomics and differential Cys labelling allowed the relative quantification of the redox state of specific Cys residues in the context of the protein's abundance. Once redox sensitive Cys residues have been identified a targeted redox proteomic approach using parallel reaction monitoring, can accurately quantify the reversible oxidative state of individual Cys residues. Results indicate that muscles from old mice have a reduced redox flexibility and that muscle types respond differently to the effects of ageing. Differences we observed in the redox proteome are a reflection of the age related changes in redox signalling within muscle types.

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OP38

The cell signalling disruption theory of ageing: importance in Alzheimer's disease and sarcopenia

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Abstract not provided by speaker

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OP39

How does vitamin E intake correlate with concentrations of tocopherols and their metabolites? Genetic variants involved in interindividual variability in vitamin E bioavailability

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Purpose: Vitamin E (VE) is essential for human health and may play a role in the aetiology of cardiovascular diseases. However, its absorption efficiency is widely variable. This high interindividual variability is assumed to be due to both dietary and genetic factors. Several polymorphisms have been identified but existing studies have so far focused on single SNPs that only explained a minor fraction of the variability of such a complex phenotype. This study thus aimed to identify a combination of SNPs that could explain a significant part of the variability in VE bioavailability.

Methods: Thirty-eight healthy male volunteers consumed a meal containing a VE supplement (134 mg a-tocopheryl acetate). Volunteers were genotyped using whole-genome microarrays. a-tocopherol (TOL) concentration was measured in plasma chylomicrons (CM) isolated at regular time intervals over 8 h postprandially. The association of SNPs in or near candidate genes (59 genes representing 4475 SNPs) with the postprandial CM TOL response was assessed by partial least squares regression.

Results: The postprandial CM TOL response to the meal was highly variable (CV of 81%). Data obtained allowed us to generate a validated significant model (P=1.8.10-8) that included 28 SNPs in 11 genes (ABCA1, ABCG1, APOB, BET1, IRS1, LIPC, NAT2, PNLIP, SLC10A2, SREBF2, ZNF664). This model explained 82% of the variance and allowed us to accurately predict a subject's VE absorption (Spearman Rho=85% between the measured and predicted VE response). For all these SNPs, univariate analysis showed that subjects who bore different genotypes exhibited a significantly different VE absorption.

Conclusions: The ability to respond to VE appears to be at least in part genetically determined. A combination of SNPs in 11 genes related to both VE and CM metabolism can explain a large part of the variability in VE absorption in males. These results could allow a better design of future clinical trials aiming at studying the association of VE supplementation with the prevention of cardiovascular diseases.

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OP40

Oxygen sensing by the Na,K-ATPase: the cellular mechanism unraveled

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Reduction in ATP generation under conditions of limited oxygen supply triggers acute decrease in ATP consumption by turning off the Na,K-pump. This adaptive response is effective when followed by "channel arrest", but may be lethal when not associated with reduction in passive Na and K permeability. Oxygen sensing by the Na,K-ATPase is mediated by way of reversible thiol modifications. We have identified the four regulatory cysteine residues within the ATP binding site of the catalytic α subunit that, when S-glutathionylated, prevent binding of ATP to the enzyme. S-glutathionylation may only occur under conditions of mild ATP depletion associated with GSSG accumulation in the enzyme proximity. Identification of the residues was performed by mass spectrometry on purified Na,K-ATPase which was completely inactivated by treatment with GSSG. In silico modelling and titration experiments using isothermal scanning calorimetry confirmed competition of GSSG and ATP for the ATP binding domain. Finally, a set of HEK293 cell lines transfected with murine α1 subunit protein with point mutations of the candidate cysteines for alanines was produced and the oxygen-insensitivity of mutant murine Na,K-ATPase was proven. The lack of oxygen-sensitive made the transfected host cells less hypoxia-tolerant. We furthermore advanced in identification of the source of hypoxia-induced free radical production triggering GSSG production which is required for making the Na,K-ATPase oxygen-sensitive. A burst of superoxide anion production originating from the mitochondria was observed in several cell types including cerebellar granule cells during the first 10-15 minutes of hypoxia. The latter could be inhibited by silencing or pharmacological inhibition of the mitochondrial Na/Ca exchanger by CGP-37157. Administration of CGP-37157 preserved the Na,K-ATPase function in hypoxic cerebellar granule cells, decreased the intracellular GSSG levels and reduced S-glutathionylation of the α subunit.

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OP41

Methylation at positon 7' but not 5' and the isoprenoid chain are required for vitamin E to induce P-glycoprotein protein expression and activity in the human colon carcinoma cell line LS180

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The eight congeners of vitamin E differ in their methylation pattern and either possess an alkyl (tocopherols, T) or an isoprenoid (tocotrienols, T3) side chain. The different chemical structures influence the biological activity of the vitamin, like the binding to the nuclear pregnane X receptor (PXR). P-glycoprotein (P-gp), a PXR target gene, is an efflux transporter expressed at various barriers in the body including small intestine, bile ducts and the blood-brain-barrier. We therefore investigated the impact of methylation of the chromanol ring (position 5' and 7' = α; position 7' = γ) and the structure of the side chain on the expression and activity of P-gp. LS180 cells, which have a low basal P-gp expression, are a good model to study the induction of P-gp protein expression and activity.

LS180 cells were either treated with the positive control rifampicin (25 μmol/L), a known P-gp inducer, the appropriate solvent control, or the respective vitamin E congener for 48 hours. αT was investigated in the range of 10–100 μmol/L, γT 10-50 μmol/L, αT3 5-50 μmol/L and γT 5-25 μmol/L due to differences in cell toxicity. Protein expression was determined by Western blot analysis and P-gp activity via the efflux of the fluorescent substrate rhodamine 123 in the presence or absence of the P-gp specific inhibitor elacridar.

αT, γT and αT3 neither influenced the protein expression nor the activity of P-gp. γT3 induced P-glycoprotein activity and protein expression which is in accordance with the literature. To induce P-glycoprotein, both methylation at position 7' but not 5' of the chromanol ring as well as an isoprenoid side chain are required as none of the two structural features individually showed an effect.

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OP42

Precision control of dissolved oxygen in mammalian cell culture media impacts on in situ volatile generation and promotes improved mesenchymal stem cell yield accompanied by reduced transcriptional variability

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Mammalian cell culture is reliant on culture media to support in vitro growth and proliferation. Culture media contains salts, vitamins, and minerals at precise levels where subtle variations result in novel media formulations. Oxygen is a bioactive molecule which impacts strongly on intracellular signalling, mitochondrial function, and metabolic pathway choices. We sought to determine the levels of dissolved oxygen in cell culture media and to design systems to support precision control of those levels.

Dissolved oxygen levels in culture media displayed substantial variability ranging from 6 – 11 mg/L (~10% O_2 - 21% O_2) in all medias tested (DMEM, MEM, IMDM). In addition to this variability it was apparent that deoxygenation of cell culture media required incubation in hypoxic (1% O2) environments for >24 hours. The deoxygenation process could be accelerated to 3 hours via a combination of cooling (4 °C), agitation, and provision of a hypoxic headspace (1% O_2, 5% CO_2). Deoxygenation rates were volume dependent but were significantly more rapid than passive approaches. Selected Ion Flow Tube (SIFT)- MS) was then used to measure changes in volatile organic compound (VOCs) in media incubated overnight in either 21% O_2, 2% O_2, and 2% O_2 following rapid deoxygenation. Reduced variation was apparent in pre-deoxygenated media where reduced standard deviations revealed significant differences to base media in 40% of VOCs tested.

Finally we sought to determine the impact of pre-deoxygenated media on clonogenicity and transcriptome of MSCs isolated from fresh marrow. Precision deoxygenation (2% O_2) of culture media associated significantly with increased yields of MSCs. Microarray analysis of MSCs isolated in all conditions further demonstrated reduced variability in global transcriptome where differentially expressed transcripts associated strongly with defined pathways.

In summary precision control of dissolved O_2 levels reduces media-based chemical reaction variability and promotes enhanced cell isolation from primary materials.

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OP43

Nitrite supports neurovascular coupling via redox cycling with ascorbate

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Upon glutamatergic stimulation in hippocampus, neuronal NOS produces nitric oxide (NO) in a transitory manner. NO may diffuse a few hundreds of microns, mediating neurovascular coupling in rats and mice (Lourenço FRBM, 2015). In addition NO may be produced in vivo via one-electron reduction of nitrite. Given, the abundance of ascorbate in neurons (mM range), the reduction of nitrite to NO by ascorbate is both thermodynamically and kinetically favored in the brain. Thus, further considering the nitrate-nitrite-NO pathway, we aimed at quantitatively assessing the redox interaction of nitrite and ascorbate in hippocampus in vivo and whether this underlined neurovascular coupling.

Using microarrays for stereotaxic insertion in the brain of living rodents, consisting of microinjection pipettes, laser Doppler blood flow probes and microelectrodes selective for NO and ascorbate, we have followed the dynamics of the neurometabolites in the hippocampus of anesthetized animals.

Glutamate stimulation evoked transient extracellular ascorbate from a background in a way that the respective dynamics correlate quantitatively with that of NO in terms of maximum flux, signal area and duration. Under these conditions, locally applied or i.p. injection of nitrite augments NO signals and CBF. Nitrite-driven NO formation is accompanied by a decrease of extracellular ascorbate. Nitrite effects on NO and CBF increases are more pronounced under metabolic acidosis (pH 6.5) and are abolished in the presence of ascorbate oxidase.

Overall, these results support a redox cycle of nitrite and ascorbate in the hippocampus, which is translated into NO and CBF transitory increases. This mechanism, supporting NVC, may be of particular relevance under hypoxia, condition in which nNOS is impaired because O2 is a substrate for the enzyme. Moreover, the results support the possibility of a dietary nitrate/nitrite modulation of NVC.

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OP44

Systems biology of oxidative stress: first insights in lipid oxidation and protein modification cross-talks

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Recent research indicates that oxidative stress (OS) affects all levels of cellular organization and induce perturbation in gene expression, epigenetic regulation, mRNA and protein levels, and cellular metabolism. Oxidative modifications of proteins and lipids represent another level of complexity in cellular regulation supported by numerous studies demonstrating how these modifications regulate gene expression and cellular signaling pathways. However, it is almost impossible to specify the input and (patho)physiological consequences of OS using data from a single modification level. Nowadays, it is clear that only a holistic approach, as suggested by systems biology, will provide an integrated view on OS-related cellular regulation and pathologies by considering all known OS determinants, identifying new ones, and establishing functional links among them.

As a first effort to study OS via redox systems biology, we applied a multi-omics approach to characterize the impact of OS on cardiomyocytes (CM) using a dynamic model of nitrosative stress. Reactive carbonylated lipids, hydroxylated and truncated phospholipids, nitrated fatty acids, and oxysterols were quantified relative to a control in CM lipid extracts after 15, 30, 70 min, and 16 h of OS. Simultaneously, the protein fraction was analyzed for a wide range of post-translational modifications, such as phosphorylation, carbonylation, cysteine oxidation, and nitrosylation. Omics data were combined and supported by biochemical and microscopy studies on oxidation dynamics, spatial distribution, and functional effects. Thus, the combination of lipidomics, data-driven proteomics, and systems biology integration allowed the identification of more than 200 proteins modified by reactive lipid peroxidation products of which many were involved in calcium signaling pathways, regulation of actin cytoskeleton, focal adhesion, and phosphatidylinositol signaling system. Biochemical and microscopy studies further confirmed OS-derived impairment of Ca-signaling and cytoskeletal protein distribution.

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OP45

Sulphur and selenium species as targets and protective species in inflammatory diseases
Why speed matters: understanding key mechanisms of protein modification

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Activated leukocytes generate multiple oxidants to kill invading pathogens including $O_2^{\bullet}$, H_2O_2, $NO^{\bullet}$, peroxynitrous acid (ONOOH) and hypohalous acids (HOCl, HOBr, HOSCN) via the activity of NADPH oxidases, nitric oxide synthases (e.g. iNOS) and myeloperoxidase (MPO). Inappropriate generation can however result in tissue damage, with this associated with multiple human inflammatory pathologies (e.g. cardiovascular disease, some cancers, rheumatoid arthritis, asthma, cystic fibrosis, neurodegenerative conditions).

Sulphur residues in peptides and proteins are key targets. Competition kinetic methods have yielded rate constants, k, for reaction of HOCl with Cys and GSH of 3.6 x 10^8 and 1.2x10^8 $M^{-1}s^{-1}$, respectively. These values are ~10-fold higher than reported previously, and are some of the fastest reactions of non-radical oxidants, emphasizing the key role of thiols in protection. N-Ac-methionine also reacts very rapidly (k 1.7x10^8 $M^{-1}s^{-1}$). ONOOH reacts rapidly with Cys and Met with k ~ 2x10^3 and 2x10^2 $M^{-1}s^{-1}$ respectively.

Selenium compounds should react faster than the sulphur analogues. Seleno-compounds, including novel seleno sugars react with HOCl with k ~10^8 $M^{-1}s^{-1}$ and ~ 100-fold faster than the sulphur analogues. A similar rate enhancement is seen with ONOOH, with k for ONOOH and selenocysteine ~2x10^5 $M^{-1}s^{-1}$. The selenoxides formed on oxidation of these species, can be readily reduced by thiols (e.g. GSH) and some enzyme systems, making these catalytic protective agents.

The seleno-sugars decrease oxidant-mediated damage to isolated proteins and human plasma at low concentrations. Examination of these seleno sugars in an animal model of wound healing has shown that topical application markedly enhances wound closure in both normal and diabetic animals.

These studies demonstrate that novel selenium-containing molecules may be potent modulators of damage at sites of acute and chronic inflammation, and be beneficial in multiple human pathologies.

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OP46

Superoxide-mediated post-translational modification of tyrosine residues

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Superoxide is a byproduct of aerobic respiration. Its in vivo formation is associated with many inflammatory diseases, oxidative stress and aging. Regardless of the rapid spontaneous disproportion of superoxide to give hydrogen peroxide and oxygen, aerobic organisms cannot survive without further catalyzing the rate of this reaction by superoxide dismutase. Therefore, chemical reactions, which contribute to the detrimental effects of superoxide, have to be fast enough to compete with the catalytic dismutation process. The reaction of superoxide with tyrosyl radicals fulfills this criterion and we have shown in a series of publications (see below) that radical addition reactions to tyrosine residues may represent a possible mechanism for superoxide toxicity. Tyr residues are major sites of radical attack on proteins and they also form at active sites of pivotal enzymes (such as cyclo-oxygenase or ribonucleotide reductase) during turnover.

This presentation will focus on the mechanisms of the reaction of superoxide with tyrosyl radicals in peptides and proteins in a wide range of model systems. We will present that addition of superoxide to tyrosyl radicals result in the formation of novel Tyr-hydroperoxide species, which can further react with 1) Met residues via intramolecular oxygen transfer to form Met sulfoxide or 2) Cys residues to generate the corresponding disulfide species. These reactions produce a modified bicyclic Tyr monoxide species, which favorably undergo a Michael addition reaction with thiols (such as glutathione) to form novel Tyr-Cys cross-linked adducts.

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OP47

Radical addition, a forgotten path to post-translational modification

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Radical addition is a long known synthesis strategy in preparative chemistry but largely ignored in the biochemical community. Therefore, radical induced protein crosslinks are usually perceived as caused by radical recombination. Such a mechanism is theoretically possible but does require that either (1) two radicals meet during their very limited lifetime or (2) that they are produced simultaneously side by side. With the low production rates of radicals in a biochemical setting, the probability of (1) is very low if the radicals are not extraordinarily long-lived. Mechanism (2) would need a high amount of energy: Production of radicals is energy-demanding and *simultaneous* production of *two* radicals much more so.

Four years ago we found that the labelling step in the production of a PET-Tracer, *i.e.* a radioactively labelled protein drug, causes formation of high amounts of impurities. Their chemical analysis let us speculate, that the underlying reaction mechanism was radical addition. This prompted the investigation of our hypothesis for the case of aromatic amino acids. Supporting data from our subsquent work will be shown. We argue that several known post-translational modifications, for example protein crosslinks, are likely to be formed *via* radical addition.

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OP48

The good and bad sides of thiols as one-electron donors in free radical processes

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Redox reactions of thiols play important roles in many biologic processes including signaling, enzymatic reactions and the defense against reactive oxygen species. These redox reactions proceed via one-electron or two-electron oxidation and reduction processes. Especially the one-electron redox reactions lead to free radical species such as thiyl radicals, and a potential role for thiyl radicals in signaling, the formation of S-nitroso-thiols, and higher oxidation products of thiols has been discussed. However, thiyl radicals are generally quite reactive towards many classes of biomolecules, including polyunsaturated fatty acids, carbohydrates, and proteins. This presentation will focus on deleterious reactions of thiyl radicals, particularly hydrogen transfer reactions within peptides and proteins, which can lead to the generation of a manifold of different products, including electrophiles, D-amino acids, cross-links and fragmentation products. Examples will be provided for glutathione (GSH), GAPDH, SERCA and a number of antibodies. These reactions proceed with some sequence specificity. Rate constants for these hydrogen transfer processes have been derived by NMR spectroscopy, mass spectrometry, and pulse radiolysis, indicating that they can compete against the reaction of thiyl radicals with oxygen, thiols and ascorbate under specific biologic conditions.

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