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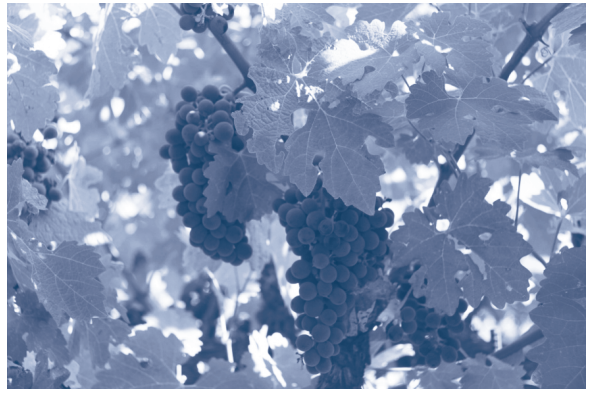
Oxidants and Antioxidants in Biology

Nutrition and Redox Biology
in
Development And Health

Abstracts



7-10 May 2014
University of California Davis Conference Center
Davis, California

Oxidants and Antioxidants in Biology
Nutrition and Redox Biology in Development and Health

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University of California Davis Conference Center

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SESSION I
THE FIRST 1000 DAYS:
FROM METABOLIC SIGNAL TO SOCIETAL IMPACT

The first 1000 days: from metabolic signal to societal impact

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From conception through age two, a period roughly 1,000 days in length, a plethora of developmental events occur in concert to prepare the infant to face the world. Adverse environmental conditions during this time period, such as nutrient deficiencies or excesses, exposures to infection or inflammation, or other metabolic stressors effect the fetus and infant in ways that can be measured in immediate physiologic or functional insults. Moreover, they may also result in alterations in metabolism, morphology, or growth patterns that have impacts well beyond the infancy period into adulthood. In our changing global world, malnutrition, encompassing micronutrient deficiencies, wasting, child stunting, overweight, and obesity, crosses borders and coexists in complicated and overlapping distributions within and among individuals, households, and populations. There have been enormous breakthroughs in our understanding of the basic biology of human development, the sensitive regulation of human growth, the consequences of exposure to chronic inflammation, and the degree to which nutrition can influence these processes. Yet, there is clearly still a need for a greater scientific understanding of potential metabolic targets for nutrition intervention and the degree to which these interventions may impact health of individuals and societies at large.

Gut microbiota: Nutritionally and Environmentally Sensitive Ecosystems that Influence the Host Biology

Mark Manary

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The microbiome is the ecological community of commensal, symbiotic, and pathogenic microorganisms within our bodies. Housed primarily in the small intestine, it contains over 100 trillion microorganisms, 100-fold more genes than the human genome. The microbiome facilitates the absorption of food and plays a role in homeostasis, micronutrient synthesis, detoxification and immune function. Each microbiome has a "finger-print" like specificity to the host, made up of a unique profile of bacteria, archaea, eukaryotes and viruses which develops in the first years of life; by age three the microbiome has reached an adult-like state. The species present in adult microbiomes are Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteroa, Verrucomicrobia, and non-bacterial Methanogenic archaea, eukaryotes and viruses. The ratios and expression of these organisms is host specific, dependent on diet, environment and exposure.

In-utero and at birth the microbiome is sterile and all host bowels are basically identical. The first factors affecting the infant microbiome are gestational age, delivery mode, the hospital and clinic environment and breast milk intakes. Breast milk is the child's primary source of nutrition for the first six months and acts as the primary source of an infant's adaptive immunity to a multitude of pathogens and infections. As the child grows and is introduced to complementary foods, different external environments and infections the microbiome adapts to help the child to absorb nutrients.

In findings observed in a comparative metagenomic study of the gut microbiomes of 531 healthy infants, children, and adults living in the USA, Venezuela, and Malawi, the representation of genes related to micro- and macronutrient biosynthesis and metabolism changed during development and based on environment. Their was significant geographic variation, children in the US had

microbiome expression profiles much different than those from the Malawi and children fed diets heavy in protein exhibited different bacterial colonies and pathogens than those fed diets heavy in carbohydrates. Similar results were observed in a study conducted comparing the microbiomes of children in Burkina Faso to those in Europe.

Emerging evidence suggests that the microbiome is not simply an environmental response to the diet, but exerts health effects by modulating the gut's absorptive capacity and immunological response to microbes. It is not simply the species of microorganisms present that constitute the effect of the microbiome in the first years of life, but the expression pattern of this microbiome collectively, called the metabolome.

Understanding the developing microbiome in the first three years of life is crucial to furthering the health and development of all children, particularly children in the developing world at risk for malnutrition, enteropathy and stunting. A thorough understanding of the development microbiome is critical to creating strategies to improve child health and nutrition.

The damaged gut: Enteropathy, enteric infection, and malnutrition

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Children living in low-income countries continue to suffer from undernutrition. Environmental enteropathy likely inhibits the efficacy of nutritional and oral vaccine interventions. Environmental enteropathy is a highly prevalent disorder among children living in low-income countries featuring small bowel inflammation, increased intestinal permeability, and decreased absorptive capacity. Environmental enteropathy is potentially caused by widespread fecal contamination and chronic insults to the gastrointestinal tract. We assessed the relationship of fecal environmental contamination and environmental enteropathy. We compared markers of environmental enteropathy, parasite burden, and growth in 119 Bangladeshi children (≤ 48 months of age) across rural Bangladesh living in different levels of household environmental cleanliness defined by objective indicators of water quality and sanitary and hand-washing infrastructure. Adjusted for potential confounding characteristics, children from clean households had 0.54 SDs (95% confidence interval [CI] = 0.06, 1.01) higher height-for-age z scores (HAZs), 0.32 SDs (95% CI = − 0.72, 0.08) lower lactulose:mannitol (L:M) ratios in urine, and 0.24 SDs (95% CI = −0.63, 0.16) lower immunoglobulin G endotoxin core antibody (IgG EndoCAb) titers than children from contaminated house-

holds. After adjusting for age and sex, a 1-unit increase in the ln L:M was associated with a 0.33 SDs decrease in HAZ (95% CI = −0.62, −0.05). These results are consistent with the hypothesis that environmental contamination causes growth faltering mediated through environmental enteropathy. Randomized trials that modify environmental enteropathy risk factors and animal studies to determine the pathogenesis of environmental enteropathy will improve the development of interventions to prevent and alleviate environmental enteropathy.

Early nutrition: consequences for the immune system

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The immune system in early infancy must deal with the dual challenge of exposure to pathogenic microorganisms and initial colonization with commensal microflora. These challenges require the development of protective as well as regulatory responses systemically and at mucosal surfaces. Exclusive breastfeeding is recommended early in infancy because human milk provides ideal nutrition for the infant and prebiotic and immune factors that promote the development of a health-promoting commensal microflora in the intestine, including the genus *Bifidobacterium*. Recent work in neonates shows that gut *Bifidobacterium* levels are associated with mucosal immune responses, as expected, but also shows strong associations with responses to systemically administered vaccines. The micronutrient status of the mother will also affect the nutritional status of the infant *in utero* and during the period of breastfeeding. As an example, vitamin A deficiency is common in pregnant women in many areas of the world and newborn infants in these areas are at high risk of vitamin A deficiency. Vitamin A plays a role in the development of both protective ("pro-inflammatory") and regulatory ("anti-inflammatory") responses by the innate and adaptive immune systems, thus vitamin A deficiency may impair crucial aspects of infant immune function. Recent work in neonates at risk of vitamin A deficiency shows that high-dose supplementation can modulate vaccine responses in the neonatal period, though effects appear to be transient. Early life nutritional and microbiota status is also associated with immune-mediated diseases that develop later in childhood, though cause-effect associations are still being evaluated.

Developmental programming for obesity: Epigenetic modifications in the hypothalamus

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Altered nutritional experiences in the immediate postnatal life via the phenomenon of metabolic programming have been recognized as one of the components in the etiology of obesity. Employing a rat model with an altered dietary experience (a high carbohydrate milk formula, HC) results in the onset of hyperinsulinemia associated with alterations in pancreatic islet structure and beta cell function including an increase in glucose-stimulated insulin secretory response of HC islets. Additionally, alterations in the expression of neuropeptide Y (Npy) and pro-opiomelanocortin (Pomc) in the hypothalamus of HC pups may contribute to hyperphagic response of HC rats. Altered methylation status of specific CpG dinucleotides in the *Npy* gene promoter but not in the *Pomc* gene promoter was observed. A decrease in acetylation of histone H3K9 for the *Pomc* gene and an increase in acetylation for the same residue for the *Npy* gene were observed without changes in histone methylation (H3K9) in both genes in HC rats. Several of these changes in HC pups persist into the post-weaning period resulting in chronic hyperinsulinemia and development of adult-onset obesity in HC rats. Pair-feeding of HC rats (HC/PF) to control animals reduced body weight gains and serum insulin levels but these parameters were restored to HC levels in the HC/PF/AL rats after *ad libitum* feeding. The expression of Npy and Pomc in the hypothalamus was not different HC, HC/PF, and HC/PF/AL rats, indicating that calorie restriction cannot erase the programmed predisposition for hypersecretory capacity of islets and hypothalamic hyperphagic response in obese HC rats.

Hormonal regulation of infant and child growth

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The identification of useful biomarkers influencing growth as a result of nutrient factors would provide potential targets for therapeutic interventions and expand our understanding of normal growth. Identification of underlying unique hormonal, substrate, genetic and/or epigenetic factors would be important. Currently, the only effective biomarker for linear growth is accurate measurements of a child's length or height. This, plus similar ones of the parents, will identify children at risk who could be ed. Linear growth is achieved largely in the spine and the epiphyses in the distal femur and proximal tibia and final adult height is largely genetically determined. Auxologic measures are epidemiologically inexpensive to obtain but the precision and accuracy of such measures overtime is not trivial but only from such longitudinal tracking can we obtain critical measures of growth velocities. Linear growth measures paired with measu-rements of bone age and a limited number of hormone measures have been the traditional mainstay of pediatric endocrino-logists. The observed increase in second generation heights upon children migrating to western societies is well documented but poorly understood. The factors responsible are most likely multifactor and a variety of biomarkers might be considered including markers of inflammation, hormone secretion, environ-mental stress and pollutants, macro and micronutrient defi-ciencies. Timing of the "nutritional" insult(s) and relief from such factors are most likely critical. Thus, evidence of the offending factor(s) at a later time may be difficult to identify. Timing of such events may influence the degree of recovery and ultimately the individual's final adult height.

Carotenoid and retinoid signaling in early development: Priming of metabolic diseases?

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Vitamin A is an essential nutrient that is required for many biological processes including vision, cell differentiation and development. This micronutrient is the precursor for at least two critical compounds, 11-*cis*-retinal, the chromophore of visual G-protein-coupled receptors, and retinoic acid, a ligand for transcription factors. Since retinoids cannot be synthesized *de novo*, dietary precursors must be absorbed by the intestine. In the diet two distinct sources for vitamin A exist: preformed vitamin A, mainly retinyl esters from animal products, and provitamin A carotenoids, mainly β-carotene from vegetables and fruits. These compounds must be metabolically converted and transported within the body to target tissues. In recent years, much progress has been made in identifying molecular components that mediate this process. Mutations in their corresponding genes impair vitamin A homeostasis and induce various pathologies in rodent models. Polymorphisms in these genes alter carotenoid and retinoid homeostasis in humans as well. This presentation will summarize the advanced knowledge about this metabolism and will especially emphasize on different role of β-carotene and vitamin A in adipocyte physiology and the regulation of body fat reserves. Understanding the molecular and regulatory basis of this process, in genetically accessible rodent models, may relate to human physiology as well. Translation of this knowledge should help to establish effective public health interventions to combat related disease. Appraisal of the consequences of common genetic polymorphisms in the involved genes will constitute an important step towards personalized nutrition for optimum health.

Integration of basic biology and public health research: Bringing research disciplines together

Lindsay H. Allen

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The speakers in this session have presented information that relied substantially on understanding the basic biology and the mechanisms underlying development during the first 1000 days. They included research on the gut microbiota and intestinal integrity, immunology and infection, endocrinology and epigenetics, and metabolic signaling. The purpose of this complex, multidisciplinary research is, in theory, to optimize and minimize risks to child health and development. Thus the potential applications of this research to public health interventions should always be borne in mind. Is it possible to apply this information and what impact could it have? What relevance does it have to potential public health interventions and as part of the research design have efforts been made to ask the relevant questions? This requires knowing what the public health concerns actually are. Useful summaries of these and the potential benefits of specific interventions have become available in recent years, for example in "The Lancet" series on Maternal and Child Nutrition, and WHO's eLENA (e-Library of Evidence for Nutrition Actions). Even so there remain many gaps in our information, some of which will be summarized in this lecture. In part these exist because the methods that have been used to evaluate the efficacy and effectiveness of public health programs in maternal and child nutrition have rarely applied an inclusive, systems biology approach and its cutting-edge methodologies. Opportunities exist to better combine systems biology assessment tools with public health nutrition interventions.

SESSION II
FLAVONOIDS: FROM METABOLISM TO DIETARY GUIDELINES

[2-^{14}C](−)-Epicatechin metabolomics: Absorption and metabolism in the small intestine and colon

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[2-^{14}C](−)-Epicatechin (EC; 60 mg, 300 mCi) was fed orally to eight healthy male subjects after which plasma, urine and feces collected for a 72 h period were analysed by HPLC with MS^2 and on-line radioactivity detection, and NMR. More than 25 radiolabelled metabolites were identified. Indicative of absorption in the small intestine, twelve glucuronidated, sulfated and methylated EC metabolites reached 19-357 nM peak plasma concentrations (C_{max}) 0.8-1.4 h after supplementation (T_{max}) and the levels declined rapidly thereafter with elimination half life times of 1.0-2.0 h. Gut microbiome-derived metabolites in the form of glucuronidated and sulphated phenylvalerolactone and phenylvaleric acids reached 39-272 nM C_{max} values after 5.5-6.8 h and were present in plasma for a more prolonged period of time than EC metabolites absorbed in the small intestine. Total radioactivity in plasma was never exceeded 2% of intake, although 82% of the ingested radioactivity was recovered in urine as a mixture of small intestine and colon-derived metabolites. A further 12% of radioactivity was expelled in feces. The study provides important information on the identity of EC metabolites in the gastrointestinal tract and passing through the circulatory system that are potentially associated with the protective effects of EC intake.

miRNA as molecular targets of flavonoid metabolites involved in preservation of endothelial cell function

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Consumption of flavanol-rich foods is associated with a reduced risk of cardiovascular diseases, which was linked to improvements in endothelial function. The specific flavanols involved in these beneficial effects and underlying molecular mechanisms is still largely unknown. We have shown that exposure of TNFα-activated endothelial cells to flavanol metabolites (4'-O-methyl(−)-epicatechin, 4'-O-methyl(−)-epicatechin-7-β-D-glucuronide and (−)-epicatechin-4'-sulfate) at physiologically-relevant concentrations decreased the adhesion of monocytes to endothelial monolayers. Nutrigenomic analysis showed that these metabolites modulate expression of genes involved in the regulation of cell adhesion/junctions, focal adhesion or cytoskeleton remodeling, and this by affecting phosphorylation levels of p65 and p38 of NF-κB and MAPK cell-signaling pathways respectively. Together with cell signaling pathways, microRNAs (short, endogenous, noncoding, single-stranded RNAs) represent another class of molecular post-transcriptional regulators of gene expression. Our nutrigenomic studies have shown that exposure of endothelial cells to the same metabolites can also modulate the expression of miRNAs. Among differentially-expressed miRNAs are those involved in the regulation of inflammation or cell adhesion, such as miR-221 and miR-181. Bioinformatic analysis shows that the potential target genes these miRNAs are also involved in regulation of cell adhesion, cytoskeleton, focal adhesion, transendothelial migration. These miRNAs could exert post-transcriptional regulation by inhibiting protein synthesis, as BIRC2, or by inducing mRNA degradation, as WASP1.

These data suggest that miRNAs are important molecular targets of flavanol metabolites involved in the regulation of expression of genes controlling adhesion and transendothelial migration processes. This original result contributes to increase the knowledge about the mechanisms underlying the protective effect of flavanols on vascular endothelium.

Flavanols and cardiovascular health: Clinical outcomes of the FLAVIOLA project

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Accumulating evidence from dietary intervention studies suggest that diets rich in flavanols are causally related to cardiovascular health benefits in humans. In order to consider flavanols as potential candidates for inclusion in future dietary recommendations, it is necessary to assess the efficacy, relevance, and applicability to the general public. Therefore, the pan-European research consortium FLAVIOLA aimed at investigating the cardiovascular effects of cocoa flavanol (CF) intake in various cohorts representing a broader segment of the healthy population. In particular, we investigated the efficacy of CF from a gender- and age perspective in healthy people. Our data demonstrate that CF intake exerts beneficial effects on various accredited cardiovascular endpoints in healthy men and women across all the ages and groups investigated. Taken together, we provide direct evidence for population-based health benefits, thus supporting a potential role for cocoa flavanols in primary cardiovascular disease prevention and future dietary guidelines.

Anthocyanin intake and risk of cardiovascular disease

Aedin Cassidy

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Our understanding of the metabolism and health effects of anthocyanins has increased significantly recently. Data from prospective cohort studies highlight the beneficial impact of habitual intakes of anthocyanins on both biomarkers of cardiovascular (CV) risk and disease outcomes including myocardial infarction and type 2 diabetes. Currently there are limited validated biomarkers to integrate intake with the extensive metabolism these compounds undergo in vivo, so our current knowledge is based on dietary intake. We recently established that anthocyanins are absorbed and metabolised to a greater extent than had been previously reported. Specifically, using a ^{13}C-labelled anthocyanin (cyanidin-3-glucoside, C3G) and IRMS, we established that breakdown products and conjugates of C3G were absorbed, metabolised and excreted; 12% of the ingested dose of ^{13}C was recovered in urine. In the circulation, the majority of the ingested anthocyanins were present as lower molecular weight ^{13}C-labelled phenolic metabolites. However proof of efficacy and the impact of metabolism on health from longer-term randomised controlled trials is distinctly lacking. To date, few carefully controlled human intervention trials have examined the effects of anthocyanins on insulin resistance and vascular health; those that have are of relatively short duration (< 2 months). In one trial both systolic and diastolic blood pressure (BP) decreased, while in another study, berry consumption (two portions daily) resulted in favourable changes in platelet function, BP and HDL-cholesterol levels. In another intervention, daily intakes of anthocyanins over 4 weeks resulted in a significant decrease in pulse wave velocity, a measure of vascular function. To date, only one study has examined the impact of anthocyanins on insulin sensitivity as assessed by the hyperinsulinemic-euglycemic clamp technique, and of the three studies that assessed fasting glucose and HbA1c levels, two observed beneficial effects. To date no long term studies have investigated the dose-response effects of anthocyanin-rich foods on insulin resistance and vascular function. These compounds are present in red/blue colored fruits and vegetables, are readily incorporated into the habitual diet, and simple dietary change could have a significant impact on prevention efforts.

Flavonoids and neuro-cognitive improvements: The involvement of the vascular system as a mediator of benefits

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Evidence suggests that dietary phytochemicals, in particular flavonoids, may exert beneficial effects on the central nervous system by protecting neurons against stress-induced injury, by suppressing neuroinflammation and by improving cognitive function. Historically, they were believed to do this via an ability to express classical antioxidant activity in the brain. However, their poor brain bioavailability and extensive metabolism means that this is unlikely. Instead, their actions on the brain appear to be mediated by effects on both the peripheral and cerebro-vascular system that lead to changes in improve blood flow to the brain capable of inducing angiogenesis, neurogenesis and changes in neuronal morphology. Such vascular effects may lead to the activation of critical protein and lipid kinase signalling cascades in the brain, leading to a suppression of neuroinflammation and the promotion of synaptic plasticity. This paper will focus on the acute effects of flavonoids and flavonoid-rich foods on human executive function (attention, sustained attentiveness and task responsiveness) and episodic memory and how such effects may be mediated by changes in peripheral and cerebrovascular blood flow, measured using flow-mediated dilatation and fMRI. Through such a mechanism, the consumption of flavonoid-rich foods throughout life holds the potential to limit neurodegeneration and to prevent or reverse age-dependent loses in cognitive performance. In addition, flavonoids may represent important precursor molecules in the quest to develop a new generation of brain enhancing drugs.

Dietary guidelines on flavonoids? Where are we today?

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Since Dietary Guidance is based on Dietary Reference Intake Values (DRIs) the first step in achieving mention in Dietary Guidance is likely obtaining a DRI value. It is important to have a DRI-like process for evaluation of bioactives because these substances are important to human health; considerable research dollars are being spent on these substances; and consumers are purchasing foods containing them, yet there are no "standards" set for determining how much science is behind purported benefits of bioactives, or how much we need to eat in order to achieve these benefits. A major challenge to establishing such a system is that the absence of a specific bioactive in the diet does not result in a deficiency disease as it does for essential nutrients. Thus different types of endpoints (such as decreased risk of disease or increased optimal health) are needed. Methodology for evaluating these endpoints is not as established as the methodology for determining nutrient deficiency disease endpoints. There are also issues of safety (particularly for fortified foods) and questions on who has "ownership" of the system and how decisions are made. Despite these issues, China has established a DRI process for the evaluation of bioactives and the US has made great progress in this direction. This talk will outline potential criteria for a bioactive to meet in order to achieve a DRI value, and suggest which criteria could or could not be would be met with flavonoids at this time.

Hydroxytytrosol is a rising star of nutraceutical after resveratrol on targeting mitochondria to prevent age-associated diseases

Jiankang Liu

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The nutraceutical resveratrol has become a star of nutraceutical because of the discovery of its effect on Sirtuin regulation by Sinclair's lab at Harvard. Nowadays, resveratrol has been widely studied on retarding aging and preventing/ameliorating numerous disorders in cellular and animal models as well as clinical studies. We have recently investigated a number of naturally occurring nutraceuticals and found that hydroxytyrosol (HT) has a unique and potent effect on regulating mitochondrial metabolism (biogenesis and degradation). HT is abundant in olives and virgin olive oil, which are widely used in Mediterranean diets. Epidemiologic studies have demonstrated that the people of the Mediterranean basin enjoy a healthy lifestyle with decreased incidence of heart disease, prostate and colon cancer, and rheumatoid arthritis. One of the possible reasons is that Mediterranean people have a high intake of HT from olives and olive oil. The beneficial effects of HT have been mainly attributed to its antioxidant effect. However, we have demonstrated that HT is an effective mitochondria-targeting nutraceutical in various cellular and animal models, including age-associated macular degeneration, metabolic disorders, and strenuous exercise. Because mitochondrial dysfunction has been implicated in aging and various diseases (such as cardiovascular diseases, metabolic disorders, age-associated macular degeneration, and cancer), the potent effects of HT on regulating mitochondrial metabolism suggest that HT is a rising star of nutraceutical after resveratrol and will be widely used for delaying aging and preventing/ameliorating age-associated diseases.

Pomegranate consumption, the microbiome, and urolithin production

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Although pomegranate ellagitannins are highly bioactive in vitro, they are not absorbed intact but are hydrolyzed in the intestine and absorbed as ellagic acid. There are known interindividual differences in the production of urolithins from pomegranate ellagitannins such that about 30 percent of individuals do not excrete urolithins in their urine after consuming pomegranate juice or extract. These differences most likely are related to the difference in the intestinal microflora. Our lab has studied both the effects of pomegranate extract on colonic microflora (the microbiome) and whether differences in the microbiome prior to pomegranate ellagitannin consumption alter production of urolithins and whether pomegranate extract consumption induces changes in the microbiome to enhance production of urolithins. In healthy volunteers, urinary urolithin A was significantly increased after 4 weeks of consumption of pomegranate and the increase is positively associated with presence of bacteroidetes and negatively with firmutues. There was trend in urolithin producers that firmcutes/bacteroidetes ratio was decreased after 4 weeks supplementation of pomegranate extract. Probiotic bifidobacteria were generally not affected by pomegranate extract, while the growth of many strains of bifidobacterium was significantly enhanced with pomegranate juice. It is evident that there are complexity of interactions between ellagitannins and microbiota in the intestinal environment.

SESSION III

REDOX SIGNALING AND SYSTEMS BIOLOGY IN HEALTH

Thiol signaling, redox circuits, and systems biology in health status

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Redox systems biology provides a framework to understand and model complexities of oxidative stress in human disease. This presentation will briefly address the redox proteome and metabolome as a central interface between the functional genome and the exposome, where the functional genome includes the genome, epigenome, transcriptome and translated proteome, and the exposome collectively includes lifelong environmental exposures. The interface is a system of reversible and irreversible modifications of the proteome, termed the redox proteome, and supportive redox-active small molecules, termed the redox metabolome. Oxidative (H_2O_2 and O_2) and reductive (GSH and thioredoxins) hubs maintain a network of kinetically controlled redox circuits to support differentiation of cell functions, sense dietary and environmental challenges, defend against these challenges, and signal adaptive responses. Powerful analytic methods provide unprecedented detail about the compartmentalization and integration of these redox elements. Knowledgebase resources for redox systems biology have lagged behind other fields, but computational tools and databases for proteomics and metabolomics create an opportunity for rapid development of redox systems biology. Examples will be provided for the integrated use of molecular biology, transcriptomics, redox proteomics and metabolomics to elucidate toxicologic mechanisms related to environmental exposures. Redox proteomics and metabolomics identify antioxidant selenoproteins as primary targets for cadmium toxicity. Transcriptomics and metabolomics identify transporters as primary targets for the combined exposures to a fungicide and herbicide. Translation of findings from cell and animal models to human research is especially facilitated by the high-resolution metabolomics methods, which now support measurement of thousands of biochemicals in single analyses.

Coupling of metabolism to local circadian clock via reversible hyperoxidation of peroxiredoxin III in the mitochondria

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Peroxiredoxins (Prxs) are a family of peroxidases that reduce peroxides, with a conserved cysteine residue serving as the site of oxidation by peroxides. Mammalian cells express six different Prx isoforms (Prx I to VI) that are localized in various cellular compartments, with Prx I, II, and VI being localized mainly in cytosol**,** Prx III being restricted in the matrix of mitochondria**,** Prx IV being found mainly in the lumen of endoplasmic reticulum**,** and Prx V being in cytosol, mitochondria and peroxisomes. Peroxides oxidize the catalytic Cys–SH to Cys–SOH, which then reacts with another cysteine residue to form a disulfide that is subsequently reduced by an appropriate electron donor like thioredoxin. Reducing equivalents for Prx I to IV are provided by thioredoxin. During catalysis, the catalytic Cys–SOH of Prx I to IV is occasionally further oxidized to Cys–SO_2H before disulfide formation, resulting in inactivation of peroxidase activity. Kinetic analysis of Prx I inactivation indicated that 7 molecules of PrxI are hyperoxidized during reduction of every ten thousands molecules of H_2O_2. This hyperoxidation is reversed by sulfiredoxin (Srx) in a very slow process (k_{cat} = 0.18/min) that requires ATP hydrolysis and reducing equivalents such as thioredoxin and GSH. Given that disulfide bond is insensitive to oxidation by H_2O_2 and needs to be reduced to Cys-SH before oxidized to Cys-SOH, hyperoxidation of Prx occurs only during catalytic cycle. Except some phototropic bacteria, prokaryotic Prx enzymes are insensitive to oxidative inactivation, and prokaryotes do not express Srx. Reversible inactivation through hyperoxidation has therefore been speculated to be a eukaryotic adaptation that allows H_2O_2 to accumulate to substantial levels under certain circumstances.

To gain insight into the role of the reversible hyperoxidation of 2-Cys Prx under physiological conditions, we examined various mouse tissues for the presence of 2-Cys Prx-SO_2H. Sulfinic form of Prx III, a mitochondria-specific enzyme, was found to be abundant especially in the

cortex of adrenal gland, and detected weakly also in brown adipose tissues, ovary, heart, muscle, and lung. The sulfinic form of other 2-Cys Prxs was not detected in any of the tissues examined. Given that hyperoxidation of 2-Cys Prxs occurs only during the catalytic cycle, these results suggested that the amount of H_2O_2 produced in the tissues under normal physiological conditions is sufficient to maintain the abundant Prx III continuously engaged in H_2O_2 reduction and that the level of mitochondrial Srx is not enough to fully counteract the hyperoxidation of Prx III.

Detailed studies in adrenal glands revealed that the conversion of cholesterol to corticosterone is accompanied by H_2O_2 generation as the result of leaky cytochrome p450 (CYP11B1), which results in the hyperoxidation and inactivation of Prx III. When the level of inactivated Prx III increases above a certain level, mitochondrial H_2O_2 accumulates and then overflows into the cytosol, where it triggers p38 MAPK activation. The activated p38 inhibits cholesterol transport, resulting in down-regulation of coticosterone production and consequently reduction in H_2O_2 production. In separate events downstream of cAMP signaling, Srx synthesis is increased, allowing more Srx molecules to translocate into the oxidatively stressed mitochondria to reactivate Prx III-SO_2H. It has been known that the levels of corticosterone in blood undergo a circadian change. We found that the amounts of PrxIII-SO_2H and Srx in the mitochondria if adrenal gland also show daily variation. These results suggest that full circadian oscillation of corticosterone controlled by both the master and adrenal peripheral clocks requires the participation of the signaling pathway involving the reversible Prx III hyperoxidation. The reversible Prx III hyperoxidation appear to play critical role in linking metabolism to peripheral clocks.

Studies on the mechanism underlying mitochondrial translocation of Srx revealed that N-terminus of Srx represents a noncanonical signal sequence, which interacts with the translocase of the outer membrane (TOM 20). The translocation requires the formation of a disulfide linkage between HSP90 and Srx, which is promoted by H_2O_2 released from the mitochondria. Imported mitochondrial Srx remains stable in the presence of PrxIII-SO_2H but degraded by Lon protease when released free from PrxIII-SO_2H.

Role of selenium and selenoproteins in insulin-regulated energy metabolism

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An assumed link between the micronutrient selenium (Se) and the pathogenesis of type 2 diabetes mellitus (T2DM) is discussed controversially. While Se at doses ingested through the habitual diet or dietary supplements is probably not sufficient to induce T2DM, a majority of cross-sectional studies found a correlation of high Se plasma levels with T2DM in humans. Both abundant and deficient expression of antioxidant selenoproteins resulted in hyperinsulinemia and decreased insulin sensitivity in animal models. A probable rationale for these findings derives from the positive and negative regulation of both glucose-induced insulin secretion and insulin-induced signaling by hydrogen peroxide. Dietary Se levels and two selenoproteins, the hydrogen peroxide-reducing selenoenzyme glutathione peroxidase 1 (GPx1) and the plasma selenium transporter selenoprotein P (Sepp1), have been shown to affect pancreatic insulin secretion and/or the activity of key proteins in the insulin signal cascade (e.g. PTP-1B, PTEN, Akt, FoxO1a/3) in liver and skeletal muscle. We have shown that the dietary Se compound sodium selenite delayed insulin-induced Akt phosphorylation and glucose uptake in myocytes. We also found molecular alterations in expression and/or phosphorylation of several proteins related to the energy metabolism in skeletal muscle and visceral adipose tissue of pigs fed a supranutritional Se diet compared to pigs fed a Se-adequate diet. Conversely, the dys-regulated carbohydrate metabolism in T2DM patients might affect plasma Se and Sepp1 levels, as the hepatic biosynthesis of Sepp1 is suppressed by insulin and stimulated under hyperglycemic conditions.

Brain cholesterol oxidation and 4-hydroxynonenal in the pathogenesis of Alzheimer's disease

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Whereas abnormalities in cholesterol metabolism are tied to a derangement of cholesterol synthesis and uptake in the peripheral tissues, leading to increased "total" plasma cholesterol (hypercholesterolemia), often they also appear to involve oxidative modification of cholesterol and/or altered cholesterol homeostasis within the brain. Among the cholesterol oxidation products (oxysterols) detectable in the brain, the so far most investigated ones are 24-hydroxycholesterol (24OH) and 27-hydroxycholesterol (27OH). Several experimental findings support a pathogenic role of oxysterols in Alzheimer's disease (AD): i) a biocompatible amount of 24OH and 27OH (1μM) definitely accelerated APP processing toward β-amyloid production by up-regulating APP and BACE1 protein levels in human differentiated neuronal cells (SK-N-BE); ii) both oxysterols were enhancing $A\beta_{1-42}$ peptide binding and internalization in different human neuronal cell lines by inducing CD36 and β1-integrin and in particular 24OH potentiated $A\beta_{1-42}$ neurotoxicity; c) both 24OH and 27OH show a remarkable pro-oxidant and pro-inflammatory action. A redox state impairment in the brain developing AD seems to play a central role and oxysterols might contribute to its induction and over-expression. Dependent upon an oxidative redox imbalance is the accumulation of another lipid oxidation product, 4-hydroxynonenal (HNE), whose pro-oxidant, pro-inflammatory and neurotoxic properties were consistently proved by the experimental studies carried out thus far. As for the two considered oxysterols, in neuronal cell lines challenged with biocompatible amounts of HNE (1μM): i) both BACE-1 expression and $A\beta_{1-42}$ synthesis were up-regulated; ii) neuronal $A\beta_{1-42}$ binding and neurotoxicity were potentiated. Targeting these lipid oxidation products in the brain might help fight AD.

Allopregnanolone as a regenerative and mitochondrial regulator: Therapeutic implications for Alzheimer's disease

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Previously, we demonstrated that the neurosteroid allopregnanolone (Allo) promoted neural stem regeneration, restored cognitive function and reduced AD pathology in the triple transgenic Alzheimer's mouse model (3xTgAD). To investigate mechanisms whereby Allo could promote neural stem cell function, promote cognitive function and simultaneously reduce AD pathology, we investigated Allo regulation of the bioenergetic system of the brain.

In vitro, Allo significantly potentiated mitochondrial respiration in primary neuronal, glial and neural stem cell (NSC) cultures while significantly increasing mitochondrial velocity and dynamics. Further, Allo promoted neural stem cell differentiation to a neuronal phenotype.

In vivo, Allo reversed deficits in mitochondrial respiration including dysregulated proton leak in female ovariectormized wildtype and 3xTgAD mice. Consistent with restored mitochondrial efficiency, Allo reversed OVX-induced increase in lipid peroxidation. Further, Allo increased activity and expression of key bioenergetic enzymes, PDH and αKGDH. Mechanistically, Allo regulated key up-stream pathways involved in brain bioenergetics and prevention of AD pathology development: activation of the insulin and PGC1a pathways to enhance brain metabolic activity; activation of ApoE pathway to promote cholesterol transport and Aβ clearance; and inhibition of presenilin pathway to reduce amyloidogenesis.

Collectively, these data indicate that Allo functions as a systems-biology regulator of regeneration, bioenergetics and cholesterol homeostasis in the brain. These systems are critical to neurological function and provide a plausible mechanistic rationale for Allo as a regenerative therapeutic for prevention and treatment of AD, which is currently under clinical investigation.

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Proteolytic cleavage of Opa1 stimulates mitochondrial inner membrane fusion and couples fusion to oxidative phosphorylation

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Mitochondrial fusion is essential for maintenance of mitochondrial function. The mitofusin GTPases control mitochondrial outer membrane fusion, whereas the dynamin-related GTPase Opa1 mediates inner membrane fusion. We show that mitochondrial inner membrane fusion is tuned by the level of oxidative phosphorylation (OXPHOS), whereas outer membrane fusion is insensitive. Consequently, cells from patients with pathogenic mtDNA mutations show a selective defect in mitochondrial inner membrane fusion. In elucidating the molecular mechanism of respiration-stimulated fusion, we uncover that real-time proteolytic processing of Opa1 stimulates mitochondrial inner membrane fusion. OXPHOS-stimulated mitochondrial fusion operates through Yme1L, which cleaves Opa1 more efficiently under respiratory conditions. Artificial cleavage of Opa1 is sufficient to mediate inner membrane fusion, regardless of respiratory state. Proteolytic cleavage therefore stimulates the membrane fusion activity of Opa1, and this feature is exploited to dynamically couple mitochondrial fusion to cellular metabolism.

Molecular basis of mitochondrial behaviors

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Mitochondria are double membrane-bounded organelles that perform a myriad of diverse and essential functions in cells. These functions are dependent on the collective intracellular behavior of the organelle. We have characterized key features of mitochondrial behaviors. Our work has addressed the physiological functions and mechanisms of mitochondrial division and fusion, which are important determinants of overall mitochondrial shape and distribution. We have uncovered contact sites that intimately link mitochondria with the ER and determined their roles in mitochondrial positioning, dynamics and mtDNA transmission. We have also addressed the fundamental question of how mitochondrial membranes are sub-compartmentalized to reveal how the complex internal architecture of the organelle is generated. Our current focus is to determine how mitochondrial behaviors are integrated with one another and physiologically regulated within cells and organisms.

Exercise and mitochondrial dynamics remodeling

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Moderate physical exercise has been correlated with a number of health benefits, including a reduced risk of developing age-related pathologies, such as sarcopenia, cardiovascular disease, and diabetes. Mitochondria are crucial organelles for the production of energy by efficient aerobic energy metabolism and for the control of signaling cascades. It is well established that regular exercise exerts profound influences on mitochondrial quality and quantity remodeling, such as altered fusion and fission dynamics, upregulation of mitochondrial biogenesis, and enhanced mitophagy. Moreover, it was suggested that exercise could increase the formation of reactive oxygen species (ROS) to a level that may induce significant, but tolerable, oxidative stress that in turn induces beneficial adaptations. However, there are still unanswered basic scientific questions in the health benefits of exercise, such as how increased ROS production during physical exercise could be involved in mitochondrial remodeling. In the present presentation, I will introduce our recent work about the response of mitochondrial dynamics remodeling to acute and endurance exercise and its regulation to mitochondrial and cellular homeostasis. In addition, the redox-sensitive signal transduction pathways associated with exercise-induced health benefits will also be discussed. Understanding the mechanisms of action of exercise will allow us to develop new therapies that mimic the protective actions of exercise.

Hypoxia activates receptor-mediated mitophagy

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Mitochondrial autophagy or mitophagy is a major mechanism for mitochondrial quality control which selectively removes damaged or unwanted mitochondria to ensure the well-being of the cell. We recently have identified that mitochondrial outer membrane protein FUNDC1 harbors LC3-interacting region (LIR) and interacts with LC3 to mediate mitophagy upon hypoxic treatment. Under normoxic condition, FUNDC1 is highly phosphorylated by Src kinase and CK2 at Try^{18} and Ser^{13}, respectively. Hypoxic treatment leads to the inactivation and dissociation of both kinases from mitochondria. Also, we have identified that mitochondrially localized phosphatase PGAM5 is able to dephosphorylate FUNDC1 at Ser^{13}. Dephosphorylation of FUNDC1 enhances its interaction with LC3, which is abrogated by a cell permeable unphosphorylated peptide encompassing Ser^{13} and LIR of FUNDC1. We also found that Bcl_{xL}, but not Bcl-2, interacts with PGAM5 to inhibit its phosphatase activities and prevents mitophagy. Hypoxia led to the degradation of Bcl_{xL} and thereby the activation of PGAM5 for mitophagy. Our results reveal a mechanistic signaling pathway linking hypoxia signaling towards the activation of mitophagy.

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Measuring and manipulating mitochondrial ROS signals

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Over the past few years myself and collaborators have developed mitochondria-targeted bioactive and probe molecules. These have included antioxidants that selectively block mitochondrial oxidative damage. Among these molecules are derivatives of the natural antioxidants ubiquinone (MitoQ) and Vitamin E. The antioxidant efficacy of these molecules was increased considerably by targeting them to mitochondria, which are the major source of oxidative stress in mammalian cells. This was achieved by covalent attachment of the antioxidant to a lipophilic cation. Due to the large mitochondrial membrane potential, these cations accumulate several hundred fold within mitochondria, protecting them from oxidative damage far more effectively than untargeted antioxidants. This was extended to develop the related mitochondria-targeted nitric oxide donor, MitoSNO, which is protective against cardiac ischemia-reperfusion injury. In parallel work we have developed the mitochondria-targeted hydrogen peroxide probe MitoB that enables us to utilise ex vivo mass spectrometry to assess mitochondrial hydrogen peroxide production in vivo. Here I will focus on how the used of these probes and bioactive molecules enabled us to determine a mechanism by which mitochondrial S-nitrosation of a particular cysteine residue on complex I led to prevention of cardiac ischemia-reperfusion injury in vivo.

Metabolomic analyses of mitochondrial metabolism

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Collaborative research with Dr. Sean Adams (WHNRC, Davis) and other research groups was directed toward the identification of novel single metabolites or metabolite signatures that reflected differences in mitochondrial metabolism with special emphasis on skeletal muscle tissue, for example, for metabolic shifts in tissue-specific long-chain fatty acid (LCFA) combustion, branched chain amino acids, carbohydrate and TCA metabolism and other areas of energy metabolism. Inefficient or incomplete ß-oxidation fatty acid metabolism is associated with insulin-resistance, although the mechanisms underlying this link remain controversial. There is evidence that specific small molecules whose cytosolic abundance is elevated when muscle delivery/accumulation of LCFA is excessive (*e.g.,* by analyzing ceramides, diacylglycerols and acyl-carnitines) may decrease signaling from the insulin receptor. Since insulin resistance and type 2 diabetes mellitus can manifest even with no changes in muscle levels of ceramide or diacylglycerols, we hypothesized that additional factors must be involved to trigger the insulin resistance phenotype in muscle. To this end, our studies used organelle, cell, animal, and human models coupled to comprehensive analytical technologies to characterize small molecules that are altered in response to LCFA β-oxidation and that are muscle abundant or muscle-specific. Since it is not requisite that metabolites shifted under these conditions be derived directly from the LCFA themselves, we were examining muscle metabolites spanning many chemical classes, *i.e.,* metabolomic techniques. We explain the current status of metabolomic techniques, including lipidomics and use of various databases, and give an overview on how metabolomics has guided us finding novel markers for mitochondrial dysfunction.

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SESSION IV

VITAMIN E: TOCOPHEROL AND TOCOTRIENOLS IN TRANSLATIONAL MEDICINE

α-Tocotrienol: multimodal protection against stroke

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Vitamin E is a generic term for tocopherols (TCPs) and tocotrienols (TCTs). Although TCPs have been widely studied for decades, the significance of α-TCT as the most potent neuroprotective forms of natural vitamin E has been uncovered recently. In 1999, we first reported that nanomolar concentrations of α-TCT, not α-TCP, prevent stroke-associated neurodegeneration. Since then several other laboratories have reported potent neuroprotective effects of tocotrienol, superior than tocopherol, in several experimental systems. During the course of the last decade we have systematically elucidated five major mechanisms by which palm tocotrienol may rescue affected neural tissue during stroke: inhibition of c-Src and 12-Lox pathways of neurodegeneration, induction of MRP-1 – a survival factor, promotion of stroke-site perfusion by enhancing collateral flow as detected by video angiography, and prevention of loss of neuroprotective miR29b. Recent research efforts are focused on clinical testing. In 2012, we published first evidence that oral tocotrienols are delivered to human brain tissue at nano-molar concentration known to protect against experimental stroke injury in pre-clinical models. In light of more than 7 million stroke survivors living in the United States today, ongoing Phase I (NCT01578629) and Phase IIb (NCT01858311) clinical trials are designed to test the safety and efficacy of tocotrienol vitamin E in secondary stroke prevention.

Lipidomic insights into actions of vitamin E in a diet-induced mouse model of NAFLD

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Background –Vitamin E (VitE) has been shown to improve nonalcoholic steatohepatitis (NASH). While VitE is widely believed to work through its anti-oxidant effects, this has not been experimentally verified. Specifically, the effect of VitE on various lipid classes and their relationship to disease phenotype remain unknown.
Aim – To use an unbiased Lipidomic approach to evaluate the impact of VitE on hepatic lipid metabolism.
Methods – 129SI/SvlmJ;B6 female mice were fed chow diet (n = 5), high fat diet (HFD) alone (n = 5) or HFD with vitE (n = 5) supplementation (0.1 IU/g/day) for the last 6 weeks of a 52 week feeding regimen. Individual molecular species level lipidomic analysis was performed on liver tissue at the end of the study period using mass spectrometry. Students T-test was used to assess significance (p-value <0.05).
Results – HFD led to development of steatosis with inflammation and fibrosis in all mice. Compared to HFD alone, VitE administration produced a modest non-significant decrease in total fat, diacylglycerol and cholesterol esters while increasing total phospholipid. The MUFA:PUFA ratio decreased non-significantly with VitE + HFD compared to HFD alone. VitE also reversed HFD-associated increase in lysophospholipids. VitE decreased several ceramide classes with the greatest effect on lactosylceramide and GB3. Sphingomyelins increased in VitE + HFD versus HFD mice. VitE also reversed HFD-associated increase in 12 HETE while increasing PGD2 and 6keto-PGF1a a marker of PGI2 activity.
Conclusions –VitE has a widespread impact on lipid metabolism in the liver. It decreases pro-apoptotic ceramides and pro-inflammatory GB3. It decreases pro-inflammatory- while increasing anti-inflammatory-eicosanoids.

Isoforms of vitamin E differentially regulate inflammation

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Vitamin E regulation of disease has been extensively studied but most studies focus on the α-tocopherol isoform of vitamin E. Reports on vitamin E indicate contradictory outcomes for anti-inflammatory functions of the α-tocopherol isoform of vitamin E with regards to animal and clinical studies. The seemingly disparate results are consistent with our studies demonstrating that two purified natural forms of vitamin E, α-tocopherol and γ-tocopherol, have opposing regulatory functions during inflammation. We demonstrated that α-tocopherol inhibits whereas γ-tocopherol elevates allergic lung inflammation in mice, airway hyperresponsiveness, leukocyte transendothelial migration, and endothelial cell adhesion molecule signaling through protein kinase Cα (PKCα). Tocopherols directly regulated PKCα. We demonstrated that α-tocopherol is an antagonist and γ-tocopherol is an agonist of PKCα through direct binding to the C1a regulatory domain of PKCα. Moreover, these tocopherols compete with diacylglycerol and retinoic acid for binding to C1a. To translate these findings in experimental models to humans, we conducted analysis of 4526 adults in the Coronary Artery Risk Development in Young Adults (CARDIA) multi-center cohort with available spirometry and tocopherol isoform data. In this cohort, increasing serum concentrations of γ-tocopherol associated with worse lung function, while increasing serum concentrations of α-tocopherol associated with better lung function. In summary, we have determined mechanisms for opposing regulatory functions of α-tocopherol and γ-tocopherol on inflammation. Information from our studies will have significant impact on the design of clinical studies on inflammation and on vitamin E consumption.

Anti-inflammatory activities of vitamin E forms and their long-chain metabolites

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Specific forms of vitamin E such as γ-tocopherol (γT), δ-tocopherol (δT) and γ-tocotrienol (γTE) have anti-inflammatory properties that are relevant to prevention and therapy against chronic diseases. This paper will discuss anti-inflammatory actions of vitamin E forms via inhibition of cyclooxygenase- and 5-lipoxygenase-catalyzed eicosanoids. Furthermore, these vitamin E forms are significantly metabolized to various carboxychromanols via cytochrome P-450 (CYP4F2)-initiated side-chain oxidation. Long-chain carboxychromanols, *esp.*13'-carboxychromanol, have recently been shown to have stronger anti-inflammatory effects than un-metabolized vitamins and may therefore contribute to beneficial effects of vitamin E forms *in vivo*. Consistent with mechanistic findings, these anti-inflammatory activities have been demonstrated in preclinical models related to inflammation and cancer.

Vitamin E and apoptosis: free radical scavenging versus regulation of enzymatic lipid oxidation

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In contrast to random non-enzymatic chemical reactions of lipid peroxidation, enzyme catalyzed peroxidative modifications of polyunsaturated fatty acyls in phospholipids is mostly associated with signaling processes. While vitamin E has an undisputable reputation of a lipid-soluble antioxidant, its role as a regulator of enzymatic oxidative reactions is much less clear. Among the latter, oxidative modification of cardiolipins in mitochondria are now thoroughly investigated. Cardiolipins [CLs] are ancient and unusual dimeric phospholipids localized in the plasma membrane of bacteria and in the inner mitochondrial membrane of eukaryotes. In this talk, I will document the signaling function of externalized on the surface of damaged mitochondria CLs as an elimination signal for mitophagy realized through the recognition by autophagy related microtubule-associated light chain 3 protein. I will review interactions of CLs with an intermembrane space hemoprotein, cytochrome c [cyt c], yielding a complex with the peroxidase competence, and present redox reaction mechanisms of cyt c-catalyzed CLs peroxidation as a required stage in the execution of apoptosis. I will consider possible role of vitamin E as a mild regulator of these reactions, particularly under the conditions associated with the "leakage of oxidative equivalents" into the bulk lipid phase of membranes. I will also illustrate the role of CLs oxidation by cyt c as a possible source of oxygenated lipid mediators.

α−Tocopherol in model membranes: A structure-function relationship describing its position and activity as an antioxidant

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We present neutron scattering, nuclear magnetic resonance and UV spectroscopy data of α-tocopherol's (most biologically active form of vitamin E) location in different lipid bilayers. These data suggest that the reduction of reactive oxygen species and lipid radicals occurs at the membrane's hydrophobic−hydrophilic interface. The latter is made possible when lipid acyl chains "snorkel" to the membrane interface, from the hydrocarbon matrix. Moreover, not all membranes are equal in this regard, as the vitamin locates differently in the lipid bilayers studied. In summary, the present results are a clear example of the importance of lipid diversity in controlling the dynamic structural properties of biological membranes. Importantly, our results suggest that measurement of α-tocopherol's oxidation kinetics and the diversity and regiochemistry of the peroxidation products so formed, should be revisited by taking into consideration the physical properties of the membrane in which the vitamin resides.

Antioxidants and skeletal muscle health

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Vitamin E (VE) deficiency can result in pronounced muscle weakness and atrophy. Membrane repair is a cell survival response evoked by a disruption in cell surface integrity, a frequent injury in skeletal muscle cells undergoing high-stress, eccentric contractions. We previously found that VE promotes membrane repair, and that oxidants strongly inhibit repair. We now directly test the hypothesis that VE deprivation impairs membrane repair in skeletal muscle. Skeletal muscle from rats deprived of VE for 4 months displayed the classical hallmarks of disease: central nucleation, necrotic fibers and an increase in connective tissue and fat. When VE deprived rats were challenged with downhill running, which induces eccentric contractions and consequent plasma membrane disruptions in leg muscles, they were profoundly deficient. Uptake of an indicator of membrane repair failure, Evans blue dye uptake, by myocytes in eccentrically exercised muscle was significantly elevated by VE deprivation. Direct assay of repair function in whole muscle, using a laser assay, revealed a striking deprivation-induced repair defect. VE is not however the only anti-oxidant that prevents lipid peroxidation: an enzyme, glutathione peroxidase 4 (GPx4) shares this role. We also show here that when GPx4 levels are knocked down in a conditional mouse embryonic fibroblast KO, repair fails catastrophically, and that this repair failure can be avoided with VE supplementation. We conclude that the lipid directed antioxidants, VE and GPx4, promote muscle health as essential components of the membrane repair response, and that maintenance of lipids in an unoxidized state is mechanism action.

ORAL PRESENTATIONS FROM SELECTED POSTERS

The metabolic triad in brain aging: Mitochondria, insulin/IGF-1 signaling, and JNK signaling

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Brain aging and the progression of neurodegenerative diseases such as Alzheimer's disease (AD) are associated with a hypometabolic state and cognitive decline induced by decrease in energy metabolism –indicating the critical role of mitochondria. As suggested by our aging- and caloric restriction studies, age-related decline in mitochondrial bioenergetics cannot be viewed independently from their redox-modulating function, but rather as an interdependent relationship reflected in the mitochondrial energy-redox axis. Moreover, as important second messenger generators, mitochondrial function is deeply integrated with both the coordinated signaling responses they trigger and the cytosolic signaling molecules they are recipients of, which establishes an intricate signaling network with close connections to mitochondrial bioenergetics and redox homeostasis. Our studies have shown that mitochondrial energy-redox axis is functionally integrated by a mitochondrial NADPH-producing enzyme – Nicotinamide nucleotide transhydrogenase (NNT). NNT dysfunction disrupts the electron flux from fuel substrates to redox components, and induces not only mitochondrial dysfunction but also impairment of redox sensitive signaling that, in turn, exacerbates further mitochondrial and cellular dysfunctions. Conversely, regulation of mitochondrial function by cytosolic signaling such as JNK signaling and insulin/IGF1 signaling (IIS) was further demonstrated by studies assessing the insulin-mimicking properties of lipoic acid: *in vivo* and *in vitro* studies have shown that the effect of lipoic acid on restoring age- and AD-associated bioenergetic decline is IIS- and JNK dependent. Collectively, these studies further support the links among mitochondrial energy metabolism, formation of redox signaling molecules, integration of cytosolic signals, and the rate of aging.

Mitochondrial localized STAT3 modulates reactive oxygen species and apoptotic cell death in pancreatic cancer

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The Signal Transducer and Activator of Transcription 3 (STAT3), overexpressed in pancreatic cancer (PC), is a key molecular driver of its progression into advance PC. Besides its nuclear transcriptional role, STAT3 also resides in the mitochondria where it participates in the optimal function of the electron transport chain. Because the role of mitochondrial STAT3 in PC is still unclear, we explored the consequences of inhibiting mitochondrial STAT3 in PC, using pharmacological and genetic approaches. In human PC cells in culture and in PC xenografts, inhibition of mitochondrial STAT3 levels with phospho-valproic acid (P-V; a novel inhibitor of mitochondrial STAT3) led to an increase in mitochondrial levels of reactive oxygen species (ROS), as determined using MitoSOX Red, a molecular probe specific for mitochondrial superoxide anion ($O_2^{\cdot-}$). This was a mitochondria-specific effect, as P-V failed to induce other cellular ROS. Similarly, knocking-down STAT3 also increased the levels of mitochondrial $O_2^{\cdot-}$. In contrast, STAT3 overexpression, which included its overexpression in mitochondria, abrogated the increase in mitochondrial $O_2^{\cdot-}$ levels in response to P-V. The increase in mitochondrial ROS levels led to the collapse of the mitochondrial membrane potential followed by cytochrome c release to the cytosol, cleavage of procaspase-9 and activation of caspase-3. Furthermore, overexpression of mitochondrial localized STAT3 rescued animals from tumor growth inhibition by P-V, indicating the importance of mitochondrial STAT3 in P-V's anticancer effect. In conclusion, our results indicate that in the mitochondria STAT3 regulates ROS and apoptotic cell death, and establish mitochondrial STAT3 as a potential target for PC treatment.

Progressive alterations in brain Bioenergetic system during female reproductive aging: Implications for risk identification and disease prevention

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Previously we demonstrated that estrogen sustained and enhanced brain metabolism from a systems-level. Estrogen promotes glucose uptake, enhances glycolytic capacity and potentiates mitochondrial catalytic capacity. In the current study, we sought to investigate the temporal changes in brain bioenergetic system during female reproductive aging. To characterize the trajectory of brain bioenergetic shift, we conducted functional and biochemical analyses in female nonTg mice at different age groups (3-6-9-12-15 months). Parameters of investigation included substrate availabitlity, the aerobic glycolysis capacity, and the mitochondrial catalytic machinery efficiency. In aging female mice, alteration in brain bioenergetics was first evidenced by a decline in brain glucose uptake between 6-to-9 months; an age window coincided with female perimenopausal transition. Mechanistically such deficits could be at least partially attributed to decline in expression of neuronal glucose transporter, compromised hexokinase activity, and inactivation of pyruvate dehydrogenase complex. In parallel, there was an increase in plasma level of alternative fuel substrate β-hydroxybutyrate and a concomitant increase in its neuronal transporter between 6-to-9 months. Decline in mitochondrial catalytic capacity, such as decreased mitochondrial respiratory and compromised cytochrome c oxidase activity, reached statistical significance between 9 and 12 months. These data indicate that hormonal dysregulation during female reproductive aging induces progressive, step-wise, and more importantly systems-wide changes in brain bioenergetic system. In comparison, ablation of ovarian hormone by surgical ovariectomy in the same mouse model induced a systemic collapse of brain bioenergetic function within a short window. Collectively, these data provide a plausible mechanism for increased AD risk in menopausal women.

Nitric oxide promotes insulin responsiveness in endothelium through *S*-nitrosylation and inactivation of protein tyrosine phosphatase PTP1B

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Insulin delivery to the skeletal muscle interstitium plays a key role in insulin-directed glucose uptake by skeletal muscle. This insulin stimulation-dependent process is tightly controlled by endothelial barrier function of the capillaries connected to skeletal muscle. It has been shown that nitric oxide (NO), which is rapidly produced by endogenous NO synthase of endothelium when exposed to insulin, functions to promote insulin delivery through movement across endothelial barrier. However, to date the underlying mechanism of such NO-dependent insulin responsiveness in endothelium remains uncharacterized. This study investigated whether protein tyrosine phosphatase 1B (PTP1B), which acts as insulin receptor (IR) phosphatase, is targeted and inactivated by the NO produced in capillary endothelium exposed to insulin, thus allowing activation of IR signaling. To test this hypothesis, we developed a novel imaging approach that enables precise visualization of endogenous PTP1B susceptible to *S*-nitrosylation in endothelium following time-dependent treatment with insulin. Employing this analytic platform, we observed a fraction of PTP1B translocated to the plasma membrane of endothelium soon after insulin stimulation to be *S*-nitrosylated. Furthermore, this specific pool of *S*-nitrosylated PTP1B was co-localized with phosphorylated IR at the cell border of endothelium under the accelerating phase of insulin signaling, suggesting a critical function of NO that enhances IR activity through inhibition of PTP1B. We have also shown that NO-mediated inactivation of PTP1B depends on *S*-nitrosylation of the active-site Cys. Our results collectively reveal a novel role of NO in promoting endothelial insulin responsiveness, which is essential for insulin delivery to skeletal muscle, through reversible *S*-nitrosylation and inactivation of PTP1B.

SHC depletion stimulates brown fat activity *in-vivo* and *in-vitro*

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Adipose tissue is an important metabolic organ that integrates a wide array of homeostatic processes and is crucial for whole-body insulin sensitivity and energy metabolism. While white adipose tissue (WAT) is the primary site for triglyceride storage and fatty acid release, brown adipose tissue (BAT), however, is a key thermogenic tissue with a well-established role in energy expenditure. BAT dissipates energy, and protects against both hypothermia and obesity. Thus, increasing BAT activity is being proposed be an effective therapy for the looming pandemic of obesity and overweight. We and others have previously shown that mice deficient for SHC (ShcKO) are lean, insulin-sensitive, and resist obesity induced by a high-fat diet and ob/ob mutation. We investigated the contribution of BAT to this phenotype. PET imaging demonstrated that insulin-dependent BAT metabolic activity is higher in ShcKO mice. In addition, primary BAT cells from ShcKO mice exhibited higher mitochondrial respiration rates than controls. Furthermore, an increase in several mitochondrial and lipid-oxidative enzymes expression was observed in BAT of the ShcKO mice. Moreover, levels of brown fat-specific markers of differentiation, UCP1, PRDM16, ELOVL3, Cox8b, were higher in BAT of ShcKO mice. When Shc expression was silenced in brown adipocyte cell line BATCL using shRNA, it increased insulin sensitivity and metabolic activity of BAT cells, thus the Shc effect was cell autonomous. *In-vivo* pharmacological stimulation of ShcKO BAT resulted in higher energy expenditure. Pharmacological inhibition of BAT abolished the improved insulin sensitivity and glucose tolerance of

ShcKO mice. These data suggest that the improved BAT differentiation and activity contributes significantly to the improved metabolic phenotype of Shc-depleted ShcKO mice. In summary, the data demonstrate that *in-vitro* and *in-vivo* inhibition of Shc increases brown adipose activity and likely explains the increased insulin sensitivity and obesity resistance of ShcKO mice. Thus, inhibitors of Shc are reasonable targets for anti-obesity and anti-diabetic therapy.

NADPH oxidase-mediated ROS in activation of Toll-like receptors: a positive feedback regulator?

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Toll-like receptors (TLRs) are one of the major pattern recognition receptors (PRRs) expressed in innate immune cells linking NADPH oxidase-mediated ROS production. TLRs recognize conserved pathogen associated molecular patterns such as LPS and lipopeptides and mount immune responses for host defense. However, TLRs can be activated by non-microbial endogenous molecules including saturated fatty acids, and elicit sterile inflammation. Dysregulated TLR-mediated sterile inflammation is linked to increased risk of development of chronic inflammatory diseases. TLR agonists induce receptor dimerization and translocation of the receptor into the microdomain of plasma membrane (lipid rafts) where downstream signaling components are recruited and activated. Such propagation and amplification of the signals eventually lead to the expression of proinflammatory cytokines and type 1 interferons that orchestrate immune responses for host defense and tissue repair. NADPH oxidase (NOX2) is one important immediate downstream component of signaling pathways that is activated by TLR4 or TLR2. Agonists for TLR4 or TLR2 induce rapid recruitment of cytoplasmic NOX2 organizer $p47^{phox}$ into lipid rafts and resulting in ROS production in phagocytic cells such as macrophages and monocytes. A pharmacological inhibitor of NADPH oxidase (diphenyleneiodonium chloride) or N-acetyl-L-cysteine inhibited recruitment of not only $p47^{phox}$ but also dimerized TLR4 into lipid rafts in macrophages (RAW 264.7) suggesting that dimerization and translocation of TLR4 into lipid rafts are ROS dependent. Furthermore, another pharmacological inhibitor of NADPH oxidase (apocynin) inhibited dimerization of TLR2/1 (as determined by time-resolved fluorescence resonance energy transfer assay) in monocytes (THP-1). Together, these results raise the puzzling question as to how inhibition of the down-

stream component (NOX2) of TLRs can suppress the activation (dimerization) of the upstream receptor. These results suggest two mechanistic insight about reciprocal modulation of NOX2 and TLR activation: first, that NOX2 and TLR4 or TLR2/1 may form a signaling complex in the lipid rafts, and second, that NOX2-derived ROS may promote dimerization of TLR4 or TLR2/1 rendering a positive feedback potentiation. Such a mechanism would be consistent with the fact that detection of invading pathogens by blood leukocytes induces a rapid burst of ROS production to subdue the pathogen. How NOX2-induced ROS can promote dimerization of TLR4 or TLR2/1 is an intriguing question. One possibility is that NOX-induced ROS promotes the formation of disulfide bond between TLR4 homodimers and TLR2/1 heterodimers in lipid rafts as an analogy to many redox-sensitive proteins. Dimerization of TLRs involves formation of disulfide bonds, which may be sensitively regulated by the immediate redox status of cell's microenvironment. A significant corollary to these results is that dietary components and physiological or metabolic processes that affect cellular redox status can directly modulate TLR-mediated inflammatory responses and their consequences.

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Procyanidins induce colorectal cancer cell apoptosis: involvment of the epidermal growth factor receptor

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Colorectal cancer (CRC) is the type of cancer with the third highest incidence worldwide. Epidemiological studies indicate that consumption of fruit and vegetables containing procyanidins (PCA), polymers of flavan-3-ols, is associated with CRC lower risk. This study investigated the capacity of a hexameric PCAs (Hex) to induce apoptotic CRC cell death via inhibition of the epidermal growth factor (EGF) receptor (EGFR). Hex decreased proliferating, but not differentiated, Caco-2 cell viability (IC50: 20 μM), and cell colony formation in a dose (10-40 μM)-manner. Hex induced apoptotic cell death in Caco-2 cells within 24 h, as evidenced by caspase 3 and caspase 9 activation, DNA fragmentation, changes in nuclear morphology, and Annexin V (+) staining. Apoptosis occurred via the mitochondrial pathway, and involved the down regulation by Hex of Akt and of the Raf/MEK/ERK1/2 cascades. Upstream Akt and ERK, Hex inhibited EGF-induced EGFR phosphorylation at Tyr 1068 and Tyr 1045. In cells preincubated with Hex, this inhibition occurred even after Hex was removed from the medium, indicating that Hex effects are related to the interactions of Hex with membrane components. In the absence and presence of Hex, the EGFR was localized at lipid rafts, main sites of Hex-membrane interactions. In conclusion, results show that large PCAs can induce CRC apoptotic cell death through the inhibition of the EGFR and downstream signaling. These effects support epidemiological evidence of a role of PCAs-rich diets in the prevention of CRC.

**Contributed equally to this work.*

Acute Tocomin treatment improves endothelium-dependent relaxation in aortae from diabetic and western diet fed rats

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Tocotrienols, components of vitamin E, may have beneficial effects on vascular function in pathologies involving oxidant stress eg: diabetes. Tocomin is an extract of palm oil with high tocotrienol content. This study investigated the effect of tocomin (10^{-4} mg/mL) treatment on endothelium-dependent relaxation and superoxide (O_2^-) production in aortae from diabetic rats and rats fed a standard (SD) or western (WD) diet. Wistar rats were treated with vehicle (control (C)) or streptozotocin (50 mg/kg iv) to induce diabetes (D) for 10 weeks. Wistar Hooded rats were fed SD (7% total fat) or WD (21% total fat) for 17 weeks. Endothelium-dependent relaxation and O_2^- were measured using standard organ bath techniques and lucigenin enhanced chemiluminescence respectively. O_2^- production was increased in the diabetic and WD rat aortae and decreased in the presence of tocomin (C 573±75 vs D 1053±111 vs D+T 528±58 and SD 518±125 vs WD 953±114 vs WD+T 312±77 O_2^- counts/mg dry tissue, $p<0.05$). Diabetes impaired acetylcholine (ACh) induced endothelium-dependent relaxation that improved in the presence of tocomin (ACh R_{max} C 85±5% vs D 64±4% vs D+T 87±3%, $p<0.05$), and a WD reduced ACh sensitivity that improved with tocomin (ACh pEC_{50} SD 7.20±0.14 vs WD 6.80±0.09 vs WD+T 7.27±0.12, $p<0.05$). In conclusion, tocotrienol rich tocomin has an antioxidant effect through which it decreases O_2^- activity and improves endothelium-dependent relaxation associated with in aortae from diabetic and WD rats.

Alterations of proliferation rate, apoptotic signaling and long-term culture lifespan in mouse embryonic fibroblasts lacking NQO1

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NAD(P)H-quinone oxidoreductase 1 (NQO1) is a 31kDa flavoenzyme expressed ubiquitously that plays an important role in antioxidant defense. NQO1 is one of the best characterized detoxifying enzymes whose expression is regulated by Nrf2. NQO1 catalyzes the reduction of several quinone substrates using both NADH and NADPH. Besides its role as antioxidant, NQO1 has been involved in the regulation of cell growth and development. Although cells from many solid tumors contain increased levels of NQO1, low levels of NQO1 are also associated with tumor development and carcinogenesis. Our results show that the lack of NQO1 results in higher proliferation rates and extended lifespan of immortalized MEFs, which is consistent with a role for decreased NQO1 expression in tumor development. We have also observed increased nuclear translocation of Nrf2 in NQO1KO MEFs, as well as in liver of NQO1KO mice when compared to their Wt counterparts. An enhancement of Nrf2-mediated antioxidant response might constitute a hormetic response to the lack of NQO1 which, together with the activation of the Akt pathway, could result in increased growth and lifespan of NQO1KO cells. However, NQO1KO MEFs also exhibited higher apoptosis levels when compared to their Wt counterparts. This could be driven by the presence of dysfunctional mitochondria in NQO1KO cells, which causes outer mitochondrial membrane permeabilization and the release of apoptotic factors that trigger the apoptosis process. Deregulation of mitogenic pathways could surpass this increase of apoptotic signaling resulting in the overall stimulation of growth rate and extended long-term survival of MEFs lacking NQO1.

SNARE-protein, munc18c, is regulated by protein tyrosine phosphatase 1b in adipocytes

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Protein Tyrosine Phosphatase 1B (PTP1B) is currently a drug target for both obesity and diabetes due to PTP1B's role in regulating adiposity and insulin signaling. However, the tissue specific roles/substrates of PTP1B has not been fully elucidated. The adipose tissue has recently gained attraction due to the ability of adipose to regulate both adiposity and glucose disposal with several adipose specific drug candidates. In this study, we identified PTP1B as a direct regulator of syntaxin binding protein 3 (munc18c) phosphorylation in adipocytes. Additionally, we show nutritional regulation of munc18c, which, upon high fat feeding in various adipose tissues munc18c expression levels is decreased. We also demonstrate that PTP1B deficiency results in hyperphosphorylation of munc18c, and we show that the hyperphosphorylation of munc18c leads to disassociation from syntaxin 4. We identified the key interacting sites between PTP1B and munc18c; Y218/219 and Y521 are required for PTP1B-munc18c interaction. This is the first study to identify the phosphatase regulating munc18c and this study also directly links PTP1B directly to regulation of the glucose transporter, GLUT4.

**Equal Contribution*

Accumulation of oxidised peroxiredoxin2 in stored red blood cells

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Peroxiredoxin 2 (Prx2) is the third-most abundant protein in red blood cells. It is responsible for detoxification of endogenous low levels of hydrogen peroxide by cycling between its reduced and disulfide-bonded forms. The recycling of oxidised dimer is slow in erythrocytes, leading to an accumulation of oxidised Prx2 during oxidative stress. We tested whether storage of RBC leads to oxidation of Prx2 and compromises antioxidant defences by storing RBC under blood banking conditions and following the oxidation of Prx2 and its reversibility over time. We also evaluated the ability of the cells to reduce disulfide Prx2 generated by hydrogen peroxide induced oxidative stress, and whether Rejuvesol™ or other compounds could prevent and reverse Prx2 oxidation during and after storage. Prx2 oxidation in erythrocytes was detected by gel electrophoresis and immunoblotting.

We found that the disulfide of Prx2 accumulates exponentially over time in RBC stored for over 21 days. Oxidation that occurred during storage was not reversed when cells were incubated with glucose, Rejuvesol™ or other compounds, with the exception being DTT. This is in contrast with the observed reversal when Prx2 oxidation is induced in freshly isolated cells. However, the effect of additional hydrogen peroxide in already stored RBC was reversed. These findings may be an indication of impairment of all antioxidant systems in RBC after 21 days of storage. Nevertheless, we found that even though the reductive mechanisms might be compromised, they are not completely absent, since Prx2 was still capable to absorb additional oxidative stress.

Protein-tyrosine phosphatase 1B regulates pyruvate kinase M2 tyrosine phosphorylation

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Protein-tyrosine phosphatase 1B (PTP1B) is a physiological regulator of glucose homeostasis and adiposity and is a drug target for the treatment of obesity and diabetes. Herein, we identify pyruvate kinase M2 (PKM2) as a novel PTP1B substrate in adipocytes. PTP1B deficiency leads to increased PKM2 total tyrosine and Tyr^{105} phosphorylation in cultured adipocytes and *in vivo*. Substrate-trapping and mutagenesis studies identify PKM2 Tyr^{105} and Tyr^{148} as key sites that mediate PTP1B-PKM2 interaction. In addition, *in vitro* analyses illustrate a direct effect of Tyr^{105} phosphorylation on PKM2 activity in adipocytes. Importantly, PTP1B pharmacological inhibition increased PKM2 Tyr^{105} phosphorylation and decreased PKM2 activity. Moreover, PKM2 Tyr^{105} phosphorylation is nutritionally regulated, decreasing in adipose tissue depots after high fat feeding. Further, decreased PKM2 Tyr^{105} phosphorylation correlates with the development of glucose intolerance and insulin resistance in rodents, non-human primates and humans. Together, these findings identify PKM2 as a novel substrate of PTP1B, and provide new insights into the regulation of adipose PKM2 activity.

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Pancreatic T cell protein-tyrosine phosphatase deficiency ameliorates cerulein-induced acute pancreatitis

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Acute pancreatitis (AP) is a common clinical problem whose incidence has been progressively increasing in recent years. Onset of the disease is trigged by intra-acinar cell activation of digestive enzyme zymogens that induce autodigestion, release of pro-inflammatory cytokines and acinar cell injury. T-cell protein tyrosine phosphatase (TCPTP) is implicated in inflammatory signaling but its significance in AP remains unclear. In this study we assessed the role of pancreatic TCPTP in cerulein-induced AP. TCPTP expression was increased at the protein and messenger RNA levels in the early phase of AP in mice and rats. To directly determine whether TCPTP may have a causal role in AP we generated mice with pancreatic TCPTP deletion (panc-TCPTP KO) by crossing TCPTP floxed mice with Pdx1-Cre transgenic mice. Amylase and lipase levels were lower in cerulein-treated panc-TCPTP KO mice compared with controls. In addition, pancreatic mRNA and serum concentrations of the inflammatory cytokines TNFα and IL-6 were lower in panc-TCPTP KO mice. At the molecular level, panc-TCPTP KO mice exhibited enhanced cerulein-induced STAT3 Tyr705 phosphorylation accompanied by a decreased cerulein-induced NF-κB inflammatory response, and decreased ER stress and cell death. These findings revealed a novel role for pancreatic TCPTP in the progression of cerulein-induced AP.

Physical exercise up-regulates RCAN 1 potein isoform4 in rat skeletal muscle: Involvement of oxidative stress

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Regulator of Calcineurin1 isoform 4 (RCAN1-4) is an oxidative stress-responsive protein which inhibits calcineurin(Cn). Cn is a ubiquitously expressed Sr/Thr protein phosphatase under the control of Ca^{+2}/Calmodulin.So far RCAN1 expression has never been studied under exhaustive exercise (EE).

Methods: 24 Wistar rats were grouped and subjected to the same EE and sacrificed either immediately or either 3 hours or 6 hours after EE. A control group of 8 rats were also sacrificed. Soleus, rich in type 1 fibers; Extensor Digitorum Longus (EDL), rich in type 2 fibers; and Gastrocnemius (GC) which contains both type 1 and 2 fibers, were collected.

Results: EE increased the expression of RCAN1-4 protein in EDL and GC. Although the content of RCAN1-4 in soleus was highest amongst all, soleus RCAN1-4 levels remained unchanged after EE. Cn was down-regulated in all muscles and its enzymatic activity decreased significantly in EDL and GC, but remained unchanged in Soleus. PGC-1α, did not change in any muscle, but was more highly expressed in Soleus. Amongst NFATs, only NFATc4 was modified in all muscles and, as expected, was down-regulated. Protein carbonyl content increased only in EDL and GC but not in Soleus.

Conclusion: EE exerts different effects in different types of skeletal muscles, and on the relative levels of oxidative-stress. We propose that RCAN1-4 expression represents an important component of the physiological adaptation to exercise in fast fibers. Further investigations, using Electrical Pulse stimulation would be conducted along with pharmacological products on Diabetes Mellitus and Alzheimer's disease cell line models.

Blueberry consumption enhances brain function in healthy elderly

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Blueberries are rich in flavonoids that possess antioxidant and anti-inflammatory properties, which in rodent models are neuroprotective. The risk of developing dementia is reduced in people habitually consuming high flavonoid intakes, but data from human intervention studies is sparse. We therefore randomised sixteen healthy elderly (8 men, 8 women) to consume either 30 ml placebo (age 68±2 y; BMI, 25.7±4.6 $kg.m^{-2}$; ACE-III, 96±3) or blueberry concentrate (age 69±4 y; BMI, 24.8±2.4 $kg.m^{-2}$; ACE-III, 97±3) providing 12.9 $mg.ml^{-1}$ anthocyanin once per day for 12 weeks. Before and after supplementation, participants undertook a numerical Stroop test within a 1.5T MRI scanner while functional magnetic resonance images were continuously acquired. fMRI data was analysed based on massunivariate testing within the general linear model framework over the whole brain, treating each participant separately and constructing individual maps comparing the differences in response between visits. Group analysis was subsequently undertaken combining the individual responses, with differences between groups identified in regions with an uncorrected p-value < 0.001 and a cluster size threshold of 10 voxels

The change in Stroop test performance was not different between conditions. However, significant increases in brain activity were observed in response to blueberry supplementation relative to the placebo group within Brodmann areas 3/4/6/7/9/10/40/45/46, the precuneus and the posterior cingulate together with significantly faster haemodynamics. Collectively these data suggest that 12 weeks of supplementation with an anthocyanin rich blueberry concentrate improves active brain areas associated with cognitive function in healthy elderly.

Endogenous nutritive support following traumatic brain injury: peripheral lactate production for glucose supply via gluconeogenesis

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We evaluated the hypothesis that nutritive needs of injured brains are supported by large and coordinated increases in Lactate Shuttling throughout the body. To that end we utilized dual isotope tracer [6,6-$^{2}H_2$]glucose, *i.e.,* D_2-glucose, and [3-^{13}C]lactate) techniques involving central venous tracer infusion along with cerebral arterial-jugular bulb blood sampling. Traumatic brain injury (TBI) patients with non-penetrating head injuries (n = 14) were entered into the study following consent of patients' legal representatives. Written and informed consent was obtained from healthy controls (n=6). As in previous investigations, the cerebral metabolic rate (CMR) for oxygen decreased 49% from 2.45±0.63 (ml/100g/min), and glucose 47% from 4.55±1.02 mg/100g/min following TBI (both, $P<0.01$). However, near normal arterial glucose and lactate levels in patients studied 5.7±2.2 days (range of days 2-10) post-injury belied a 71% increase in systemic lactate production compared to control, that was largely cleared by greater (hepatic + renal) glucose production. Following TBI, gluconeogenesis from lactate accounted 67.1% of lactate disposal rate (Rd), which was compared to 15.2% in healthy controls. We conclude that elevations in blood glucose concentration following TBI results from a massive mobilization of lactate from corporeal glycogen reserves. This previously unrecognized mobilization of lactate subserves hepatic and renal gluconeogenesis. As such, a Lactate Shuttle mechanism indirectly makes substrate available for the body and its essential organs, including the brain, following trauma. Additionally, when elevations in arterial lactate concentration occur following TBI, lactate shuttling may provide substrate directly to vital organs of the body, including the injured brain.

Anti-inflammatory and antioxidant hepatoprotective activities of green tea during nonalcoholic steatohepatitis

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Nonalcoholic steatohepatitis (NASH) is characterized by oxidative stress and inflammation. We hypothesized that the antioxidant and anti-inflammatory activities of green tea extract (GTE) would protect against liver injury during NASH. We fed rats a low-fat (LF) diet containing no GTE or a high-fat (HF) diet containing GTE at 0, 1, or 2% (w/w) for 8 wk prior to evaluating liver injury, glutathione status, NFκB activation, and expression of pro-inflammatory cytokines. HF-feeding increased body and adipose mass, serum alanine aminotransferase (ALT), and histological evidence of steatosis. GTE normalized liver steatosis and serum ALT to that of LF controls without any dose-dependent differences or effects on energy intake. HF-feeding increased hepatic NFκB binding activity in association with greater phosphorylation of IκBα, and mRNA and protein of TNF-α and MCP-1. GTE reduced TNF-α and MCP-1 expression by inhibiting NFκB binding activity, which was accompanied by increases in hepatic glutathione. Hepatic NFκB binding activity was positively correlated to serum ALT, and inversely correlated to hepatic glutathione. In HC-04 cells, 0.1-5 μM epigallocatechin gallate (EGCG), the predominate green tea catechin, induced nuclear Nrf2 accumulation. HF diets fed to Nrf2-knockout mice exacerbated hepatic steatosis and serum ALT compared with Nrf2-knockout mice fed no GTE. GTE in Nrf2-knockout mice lowered hepatic lipid accumulation and serum ALT to levels no different from wild-type controls. These findings suggest that antioxidant activities of GTE mitigate hepatic inflammatory responses leading to NASH, and that GTE may protect against NASH, at least in part, in a Nrf2-dependent manner.

Prenylflavonoids as potential hepatoprotective compounds towards ethanol-induced liver injury: A mechanistic study

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The prenylflavonoids xanthohumol (XN), isoxanthohumol (IXN), 6- prenylnaringenin (6-PN) and 8-prenylnaringenin (8-PN) have been shown to present a broadspectrum of cancer chemopreventive activity. Beer is the most important dietary source of prenylflavonoids. These compounds are found in hops (*Humulus lupulus*), the bitter flavor ingredient of beer brewing. Alcoholic liver disease is one of the most common causes of chronic liver disease and in companion with chronic viral hepatitis are the leading causes of cirrhosis and hepatocellular carcinoma worldwide. Experimental evidences demonstrate that oxidative stress plays a major role in ethanol-induced liver injury, which is enhanced by Hepatitis C virus. Ethanol is shown to be metabolized to 1-hydroxyethyl radical (HER) in liver leading to the depletion of cellular antioxidants like GSH and to induce lipid and sensitive structures oxidation. Herein, we report the kinetics and thermodynamics of the interaction of prenylflavonoids and lipids with HER. Lipids were shown to react with HER with second-order rate constant varying from non-reactive (methyl oleate) to 5.2 10^7 L $mol^{-1}s^{-1}$ (conjugated methyl linoleate) depending on the number of bis-allylic hydrogens, conjugation, and structure. Prenylflavonoids were shown to react with HER with second-order rate constants close to the diffusion control ranging from 1.5 to 3.6 10^9 L $mol^{-1}s^{-1}$ for 6-prenylnaringenin (6PN) and xanthohumol (XN), respectively. On the other hand, quercetin is a poor antioxidant towards HER (k_2 ~ 10^4 L $mol^{-1}s^{-1}$). The major oxidation products were positional isomers formed by radical addition of HER molecule at the prenyl side chain of the flavonoid, excepted for XN which shows the formation of an adduct containing two HER molecules. DFT calculations and kinetics experiments with analogue flavonoids and in deuterated medium suggest the bis-allylic position in the prenyl

side chain as the reactive site, in which the H-atom abstraction leads to resonance-stabilized radical that further recombined with HER. An additional reactive site was verified for XN resulting in 1,4-addition of the radical to the α,β-unsaturated carbonyl group of XN. Prenylflavonoids are shown to be efficient antioxidants against HER induced oxidation of GSH (k_2 = 7.1 10^8 L $mol^{-1}s^{-1}$) and lipid sensitive structures being potential herbals drugs to ameliorate liver degeneration due to ethanol consumption.

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Effects of supplementation with pomegranate pulp on parameters of oxidative stress induced by exhaustive swimming in rats

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Free radicals are constantly formed in our body in controlled amounts and play some physiological roles. However, in amounts above those acceptable becomes a risk factor for proper functioning of the body. This can be benefited by the presence of exogenous antioxidants such as phenolic compounds found in pomegranate (Punica granatum). This study addresses the evaluation of antioxidant activity - In vivo assessment of the antioxidant potential of the alcoholic extract of pomegranate pulp through the analysis of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) in muscle tissue (gastrocnemius and soleus) and plasma after an exhaustive exercise protocol performed acute swimming with a group of 40 rats divided into control group-CO (n = 10); supplemented exercised-dose I-SGI (n = 10);-supplemented exercised II dose SGII-(n = 10) and non-supplemented group-exercised-NS (n = 10) - in vitro method ORAC, DPPH and ABTS, quantification of phenolic compounds from fruit samples. Muscle tissues and plasma showed a significant decrease in the levels of lipid peroxidation. There was a decrease in enzyme activity in the supplemented groups. All results indicated a high antioxidant fruit. The chronic administration of pomegranate extract favored the protection against excessive oxidative attack.

The long-term effects of a Mediterranean diet intervention on biomarkers of vascular wall inflammation and plaque vulnerability

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Previous studies have associated a high adherence to traditional Mediterranean diet (MD) with reduced incidence of coronary heart disease and mortality. However, whether the signaling pathways involved in atherogenesis are affected by the MD at molecular and gene levels are still unknown. So, 285 participants included in the Prevention with Mediterreanean Diet trial (PREDIMED) were randomly assigned into 3 intervention groups: a MD supplemented with extra-virgin olive oil (MD+EVOO) or nuts (MD+Nuts), and a low-fat diet (LFD). At baseline and after 3 years of intervention, 13 inflammatory biomarkers (IL-1β, IL-6, IL-8, IL-12p70, IL-18, IFNg-γ, TNF-α, IP-10, ENA78, ITAC, MCP-1, MIP-1β and RANTES) were determined by Luminex and 10 gene expression

Toll-like receptors 2, 4, 6 (TLR), inflammasome (NLRP3), caspase-1 (CASP-1), Interleukin-1 receptor type 1 (IL-1R1) and chemokine receptors type (CCR2, CCR5, CXCR2 and CXCR3) were evaluated by RT-PCR using RNA isolated from blood. hs-CRP was determinated by standard enzyme-linked immunosorbent assays. Our results were that after 3 y, both MD showed a significant reduction in the plasma concentrations of IL-6, IL-8, hs-CRP, TNF-α, MCP-1, MIP-1β, RANTES and ENA78 ($P<0.05$;all) and MD+EVOO and MD+nuts showed reduced levels of IFN-γ and TNF-α ($P\leq0.04$;both), respectively. The gene expression of TLR2, TLR6, CCR2, CCR5, CXCR2 and CXCR3 for LFD also was significantly increased ($P<0.05$). We concluded, that a long-term intake of MD competes with CVDs and aging, keeping the gene expression levels and decreasing plasma levels of inflammatory molecules in older persons with high cardiovascular risk.

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Transplantation of peptide-labelled mitochondria ameliorates a loss of Purkinje cells and motor function deterioration in a mouse model of Spinocerebellar Ataxia Type 1

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Mitochondria are subcellular organelles whose major function is to generate energy and implicated on events of oxidative stresses. Mitochondria dysfunction expedites the progression of spinocerebellar or sensory ataxia but the central role is still undefined. Presently, through the peptide-mediated delivery system of mitochondria organelle, mitochondrial regulation on effect of spinocerebellar ataxia type 1 (SCA1) process will be studied. Allogeneic mitochondriaderived from kidney mesangial cell line were conjugated Pep-1 (P-Mito) prior to individual transplantation into the cerebellar white matter (WM) or Purkinje cell layer (PCL) of Spinocerebellar ataxia type 1 (SCA1, B05 strain) mice, expressing human ataxin-1 with an expanded polyglutamine (82 CAG repeats) , when they showed dramatically PC loss (30 weeks of age). After 9 weeks transplantation, the motor coordination examined by rotarod test in P-Mito-grafted SAC1 mice was improved to compare with groups of sham-treated controls, mitochondria alone and Pep-1 alone, and the better performance was observed at grafting in PCL than in the WM area. No grafted P-Mito were observed in cerebellar cortex after long-term grafting but it had the more surviving PCs relative to the non-treated contralateral control as well as the higher activity of mitochondrial electron transport chain complex III in cerebellum. Moreover, both of immunoreactivity for phosphorylated and total S6 ribosomal protein, a downstream target of the mammalian target of rapamycin (mTOR) pathway, were significantly decreased in grafted side to the non-treated contralateral control. Thus, we postulate that benefit effect from the Pep-1-mediated mitochondrial grafting could be associated with the activation of protective autophagy. Further, study of the selective autopahgy involving mitophagy and the clearance of mutant protein aggregates are ongoing.

Evaluation of antimicrobial and antioxidant activity of bioactive compounds present in vegetables and spices consumed in Brazil

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Polyphenols are present in all plant foods and their classes and levels vary among diets depending on the type and quantity of vegetables in the diet. Several population studies have reported that the consumption of these compounds has an inverse correlation with the risk of diabetes, cancer, coronary and degenerative diseases. These benefits could be associated with many biological activities that polyphenols may have, such as antioxidant, anticancer, anti-inflammation, antiaging and antimicrobial. This study aim to investigate in ethanolic extracts the antioxidant capacity and antimicrobial potential against pathogenic and probiotics microorganisms of eight vegetables (sage, white pepper, pomegranate peel and pulp, curry, ginger, nutmeg and cinnamon). The pomegranate peel had the highest total phenolic compounds and antioxidant activity measured by FRAP while ginger and white pepper had the lowest values, respectively ($p<0.05$). When determining the antibacterial activity by the disc diffusion test *Lactobacillus casei shirota* and *Lactobacillus casei defensi* were slightly inhibited by all extracts. *Staphylococcus aureus* was weakly inhibited by white pepper and *Bacillus cereus* by curry, whereas pomegranate peel and nutmeg showed strong inhibitory effects for both strains when compared to ampicillin inhibition ($p<0.05$). This difference between antimicrobial activity against pathogenic and probiotics strains suggest that phenolic compounds reacts differently towards different microorganisms. The results showed that the order of the antioxidant capacity of extracts is different than the one obtained for antimicrobial activity and phenolic content. This may indicate that the properties of phenolic compounds are more related with their types than content. In conclusion, pomegranate is the vegetable with better potential to be used in functional food development.

A possible role of mevalonate pathway in embryogenesis of G6PD-deficient *C. elegans*

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Glucose-6-phosphate dehydrogenase (G6PD) is a housekeeping enzyme, which generates NADPH for reductive biosynthesis and the maintenance of cellular redox balance. It has been known that defective lipid biosynthesis undermines embryonic development in *C. elegans*. Our previous data showed that G6PD-deficient *C. elegans* exhibits defective embryogenesis. Recently, our preliminary data suggest that certain embryonic defects phenocopy the consequence induced by impaired lipid synthesis. Specifically, G6PD-deficient *C. elegans* embryo showed increased dye permeability (60-70%, $N>3$, $p<0.001$) by Hoechst staining, defective permeability barrier indicated by abnormal localization of mCherry::CPG-2 and impaired eggshell structure shown by electron transmission microscopy. Cholesterol, produced by mevalonate pathway in human, is an essential molecule of lipid bilayer membrane and may affect the integrity of embryonic permeability barrier. Furthermore, mevalonate is produced by NADPH dependent HMG-CoA reductase (*hmgr-1*). Hence we investigated whether altered mevalonate pathway plays a role in G6PD-deficiency-induced defective embryonic permeability and barrier integrity in *C. elegans*. The knockdown of HMRG-1 showed increased dye permeability. In addition, exogenous supplement of different concentrations of cholesterol (0.05 mg/ml to 0.1 mg/ml) could partial-

ly rescue Hoechst dye permeability and permeability barrier integrity of G6PD-deficient embryo (40-50 %, $N>3$, $p<0.05$). In summary, these findings suggest a role of mevalonate pathway in G6PD deficiency-induced defective embryonic defects but the detail mechanism remains to be elucidated.

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Caveolin-1 provides palliation for adverse hepatic reactions in hypercholesterolemic rabbits

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Caveolins are an essential component of cholesterol-rich invaginations of the plasma membrane known as caveolae, including vesicular transport, cholesterol homeostasis, and signal transduction. We investigated the effects of CAV-1 on mitochondrial biogenesis and antioxidant enzymes in hypercholesterolemia-affected target organs. A total of eighteen male New Zealand white rabbits were divided into three groups: a normal-diet group, an untreated hypercholesterolemia-induced group, and a hypercholesterolemia-induced group that received intravenous administration of antennapedia-CAV-1 peptide every 2 days for 2 weeks. Circulating cholesterol and low-density lipoprotein cholesterol (LDL-C) levels differed significantly between the three groups ($P < 0.05$). Immunohistochemical staining intensity of CAV-1 was greater in AP-CAV-1-treated rabbits than in untreated rabbits, especially in the vicinity of the liver vasculature. The high levels of neutral lipids, malondialdehyde, peroxisome proliferator-activated receptor-γ coactive 1α (PGC-1α), and nuclear respiratory factor-1 (NRF-1) seen in untreated hypercholesteremic animals were attenuated by administration of AP-CAV-1 ($P < 0.05$). In addition, mitochondria in animals that received treatment exhibited darker electron-dense matrix and integrated cristae. Furthermore, the levels of ROS modulator 1 (Romo1), superoxide dismutase (SOD)-2, and catalase activity were significantly lower in CAV-1-treated hypercholesterolemic rabbits ($P < 0.05$). AP-CAV-1 treatment also restored mitochondrial respiratory chain subunit protein content (OXPHOS complexes I-V, $P < 0.05$). Furthermore, AP-CAV-1 treatment significantly suppressed apoptotic cell death, as evidenced by a reduction in the number of TUNEL-positive cells. Our results indirectly indicate that CAV-1 mediates the negative effects of PGC-1α on hepatic mitochondrial respiratory chain function, promotes the antioxidant enzyme defence system, and maintains mitochondrial biogenesis.

The correlations of heart rate variability and a number of metabolic parameters in healthy sedentary young subjects

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Sedentary lifestyle is major risk factor for diabetes, cardiovascular and other diseases. It is known observation that the heart rate variability (HRV) is dramatically reduced in conditions associated with oxidative stress (OS). We hypothesize that quantitative and qualitative changes of HRV may reflect early metabolic derangements leading to the development of disease. The aim was to evaluate HRV parameters and analyze its correlations with a number of variables including metabolic, immunological and OS parameters in sedentary subjects. 30 healthy sedentary male subjects aged 20-40, nonsmokers were enrolled. Athletes, obese and men of physical work were excluded. HRV parameters were derived from 5 min. ECG records in supine position and orthostatic test. Heights, weight, body mass index (BMI), age and body composition, estimated by bioelectric impedance were evaluated. The biochemical parameters included: lipid profile (total, LDL and HDL cholesterol, triglycerols); hormones (thyroid panel, insulin and C-peptide, aldosterone, cortisol, and testosterone); inflammatory markers (C-reactive protein, TNF-alpha, interleukins 6 and 10, and *Helicobacter pylori* status); parameters of oxidative stress (comet-assay for DNA damage in lymphocytes, 1,4-dihydroxynonan mercapturic acid in urine), blood glucose and HbA_{1C}. Linear correlation coefficient (r) and other statistics were calculated with Statistica 7.0. Expectedly the most of parameters were found to be within the normal range. However some subjects have had the levels of LDL-cholesterol, triglycerols, insulin, C-reactive protein, HbA_{1C} and DNA-damage slightly elevated (marginal clinically not significant increase). A wide panel of correlations was obtained. Moderate to strong significant mainly negative correlations were observed be-

tween some time domain and frequency domain parameters of HRV and BMI, age, fat tissue (visceral fat in particular), triglycerols, C-reactive protein and HbA_{1c}. A number of mild correlations were determined for the wide variety of parameters including OS markers. Thus, the evidence that even mild metabolic shifts may provide notable changes in quantitative and qualitative characteristics of HRV in healthy sedentary young subjects has been confirmed.

Untargeted MS/MS^{ALL} lipidomics reveals loss of a key phospholipid in vitamin E-deficient zebrafish brains

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We hypothesized that vitamin E deficient zebrafish will undergo increased lipid peroxidation and loss of specific, highly polyunsaturated fatty acids. To test this hypothesis, zebrafish were fed for 4 months defined diets without (E-) or with added vitamin E (E+, 500 mg RRR-α-tocopheryl acetate/kg diet). Dietary α-tocopherol concentrations were E- 1.6 ± 0.1 and E+ 334 ± 127 mg/kg. Fish heads were homogenized with chloroform:methanol (1:2) and extracted according to Bligh and Dyer. The untargeted lipidomics analysis (MS/MS^{ALL}) revealed significant differences in the lipid profiles. DHA-PC 38:6 (PC 16:0/22:6) was the lipid that showed the most significant fold-differences between the two groups. Another group of fish were used for specific quantification of DHA-PC in dissected brains from E+ and E- fish (n=10/group) using a triple quadruple LC-MS/MS. Brain DHA-PC 38:6 concentrations were approximately 1/3 lower in E- fish (4.3 ± 0.6 μg/mg brain, mean ± SEM) compared with E+ fish brains (6.5 ± 0.9, P=0.0413). DHA-PC 38:6 has been reported as a plasma biomarker of increased Alzheimer's disease risk in humans. Therefore, these results indicate that inadequate vitamin E status results in a specific depletion in a critical brain phospholipid that is associated with increased Alzheimer's disease risk.

(-)-Epicatechin mitigates insulin resistance by modulating redox-sensitive signals in animal models of metabolic syndrome

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The incidence of metabolic syndrome (MetS) in the world's adult population is becoming a major public health concern. Epidemiological studies show that consumption of fruit and vegetables in humans decreases the risk for MetS. The flavan-3-ol (-)-epicatechin (EC) is present in large amounts in fruit and vegetables. We previously showed that EC improves parameters of inflammation and insulin sensitivity in adipocytes in vitro. The aim of this study was to investigate, in *in vivo* models of MetS, the capacity of dietary EC to improve insulin sensitivity and the involvement of redox-sensitive signals underlying MetS-induced insulin resistance. The effects of dietary EC (20 mg/kg body weight) on the above parameters were assessed in two rodent models: A-MetS induced by high fructose (HFr) consumption in rats; B-MetS induced by high fat (HFat) consumption in mice. After 8 w, although glucose (GTT) and insulin (ITT) tolerance tests were similar among groups, HFr rats required higher insulin levels to maintain control plasma glucose levels. Mice fed the HFat diet developed significant alterations in GTT and ITT after 8 w, showing higher fasting glucose and insulin levels than controls. Dietary EC improved parameters of insulin resistance in both models of MetS. In liver and adipose tissue, EC inhibited/mitigated MetS-triggered impairment of the insulin pathway. EC acted in part inhibiting events that underly MetS-induced insulin resistance: i- oxidative stress and increased NADPH oxidase expression, ii- activation of redox-sensitive signals (JNK, IKK/NF-κB), iii- inflammation, and iv- select subarms of the unfolded protein response. Results indicate that dietary EC supplementation may provide potential benefits against insulin resistance and MetS.

Development of a high-throughput screen for discovery of leber's herediatry optic neuropathy (LHON) therapeutics

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Leber's hereditary optic neuropathy (LHON) is a maternally inherited, mitochondrial disease, causing blindness. Point mutations in the complex I-encoding genes in mitochondrial DNA, and a subsequent complex I-associated bioenergetic defect are thought to be the causal factors of LHON. Currently, no therapeutic agent is approved in the US to prevent or reverse LHON vision loss. Drug discovery efforts for a viable therapeutic treatment of LHON have been hampered by absence of a high throughput screening system. We observed a significant difference in ATP synthesis rate between the rotenone-treated (0.03 μM, 2 h) control (no mutation) and mutant (11778 mutation) cybrid cells. We hypothesized that the difference in rotenone-sensitivity between the control and mutant cells is due to the complex I defect associated with the 11778 mutation, and the drugs increasing ATP synthesis in rotenone-treated mutant cell lines would be a possible candidates for LHON therapy. Since the drug development process is expensive and time-consuming, we have chosen to 're-purpose' existing FDA-approved drugs for a potential benefit in LHON. A 96-well format cell-based assay for complex I driven ATP synthesis was developed to screen a library of 1600 FDA-approved drugs for LHON drug discovery using mutant (11778 mutation) osteosarcoma cybrids. Multiple agents reversing the rotenone sensitivity of the mutant cells (11778 mutation) have been identified. Papaverine dose-dependently reversed the effect of rotenone on the complex-I driven ATP synthesis in mutant (11778 mutation) cybrids. Further investigation of papaverine in different LHON models will be required to confirm its beneficial effect in LHON.

Antioxidant activity and phytochemical screening of some Sudanese medicinal plants

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Five Sudanese medicinal plants namely; *Grewia tennax*.(GT), *Guiera senegalensis*.(GS), *Khaya sengalensis*.(KS), *Geigeria alata*.(GA), and *Sida alba*.(SA), belong to the families *Tiliaceae*, *Combretaceae*, *Meliaceae*, *Asteraceae* and *Malvaceae* respectively, have been collected from northern Kordofan State (West of Sudan), extracted by 80% Ethanol and fractionated using petroleum ether, chloroform, ethyl acetate, n-butanol, and distilled water. Plants extracts and fractions were screened for their antioxidant activities via 2, 2-Diphenyl-Picryl-Hydrazyl Radical DPPH, and iron chelating assays. The DPPH radical scavenging assay demonstrated that, 80% ethanolic extract of GS was the most active extract showing interesting antioxidant activity (IC_{50} 195 ± 6.9 µg/ml) that was more potent than the standard antioxidant propyl gallate (PG, IC_{50} 221 ± 24.0 µg/ml). The extracts of GS, SA, GA and KS exhibited potent DPPH scavenging activity in a concentration dependent manner. The radical scavenging activities (RSA) of GS, SA, GA, and KS at concentration of 500 µg/ml were found to be 74% ± 0.02, 66% ± 0.05, 53% ± 0.04, and 52% ± 0.03, respectively. The antioxidant results of GT, GS, GA, SA, and KS plant fractions showed that, the activity against DPPH free radical were very intense in the butanol and ethyl acetate fractions. On the other hand, these plants have shown moderate activity ranged from 28% to 5 % via iron chelating antioxidant assay. The results of phytochemical screening showed that all extracts and fractions of studied plants contain flavonoids, saponins, triterpenes, steroids, cumarines and tannins.

Coenzyme Q levels adaptations to calorie restriction and dietary fat in mouse liver and skeletal muscle

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Coenzyme Q (Q) is a lipid-soluble electron carrier and a powerful antioxidant. Dietary n-6 polyunsaturated fatty acids (n-6 PUFA) increase hepatic Q levels with aging. To investigate if these effects were altered by calorie restriction (CR) we established two groups of mice fed lifelong diets containing soybean oil (high in n-6 PUFA) as the main fat source, either *ad libitum* or under CR. We also studied two additional CR groups fed diets containing lard (high in saturated and n-9 monounsaturated fatty acids) or fish oil (high in n-3 PUFA). Liver and skeletal muscle samples were taken after 6 or 18 months of dietary intervention starting at an age of 3 months. Hepatic Q_9 and Q_{10} levels increased and Q_9/Q_{10} ratio decreased with age in the control but not in the CR group. When comparing the three CR diets it was found that Q_9 and Q_{10} levels were maximal in CR-fish, and Q_9/Q_{10} ratio was the lowest in CR-soy and CR-fish. In skeletal muscle, levels of coenzyme Q_9 did not change either by CR or age but Q_{10} levels increased significantly with age in the control although not in CR group. Q_9 levels were higher in CR-soy compared with CR-lard, whereas CR-fish displayed the lowest Q_9/Q_{10} ratio. We demonstrate that: i) CR abolishes age-induced changes of Q levels in liver and skeletal muscle from mice fed PUFA-enriched diets, and ii) liver and skeletal muscle tissues adapt to a PUFA-enriched CR diet by increasing Q levels and decreasing Q_9/Q_{10} ratio.

Cardiac rehabilitation induce changes in blood plasma properties of ischemic heart disease patients

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Physical activity improve the state of patients, thus in this work we investigated the properties of blood plasma of men after physical exhaustion with coronary heart disease (CHD) before and after cardiac rehabilitation (CR). The following parameters: lipid peroxidation (TBARS), hydroperoxide, thiols level and antioxidant capacity (AC) were examined before and after four week CR.

Venous blood was taken before, immediately after and 1h after an exhaustive incremental cycling test (30 $W \cdot min^{-1}$ ramp) performed by 12 male volunteers: age (52 ± 6.4 years); height (172 ± 3 cm), weight (84.2 ± 12.9 kg), BMI (28 ± 4.2).

Model rehabilitation was established as 35 min cycling on an ergometer. Cycling was repeated three times a week for two months and modified according to specific state of each patient. Experiments were approved by Local Ethic Committee. The standard blood parameters were determined.

Lipid peroxidation was determined using thiobarbituric acid and xylenol orange (hydroperoxide) methods. The decrease in TBARS and hydroperoxide levels after CR was observed. However, we did not find changes in these parameters just after exercise and 1 hour later. On the other hand, the increase in the level of –SH groups was found after CR 1 hour after exercise. We observed also the increase in AC using FRAP and DPPH methods.

We conclude that CR improve main parameters of whole blood and investigated plasma: decrease of oxidative stress and increase of AC as well as thiols level.

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Friedeich's ataxia cell and animal models express higher levels of inflammatory lipid mediators resulting from increased cyclooxygenase 2

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Friedreich's ataxia results from an inherited deficiency of the mitochondrial protein frataxin, the mechanism by which this deficiency triggers neurodegeneration and cardiodegeneration is unclear. Microarrays of neural tissue of animal models of the disease showed decreases in antioxidant genes, and increases in inflammatory genes. Cycloxygenase-derived oxylipins are important mediators of inflammation. We measured oxylipin levels using tandem mass spectrometry and ELISAs in multiple cell and animal models of Friedreich's ataxia. Mass spectrometry revealed increases in concentrations of prostaglandins, Thromboxane B2, 15 -HETE and 11-HETE in cerebellar samples of KIKO mice. One possible explanation for the elevated oxylipins is increased cyclooxygenase 1 or 2 expression and activity, and these were investigated. While constitutive cyclooxygenase 1 was unchanged, inducible cyclooxygenase 2 (COX2) expression was elevated over 1.35 fold ($p<0.05$) in two Friedreich's mouse models and Friedreich's lymphocytes. Consistent with higher COX2 expression, its activity was also increased over 1.58-fold over controls. COX2 expression is driven by multiple transcription factors, including activator protein 1 (AP1) and cAMP response element-binding protein (CREB), both of which were elevated over 1.52-fold in cerebella. Taken together, the results support the hypothesis that reduced expression of frataxin leads to elevation of COX2-mediated oxylipin synthesis stimulated by increases in transcription factors that respond to increased reactive oxygen species. These findings support a neuroinflammatory aspect to Friedreich's ataxia, which may have both pathomechanistic and therapeutic implications.

Soluble epoxide hydrolase deficiency ameliorates cerulein-induced acute pancreatitis in mice

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Acute pancreatitis (AP) is a frequent gastrointestinal disorder that causes significant morbidity and its incidence has been progressively increasing in recent years. AP starts as a local inflammation in the pancreas that often leads to systemic inflammatory response and complications. Soluble epoxide hydrolase (sEH) is a cytosolic enzyme whose inhibition in murine models has beneficial effects in inflammatory diseases. However, its significance in AP remains unclear. In this study we investigated the role of sEH in cerulein-induced AP. sEH expression was increased at the protein and messenger RNA levels in the early phase of AP in mice. To determine whether sEH may have a causal role in AP we utilized sEH knockout (KO) mice. Amylase and lipase levels were lower in cerulein-treated sEH KO mice compared with controls. In addition, pancreatic mRNA and serum concentrations of the inflammatory cytokines IL-1β and IL-6 were lower in sEH KO mice compared with controls. Further, sEH KO mice exhibited decreased cerulein-induced NF-κB inflammatory response and decreased cell death. In summary, these findings revealed a novel role for sEH in the progression of cerulein-induced AP.

Effects of gestational zinc deficiency on placental 11β-hydroxysteroid dehydrogenase type 2

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A decreased zinc availability during gestation can affect the development of the fetal central nervous system (CNS). Exposure of the fetus to high glucocorticoid (GC) levels can affect the fetal hypothalamic-pituitary-gonad axis leading to impaired CNS development. This work investigated the hypothesis that the deleterious effects of zinc deficiency on CNS development can be due to a disruption of maternal/fetal GC homeostasis through a deregulation of placental 11β-hydroxysteroid dehydrogenase type 2 (11ßHSD2). This enzyme metabolizes maternal cortisol protecting the fetus from excess GCs. Rats were fed control or marginal zinc (MZD) diets from gestation day 0 through gestation day 19. MZD did not affect general maternal and fetal outcomes. Maternal and fetal weight, live fetuses per litter and placenta weight were similar between the groups. Placenta 11ßHSD2 protein levels were higher in the MZD group than in controls, with a trend for higher 11ßHSD2 mRNA levels. The effects of zinc deficiency on human placenta BeWo trophoblastic cells were next investigated *in vitro*. Incubation of BeWo cells in zinc deficient medium significantly decreased 11ßHSD2 expression. These results indicate that 11ßHSD2 upregulation in placentas from MZD rats can be a consequence of a stimulatory effect resulting from increased maternal GCs rather than an effect of zinc per se.

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Allopregnanolone preclinical therapeutic development in relevant aging and AD small animal models

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To develop allopregnanolone as a therapeutic for Alzheimer's disease, we investigated multiple formulations and routes of administration in translationally relevant animal models. Pharmacokinetic analyses of intravenous allopregnanolone in rabbit and mouse indicated peak plasma and brain levels (3-fold brain/plasma ratios) at 5min sufficient to activate neuro-regenerative responses at sub-sedative doses. Slow-release subcutaneous suspension of allopregnanolone displayed 5-fold brain/plasma ratio at C_{max} at 30min. At therapeutic doses by either subcutaneous or intravenous routes, allopregnanolone mouse plasma levels ranged between 34-51 ng/ml by 30 min, comparable to published endogenous human level in the third trimester of pregnancy. In rabbit and mouse, allopregnanolone elimination half-life in plasma was 3.3h and 4h, respectively. Neurogenic efficacy was achieved with allopregnanolone brain exposure of 300-500 hr/ng/g. Exposure to allopregnanolone, administered within a safe and tolerable dosage range, increased hippocampal markers of neurogenesis in young 3xTgAD and aged wildtype mice. Subcutaneous, topical (transdermal and intranasal), and intravenous allopregnanolone increased proliferation markers including PCNA. Intravenous allopregnanolone transiently and robustly phosphorylated CREB within 5min and increased levels of neuronal differentiation transcription factor Neu-

roD within 4h. In rats, the maximally tolerated dose of allopregnanolone was 8 mg/kg for both subcutaneous and intramuscular routes and 2 mg/kg for intravenous route accompanied by 30 min, 45 min, and 10min maximal sedation respectively. Compared to females, males exhibited ≥ 40% greater sedation behavior time. Allopregnanolone formulated in sulfobutyl-ether-beta-cyclodextrin at 1:6 complexation resulted in optimal, by rate and efficacy, allopregnanolone delivery to brain by all routes of administration and will advance to clinical trials for Alzheimer's disease.

PTP1B deficiency exacerbates cerulein-induced acute pancreatitis in mice

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Background: Acute pancreatitis (AP) is a common clinical problem whose incidence has been progressively increasing in recent years. The Onset of AP is trigged by intra-acinar cell activation of digestive enzyme zymogens that induce autodigestion, release of pro-inflammatory cytokines and acinar cell injury. Protein-tyrosine phosphatase 1B (PTP1B) is a physiological regulator of glucose homeostasis and energy balance. Additionally, PTP1B has been reported to regulate cytokine and inflammation signaling in vitro. However, the role of PTP1B in AP remains largely unknown.

Results: In this study we assessed the role of pancreatic PTP1B in cerulein-induced AP. PTP1B expression was increased at the protein and messenger RNA levels in the early phase of AP in mice and rats. To directly determine whether PTP1B may have a causal role in AP we generated mice with pancreatic PTP1B deletion (panc-PTP1B KO) by crossing PTP1B floxed mice with Pdx1-Cre transgenic mice. Serum levels of amylase and lipase were higher in panc-PTP1B KO mice compared with controls. In addition, the inflammatory cytokines TNFα, IL-1α and IL-6 were increased in panc-PTP1B KO mice. At the molecular level, panc-PTP1B KO mice exhibited enhanced cerulein-induced NF-κB inflammatory response, and increased ER stress and cell death.

Conclusion: Our findings revealed a novel role for pancreatic PTP1B in the progression of cerulein-induced AP.

Lipoic acid restores age-associated impairment of brain energy metabolism through the modulation of Akt/JNK signaling and PGC1α transcriptional pathway

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This study examines the progress of a hypometabolic state inherent in brain aging with an animal model consisting of Fischer 344 rats of young, middle, and old ages. Dynamic microPET scanning demonstrated a significant decline in brain glucose uptake at old ages, which was associated with a decrease in the expression of insulin-sensitive neuronal glucose transporters GLUT3/4 and of microvascular endothelium GLUT1. Brain aging was associated with an imbalance of the PI3K/Akt pathway of insulin signaling and JNK signaling and a downregulation of the PGC1α mediated transcriptional pathway of mitochondrial biogenesis that impinged on multiple aspects of energy homeostasis. R-(+)-lipoic acid treatment increased glucose uptake, restored the balance of Akt/JNK signaling, and enhanced mitochondrial bioenergetics and the PGC1α-driven mitochondrial biogenesis. It may be surmised that impairment of a mitochondria-cytosol-nucleus communication is underlying the progression of the age-related hypometabolic state in brain; the effects of lipoic acid are not organelle-limited but reside on the functional and effective coordination of this communication that results in improved energy metabolism.

Astrocytic metabolic and inflammatory changes as a function of age

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Primary astrocytes from 7-, 13-, and 18-month-old Sprague Dawley male rats were isolated to investigate age-dependent metabolic-inflammatory axis. Data showed age-dependent activation of NFκB signaling in the cytosol as well as the translocation of NFκB to the nucleus. Astrocytes also displayed augmented responses with age to inflammatory cytokines, IL-1β and TNFα. Activation of NFκB signaling resulted in increased expression of iNOS, leading to elevated NO production. Increased H_2O_2 generation was also observed with age, which was likely linked to increased NADPH oxidase 2 (NOX2) levels. Increased release of NO and H_2O_2 from astrocytes could serve as inflammatory mediators that cause neuronal damage in aging. Interestingly, primary astrocytes demonstrated an age-dependent increase in mitochondrial oxidative metabolism respiring on glucose-derived substrates, which was likely due to increased mitochondrial biogenesis. Enhanced astrocytic mitochondrial respiration is expected to limit substrate supply to neurons, and may be viewed as an adaptive response to altered cellular inflammatory-redox environment that develops with age. In line with that, IL-1β and TNFα treatment stimulated mitochondrial oxidative metabolism and mitochondrial biogenesis in astrocytes. It may be surmised that increased mitochondrial aerobic metabolism and inflammatory responses are interconnected and support the functionality switch of astrocytes, from neurotrophic to neurotoxic with age.

Ginger oil-mediated down-regulation of adipocyte specific genes inhibits adipogenesis and induces apoptosis in adipocytes

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Dietary ginger (*Zingiber officinale* Roscoe) is known to suppress body weight gain and body fat accumulation. The objective of this study was to investigate the effects of ginger oil on adipogenesis, proliferation and apoptosis of adipocytes. After treatment with ginger oil, 3T3-L1 adipocytes were stained with Oil-Red-O to visualize lipid droplets and were then measured for lipid content. Mature adipocytes were treated with ginger oil (100 and 200 μg/ml), and the expression of adipocyte-specific genes was investigated using real-time RT-PCR. The ginger oil was shown to inhibit adipogenesis and proliferation of adipocytes as evidenced by dose-dependent increase in apoptosis. The ginger oil down-regulated the expression of PPARγ (peroxisome proliferator-activated gamma), C/EBPα (CCAAT/enhancer binding protein-alpha), SREBP1c (sterol regulatory element binding proteins-1c) and AKT1 (thymoma viral proto-oncogene 1) as compared to the control. Our results suggest that ginger oil effectively suppresses adipogenesis, mainly through the down-regulation of PPARγ, C/EBPα, and SREBP1c, subsequently suppressing the expression of AKT1 gene which inhibits proliferation and differentiation and induces apoptosis in adipocytes. Ginger oil may alter fat mass by directly inhibiting adipogenesis and inducing apoptosis in adipocytes and therefore may have applications for the treatment and prevention of obesity.

The microRNA basis of palm tocotrienol neuroprotection

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Glutathione depletion and 12-lipoxygenase dependent metabolism of arachidonic acid are known to be implicated in neurodegeneration associated with acute ischemic stroke (AIS). The objective of this study was to investigate the significance of miR-29 in neurodegeneration associated with AIS. Neural cell death caused by arachidonic acid insult of glutathione-deficient cells was preceded by a 12-lipoxygenase dependent loss of miR-29b. Delivery of miR-29b mimic to blunt such loss was neuroprotective. miR-29b inhibition potentiated such neural cell death. 12-Lipoxygenase knockdown and inhibitors attenuated the loss of miR-29b in challenged cells. *In vivo*, stroke caused by middle-cerebral artery occlusion (MCAO) was followed by higher 12-lipoxygenase activity and loss of miR-29b as detected in laser captured infarct site tissue. 12-Lipoxygenase knock-out mice demonstrated protection against such miR loss. miR-29b gene delivery markedly attenuated stroke-induced brain lesion. Oral supplementation of α-tocotrienol, a vitamin E 12-lipoxygenase inhibitor, rescued stroke-induced loss of miR-29b and minimized lesion size. This work provides first evidence demonstrating that loss of miR-29b at the infarct site is a key contributor to stroke lesion. Such loss is contributed by activity of the 12-lipoxygenase pathway providing maiden evidence linking arachidonic acid metabolism to miR-dependent mechanisms in stroke.

Stable magnetic isotopes as nutrition additives: premises and promises

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The goal of this paper is to demonstrate that stable magnetic isotopes can be promising as the nutrition additives in biomedicine. Of special interest is magnesium as one of the most abundant cell elements. Among three stable isotopes, ^{24}Mg, ^{25}Mg and ^{26}Mg with natural abundance approximately 79, 10 and 11 %, only ^{25}Mg is magnetic (nuclear spin $I = 5/2$) whereas ^{25}Mg and ^{26}Mg are non-magnetic (spin-less, $I = 0$). The striking effect of ^{25}Mg was revealed in the experiments with yeast cells of *S. cerevisiae*. After UV irradiation, the recovery process of the cells enriched with ^{25}Mg was progressed more effectively than the recovery of the cells enriched with the nonmagnetic isotopes. The rate constant of post-radiation recovery was twice higher for the cells enriched with ^{25}Mg (Grodzinsky et al., 2011). With another commonly accepted cell model, *E. coli*, it was found that the bacterial cells essentially faster adapt to the growth media enriched with ^{25}Mg by comparison to the media enriched with ^{24}Mg or ^{26}Mg (Koltover, Korolev, and Kutlakhmedov, 2012; Koltover, 2013). Furthermore, it was revealed that magnetic ^{25}Mg essentially stimulates, 2-2.5 times by comparison to nonmagnetic ^{24}Mg and ^{26}Mg, the ATP hydrolysis reaction catalyzed by myosin isolated from smooth muscles (Koltover, Labyntseva, Karandashev, and Kosterin, 2014). The discovery of the nuclear spin catalysis in living cells obviously enables potential applications of the stable magnetic isotopes as the "hormetic" nutrition additives, including synthesis of novel anti-stress and radio-protective drugs. [Supported by RFBR, grant 14-04-00593a].

Antioxidant biomedicine: from free radical chemistry to systems theory of reliability

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In vivo, neither natural antioxidants like vitamin E, ascorbic acid or flavonoids, nor synthetic antioxidants like butylated hydroxytoluene (BHT) are able to operate effectively as simple free radical scavengers. The efficiency of such the scavengers in cells and tissues is negligibly low as compared with the natural antioxidant enzyme systems such as superoxide dismutase (SOD), etc. The so-called "mitochondria-targeted" antioxidants can hardly upgrade the characteristics without considerable disturbances in mitochondrial nanoreactors. As for radical $OH^{\cdot}$, it reacts with any organic molecules so rapidly that there is nothing able to intercept this radical *in vivo*. Meanwhile, the main line of keeping the high reliability of systems composed from unreliable components is the preventive maintenance or prophylaxis of failures. Antioxidants can provide exactly such preventive protection against the free radicals (Koltover, 2009; 2010). At this, particular protection mechanisms may be different for antioxidants of different types. For example, BHT performs its preventive antioxidant protection acting upon the endocrine system as a mild stress factor (Frolkis et al., 1990). Another kind of prophylactic protection is demonstrated by flavonoids and resveratrol which induce the expression of SOD and catalase (Nelson et al., 2006). Apparently, these natural antioxidants also act via the hormonal regulation. The transient dynamics deficiency in antioxidant enzymes under the stress conditions seems to be the critical factor in free-radical pathologies. Furthermore, phenol compounds modulate human microbiota while the microbiota impacts on brain. Thus, in XXI century, antioxidant biomedicine has displaced "back to the future" – Metchnikoff arises. [Supported by RFBR, grant 14-04-00593a].

Tamoxifen metabolic activation to form DNA adducts: A new possible mechanism behind tamoxifen-driven endometrial cancer

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In the United States one out of eight women will develop breast cancer in their lifetime. In the 1970's a revolutionary anti-estrogenic drug, Tamoxifen (TAM), was introduced into the market. TAM reduces the reoccurrence and decrease the risk of new developing breast cancer by 50%, proving it's self as an effect method to fight against breast cancer. However, some side effects of TAM is an increased risk of endometrial cancer and rare tumor types that are difficult to treat and associated with poor prognosis. TAM is primarily metabolized to 4-hydroxytamoxifen (4-HT) and N-desmethyltamoxifen in human as well as animals. This current study demonstrated that peroxidase mediated side chain cleavage of 4-HT to yield bisphenol (BPT). BPT can be further oxidized to form a quinone methide which can interact with adenine to form DNA adducts. Liquid choromatography tandem mass spectrometry (LCMS-MS) analysis of urine from a postmetapausal woman taking TAM showed the formation of BPT-Adenine, further confirming the metabolic activation of TAM to BPT-adenine. These results suggest that TAM is metabolized to BPT and that BPT is metabolically activated to form DNA adducts *in-vivo*. We report for the first time a new TAM metabolic pathway that could lead to carcinogenicity, and provide a potential mechanism behind the higher risk of endometrial cancer associated with TAM administration.

Flavan-3-ol intake and CVD disease risk: meta-analysis of the evidence available

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Dietary intervention studies have shown a vasculoprotective effect of flavan-3-ols, and limited data from anthropological studies suggest that a similar effect can be observed at population level. However, data from epidemiological observational studies conducted previously have failed to establish an unambiguous association between flavan-3-ol intake and the risk of cardiovascular disease. This might be explained by the lower habitual intake of flavan-3-ols in the study populations. Furthermore, these studies relied on self-reported intake which is prone to recall bias and can lead to an attenuation of results.

In order to investigate this apparent discrepancy, we have conducted a meta-analysis of intervention and epidemiological observational studies. In these analyses, we have determined the associations between intake and both, markers of cardio-vascular disease risk and actual risk. Furthermore, we have investigated habitual flavan-3-ol intake in fourteen countries of the European Union and in more than 25,000 participants of the Norfolk cohort of the *European Prospective Investigation into Cancer and Nutrition* (EPIC) using different dietary assessment instruments (diary, 24h recall and food-frequency questionnaire [FFQ]).

This data allows us to compare habitual intake with intake used in intervention studies, and also associations with different CVD risk markers and endpoints. The results will allow us to investigate if and how the findings from these studies can be translated into dietary recommendations.

Lignans, flavanols, and hydroxybenzoic acids intake decreased CVD risk in the PREDIMED Trial

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Epidemiological studies showed an inverse association between flavonoids and cardiovascular disease (CVD). However, until recently, epidemiological studies have used the USDA (US Department of Agriculture) Flavonoid Database, which captures only a subgroup of polyphenols (the flavonoids) and, therefore, does not reflect the other diversity of polyphenols found in food. The PREDIMED (Prevención con Dieta Mediterránea) study is a randomized, primary prevention trial aimed to assess the effect of the traditional Mediterranean Diet on cardiovascular events. The aim of this study was to evaluate the association between intakes of all polyphenol subgroups, and the risk of major CVD events (myocardial infarction, stroke or death from cardiovascular causes) in the PREDIMED trial. Over an average of 4.3 years of follow-up, there were 273 confirmed CVD events among the 7172 participants who completed a validated 137-item food frequency questionnaire (FFQ) at baseline. Polyphenol consumption was calculated by matching food consumption data from the FFQ with the Phenol-Explorer database on polyphenol content of each reported food. To assess long-term polyphenol intake, we calculated the cumulative average of polyphenol intake at each yearly visit as the average of

all previous available dietary questionnaires. Time-dependent Cox proportional hazards regression with updated diet and covariates information was used to estimate hazard ratios (HR) using the lowest quintile of intake as the reference group. The polyphenols with the strongest inverse associations were flavanols (HR=0.40; CI 0.23-0.72; *P*-trend=0.003), lignans (HR=0.51; CI 0.30-0.86; *P*-trend=0.007), and hydroxybenzoic acids (HR=0.47; CI 0.26-0.86; *P*-trend 0.02).

International Standard Randomized Controlled Trial Number: 35739639.

Impairment of nitric oxide-mediated neurovascular coupling in Alzheimers´s disease and aging

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By demonstrating a spatial, temporal, and amplitude association between vascular changes, O_2 tension and neural functioning we have supported the notion that neuronal-derived nitric oxide (•NO) acts as a direct mediator of neurovascular coupling. This was done by recording the sequence of events encompassing •NO signals, cerebral blood flow (CBF) and O_2 changes simultaneously and quantitatively *in vivo,* and in a way that the dynamics of all the processes was clear visible.

The impairment of the coupling in such a critical process (neurovascular coupling) for brain structure and integrity would be an early phenomenon in disease and aging. This hypothesis was tested in a triple transgenic mice model of Alzheimer disease (AD) and of aging (Fisher 344 rats).

Mechanistically, in the triple transgenic model of AD, the *co-operative signal volume* by nitric oxide reaching blood vessels located within its diffusional spread in the hippocampus is operative but a significant impairment in the corresponding CBF change was found in the later stages of the disease (12 months-old). Data supports that the impairment in coupling between active neurons and local vessels in AD likely results from a dysfunction at the transducer site (vasculature), rather than at the signal producer site (•NO-producing neurons). Likewise, with age the vasculature becomes insensitive to neuronal derived •NO. Moreover, the decoupling in terms of CBF/NO observed for middle aged (≈ 12 months) AD animals is quantitatively recapitulated by the control animals at old age (> 18 months), supporting the hypothesis of vascular impairment in the neurovascular coupling as an early dysfunctional process in the AD brain.

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Stability of whole blood antioxidants during overnight storage at 4°C

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To determine the optimal conditions for blood collection during a clinical trial where sample handling logistics might preclude prompt separation of erythrocytes from plasma, 8 subjects (6M,/2F) were recruited and non-fasting blood collected into tubes containing ethylenediaminetetraacetic acid (EDTA), Li-heparin or Na-heparin. We hypothesized that heparin, but not EDTA would effectively protect plasma and red cell vitamins E & C, cholesterol, and vitamin E metabolites (α- and γ-CEHC). To test this hypothesis, one set of tubes was processed immediately and samples stored at -80°C, while the other set was stored at 4°C and processed the following morning (~30 hours). Plasma vitamins E and C (acidified 1:1 with 5% MPA) were measured by HPLC-ECD; plasma and RBC α-tocopherol concentrations were measured by LC-MS, and LC-MS/MS. Neither time prior to processing, nor anticoagulant used had any significant effects upon plasma or red cell α-tocopherol, or plasma CEHC concentrations. Plasma vitamin C decreased by 75% with storage using EDTA as an anticoagulant, but was unchanged using heparin as an anticoagulant. Thus, whole blood refrigerated at 4°C overnight does not change either the plasma or RBC concentrations of vitamin E and its metabolite, CEHC. Vitamin C is unstable in whole blood when EDTA is used as an anticoagulant, but when whole blood is collected with heparin, it can be stored overnight and subsequently processed.

Potential involvement of reactive oxygen species and Nrf2 signaling in the inhibitory effects of flavonoids on adhesion molecule expression in human aortic endothelial cells

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Atherosclerosis, the underlying cause of ischemic heart disease and stroke, is an inflammatory disease of arteries in a hyperlipidemic milieu. Expression of adhesion molecules, such as E-selectin and intercellular adhesion molecule 1 (ICAM-1), on endothelial cell surfaces plays critical role in the initiation of atherosclerosis. Some dietary flavonoids and their metabolites have been reported to inhibit expression of adhesion molecules, but the underlying mechanisms are not completely understood. We investigated the effects of flavonoids and flavonoid metabolites on adhesion molecule expression in human aortic endothelial cells (HAEC). At concentrations of 5 to 20 μM, quercetin and its metabolites, isorhamnetin (3'-O-methyl-quercetin) and tamarixetin (4'-O-methyl-quercetin), dose-dependently inhibited lipopolysaccharide-induced protein expression of E-selectin and ICAM-1. Quercetin also inhibited ICAM-1 and E-selectin expression at the transcriptional level. Isorhamnetin imposed weaker inhibition on ICAM-1 mRNA levels than quercetin. Administration of quercetin to HAEC significantly reduced cellular levels of reactive oxygen species (ROS). In addition, quercetin caused Nrf2 activation and increased transcription of heme oxygenase 1 (*HO-1*), NAD(P)H dehydrogenase, quinone 1 (*NQO1*) and glutamate-cysteine ligase catalytic (*GCLC*) and regulatory (*GCLM*) subunits in a time- and dose-dependent manner. Interestingly, a protein kinase C (PKC) inhibitor, staurosporine, exerted similar inductive effects as quercetin on *HO-1* and *GCLM* transcription. These results suggest that ROS formation may be involved in adhesion molecule expression in HAEC. Suppression of ROS formation through Nrf2 signaling may explain, in part, the inhibitory effects of quercetin and some of its metabolites on endothelial adhesion molecule expression, and PKC might be involved in this effect of quercetin.

High-fat diet induces a systemic insulin resistance that impairs synaptic plasticity

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High fat diet (HFD) is associated with the development of insulin resistance, in instances such as obesity and type II diabetes. The experimental model consisted of C57BL/6J mice fed a HFD (60% of calorie are from fat) for 12 weeks: this paradigm was associated with a significant increase in body weight, development of fatty liver, hyperglycemia and hyperinsulinemia, and glucose intolerance. Comparisons were made against mice fed normal chow. In *liver*, HFD group showed (1) insulin resistance (decrease of phosphorylation of the insulin receptor substrate (IRS) on tyrosine 608) and increase of phosphorylation on serine 307 (indicative of inactivation of the IRS); (2) mitochondrial function impaired (a decreased cellular oxygen consumption rate), and (3) inflammatory responses (increase of iNOS and NFκB protein expressions and increase in the ratio of the phosphorylated (active) over non-phosphorylated (inactive) MAP kinases (p38 and JNK). In *brain,* HFD feeding elicited (1) impaired synaptic plasticity suggested by a substantial decrease in Input/Output (I/O) and long term potentiation in the hippocampal CA3 and CA1 region; (2) a suppression of the ERK/CREB pathway; (3) inhibition of insulin signaling through inactivation of the IRS and, as a consequence (4) a decreased expression of the insulin-sensitive neuronal glucose transporters GLUT3/GLUT4. It may be surmised that 12 weeks feeding with HFD induce a systemic insulin resistance that impacts profoundly on brain activity, *i.e.,* synaptic plasticity.

The NOX enzymes in melanoma development and drug resistance

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Reactive oxygen species (ROS) play crucial roles in all aspects of melanoma development; however, the source of ROS is not well defined. The cellular ROS pool in melanocytes can be derived from mitochondria, melanosomes, NADPH oxidase (NOX) family enzymes, and uncoupling of nitric oxide synthase. Nox1 and Nox4 are expressed in the melanocytic lineage. While there is no difference in Nox1 expression levels in primary and metastatic melanoma tissues, Nox4 expression is significantly higher in a subset of metastatic melanoma tumors as compared to the primary tumors, suggesting distinct and specific signals and effects for NOX family enzymes in melanomagenesis.. Nox1 expression level is higher in all melanoma cell lines examined than that in normal human melanocytes. Hence, we hypothesize that Nox4 plays an important role in metastasis while Nox1 may be a crucial enzyme for an early transformation event. Our experiment data support this hypothesis: the total NOX activity and Nox1 protein (as well as Nox1 activator Rac1) was induced by both UVA and UVB radiation. UVA induced higher level of NOX activity than UVB, confirming that the UVA-induced NOX activity is a major cause for UVA-induced oxidative stress. Targeting these NOX enzymes using specific NOX inhibitors may be effective for prevention of melanoma and treatment of a subset of Nox4-overexpressing tumors. Further understanding of the contribution of ROS to melanoma etiology and progression is necessary for developing new prevention strategies.

Dietary fat source modulates gene expression, longevity and mitochondrial processes in calorie-restricted mice

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Calorie restriction (CR) without malnutrition extends lifespan in a wide range of animal species. Although the underlying mechanisms are not fully understood, a decrease in ROS production and changes in membrane composition and function have been suggested to mediate CR-induced lifespan extension. In our study, 40% CR was combined with the use of different fat sources (soybean oil, lard and fish oil) to test whether such alterations affected membrane-linked metabolic processes and longevity. Lifespan was significantly altered by dietary fat in CR mice. The CR-lard group exhibited increased longevity when compared to both CR-soybean oil and CR-fish oil groups. However, the primary causes of death remained unchanged among groups. Mitochondrial fatty acid composition in skeletal muscle and liver of adult mice changed according to the polyunsaturated fatty acid composition of the diet. A transcriptomic analysis showed showed differences between diet groups in the expression of enzymes of major metabolic pathways, antioxidant enzymes and signal transducers and transcription factors regulating metabolic processes in liver. Interestingly, this transcriptional regulation in liver contrasted with the lack of changes between diet groups in skeletal muscle. Mitochondrial dynamics and cell death in both organs were influenced by dietary fat in CR mice in an age-dependent manner, with 21 month old CR-lard mice exhibiting lower rates of caspase-3 activity in skeletal muscle and DNA fragmentation in liver. The results of this study indicate that dietary fatty acids can influence life span response and metabolic adaptation to CR.

High resolution LTQ-Orbitrap-MS improves the identification of polyphenols and their metabolites in grape extracts as functional ingredients and human urine

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Grape pomace contains many polyphenols with biological activities which may be used as functional ingredients for new or functional foods. However, an exhaustive identification of the polyphenols present in this functional ingredient, grape extract, and in biological samples obtained after the consumption of this novel food is necessary. The application of high resolution mass spectrometry (HR-MS) allows the identification of phenolics using accurate mass measurements. In order to verify the oral absorption and metabolism of the polyphenols contained in grape extracts, we performed a prospective randomized, cross-over study with 12 healthy volunteers with three interventions: 500 mL of a functional beverage enriched with 100 mL of grape extract, 500 mL of the same beverage enriched with 200 mL of grape extract and 500 mL of a control beverage without grape extract. LTQ-Orbitrap-MS allowed the identification of phenolics in the grape extract and in human urine collected during the three interventions. Forty one polyphenols were identified in extract mainly procyanidins, phenolic acids and flavonols. In urine samples, more than sixty metabolites of phenolic compounds of the extract were detected including microbiota metabolites, glucuronides and sulfate derivatives.

In conclusion, the HR-MS enhances the identification of large variety of phenolic compounds and their metabolites with very good mass accuracies for all molecular ions. Moreover, HR-MS allows unambiguous assignment of all fragment ions with fewer experiments and easier interpretation than previous methods.

Involvement of the modulation of cancer cell redox status in the anti-tumoral effect of polyphenols

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Polyphenols are present in the human diet through the ingestion of vegetables and fruits. Several studies demonstrate their potential to interfere with cancer initiation, promotion and progression. This work aims to characterize how polyphenols modulate the redox status of gastric (AGS) and colon (Caco-2) cancer cell lines and its contribution to their anti-proliferative effects. Our results show that pyrogallol, gallic acid, quercetin, myricetin and delphinidin-3-glucoside significantly inhibited cellular proliferation, as assessed by SRB and BrdU assays. The IC_{50} of these compounds was similar in both cell lines, except for quercetin that had an IC_{50} two fold higher in Caco-2 when compared to AGS. The anti-proliferative effect of the polyphenols tested, except quercetin, was partially mediated by the generation of H_2O_2 in culture medium, as demonstrated by its attenuation in the presence of catalase. The analysis of reactive oxygen species in AGS cells labelled with the redox sensitive probes H_2DCF-DA or DHE showed that quercetin treatment led to a decrease of intracellular oxidation but did not affect the levels of superoxide radicals. These changes were correlated with a decrease of total glutathione levels but catalase and superoxide dismutase activities were not altered. Overall, these results suggest that the anti-tumoral effect of quercetin in AGS cells might be related to glutathione depletion.

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Fatal flaws:
Identifying the myths and artifacts of vitamin C research

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Vitamin C research in cell culture, animal models, and human clinical trials is often flawed and misleading. In cell culture, the high oxygen environment and redox-active transition metal ions in culture media rapidly degrade ascorbic acid. In serum-free media under standard culture conditions, the half-life of ascorbate is only 1.5 hours, leaving negligible amounts after 6 hours. Under the same conditions, adding metal chelators to media slows the rate of ascorbate oxidation, doubling its half-life. Conversely, increased media pH will lead to more rapid ascorbate oxidation. Studies in rodent models are complicated by endogenous ascorbic acid synthesis in liver. We observed that without vitamin C supplementation, rodents can synthesize enough ascorbate to saturate plasma and tissue levels. Long-term supplementation with high doses of ascorbic acid (3.3 g/L in drinking water) has a transient effect on plasma ascorbate levels, and negligible effects on whole-body ascorbate status. Lastly, human studies are limited by methodology that fails to appreciate the susceptibility of ascorbic acid to ex vivo oxidation. Blood collected into EDTA vacutainers have lower ascorbate levels than plasma from the same blood preserved with heparin, or in serum. Handling of whole blood and exposure to light, air, and/or heat may also increase the rate of ex vivo ascorbate oxidation. Together, these data suggest that careful attention to experimental details is necessary to avoid artifactual oxidation of ascorbate in vitro and ex vivo, and ascorbate levels need to be measured to ascertain its presence and for proper quantitative data evaluation.

The influence of simulated digestion *in vitro* on antioxidant activity of bacupari *(Garginia brasiliensis* Mart - Clusiaceae*)* from Brazilian Amazon Forest and characterization of phenolic composition by LC-ESI-MS

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Bacupari *(Garcinia brasiliensis)* is a native plant to the Amazon Region and is used for medicinal purposes in the treatment of several pathologies. As part of bioprospecting program seeking to identify new plant metabolites with biological capacities, the bioacessibility of *G. brasiliensis* was studied using a simulated gastrointestinal digestion model, chemical and cell basead antioxidant assays. The total phenolic and antioxidant activities were significantly lowered (10-50%) and degradation seemed to have occured during the *in vitro* digestion. In parallel, extracts were analyzed by liquid chromatography coupled to electrospray ionization mass spectrometry (LC-ESI-MS). Significant differences of contents of phenolic compounds between crude and digested extracts were found. In the crude extract the phenolic compounds identified were p-coumaric acid (176,15 ± 0,277 $\mu g.mL^{-1}$), cathequin (14.608,31 ± 144,28 $\mu g.mL^{-1}$), apigenin (25,214 ± 2,23 $\mu g.mL^{-1}$), luteolin (252,23 ± 8,716 $\mu g.mL^{-1}$), kaempferol (90,88 ± 25,42 $\mu g.mL^{-1}$) and narigenin (only detected, below the limit of quantification). In the digested extract, the compounds kaempferol, luteolin, apigenin, cathequin were not detected. The concentration of p-coumaric acid decreased about 70% (54,252 ± 2,711 $\mu g.mL^{-1}$) and narigenin was only detected. Our results indicated that these phytochemicals were degraded under pancreatic conditions whereas p-coumaric acid and narigenin were not. Additional tests are being conducted to confirm the activity of these remaining compounds in other bioassay systems and animal models.

Zinc deficiency disrupts Extracellular Signal-Regulated Kinase signaling leading to impaired neural progenitor cell proliferation

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Decreased zinc availability results in a downregulation of the extracellular signal-regulated kinase (ERK1/2) mitogen activated protein kinase cascade in neuronal cells. This study investigated the mechanisms underlying altered ERK1/2 signaling associated with zinc deficiency and its consequences on neural progenitor cell (NPC) proliferation during fetal brain development. Pregnant rats were fed a marginally zinc deficient or adequate diet from the beginning of gestation until embryonic day (E)14, E19, or postnatal day (P)2. Immunostaining for phosphorylated ERK1/2 (p-ERK) in the cerebral cortex was decreased at E19, and P2, but not E14. This effect was strongest in mitotic NPCs at the ventricular surface and the proportion of proliferating cells in the ventricular zone was decreased by marginal zinc deficiency at E19. Furthermore, phosphorylation of the upstream mitogen activated ERK kinases (MEK1/2) was not affected by marginal zinc deficiency, suggesting that marginal zinc deficiency increased ERK-directed phosphatase activity. Similar results were observed in rat cortical neurons and in IMR-32 cells, in which zinc-deficiency decreased p-ERK1/2 levels without affecting MEK1/2 phosphorylation. Zinc deficiency also increased the activity of the ERK-directed phosphatase, protein phosphatase 2A (PP2A), in IMR-32 cells. Furthermore, a PP2A inhibitor, okadaic acid, prevented the decrease in ERK phosphorylation and cell proliferation of zinc deficient IMR-32 cells. Together these results support the concept that decreased zinc availability causes ERK downregulation as a consequence of PP2A activation leading to a decrease in NPC proliferation.

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Molecular mechanisms in high fructose diet-induced insulin resistance

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Dietary energy intake, particularly the consumption of simple sugars such as fructose, has been increasing steadily in Western societies. Fructose, the number one commercial sweetener in Western civilization, has been implicated in contributing to obesity and metabolic syndrome. Recent studies have been able to draw associative conclusions between increased consumption of fructose and the development of Type-2 diabetes, Obesity and Non Alcoholic Fatty Liver Disease (NAFLD). However, all the factors linked to the development of these conditions are not well understood. Emerging literature suggests that insulin resistance, inflammation, fat re-deposition, abnormalities in control of reactive oxygen species and mitochondrial dysfunction could be involved in disease etiology. We investigated the role of high-fructose diet on the molecular mechanisms related to insulin resistance and the accompanying physiological changes in C57BL6/129S strain of mice.

In the present study, mice were supplemented with 15% fructose solution (HFr) in their drinking water and fed standard mouse chow for a period of 9 weeks. After 9 weeks, no change in either the body weight or overall caloric intake was observed. However, the mice developed an insulin resistant state, demonstrated by glucose tolerance test. Additionally, the mice showed increased levels of triglycerides in blood plasma. Moreover, detrimental effects on the mitochondrial function in the liver and brain were evident with increased oxidative stress. Furthermore, the effect of HFr diet on the brain is being examined using molecular techniques and functional assays such as electrophysiological techniques measuring long-term potentiation, mitochondrial respiratory control ratio measurements, and metabolic flux analysis.

Neuronal and glial metabolic alterations in the liver-specific PTEN knockout mouse model

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Phosphatase and Tensin Homologue (PTEN) is a negative regulator of the phosphatidylinositol 3-kinase/AKT pathway. Liver-specific deletion of PTEN results in increased fatty acid synthesis, accompanied by hepatomegaly and a fatty liver phenotype. Interestingly, deletion of PTEN in the liver also causes an enhanced liver insulin action with improved systemic glucose tolerance. Liver plays a major role in glucose metabolism and adequate distribution of glucose to the brain, whereas, insulin controls both glucose and lipid metabolism in the liver. Thus, a liver-specific deletion of PTEN provides a valuable model to directly probe the effect of liver glycolytic metabolism on brain function. Recent studies have reported hepatic inflammation and metabolic dysfunction as extrinsic risk factors for neurodegeneration mediated by brain insulin/IGF resistance and deficiency.

In this study, we have used the liver-specific PTEN knockout mouse model ($PTEN^{loxP/loxP}$) at the age of 4.5 months to assess neuronal and astrocytic metabolism after [1-^{13}C]glucose and [1,2-^{13}C]acetate infusion using ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy. Specifically, we studied the glycolytic and tricarboxylic acid cycle metabolism and the exchange of neurotransmitters between neurons and astrocytes in $PTEN^{loxP/loxP}$ mice. Preliminary results show an increased flux of metabolites from glucose and acetate, resulting in increased flux of the major neurotransmitter in the brain *i.e.,* glutamate. Additional studies are underway to study brain glucose uptake using *in vivo* PET-CT imaging. The functional outcome of PTEN KO on the brain will be determined by measuring synaptic plasticity through electrophysiological techniques measuring long-term potentiation and input-output relationships.

Aldosterone activates transcription factor Nrf2 in kidney cells both *in vitro* and *in vivo*

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Aim: Epidemiological studies found an increased risk for kidney cancer in hypertensive patients. These patients frequently exhibit hyperaldosteronism, known to contribute to kidney injury, with oxidative stress playing an important role. We investigated, *in vitro* and *in vivo*, the capacity of kidney cells to upregulate transcription factor Nrf2, key regulator of the antioxidative defense system, to prevent aldosterone-induced oxidative damage. Results: Aldosterone activated Nrf2 *in vivo* and led to the expression of enzymes involved in glutathione synthesis and detoxification. This activation was mediated by the mineralocorticoid receptor (MR) and oxidative stress. *In vitro*, aldosterone also led to the activation of Nrf2, associated with a rapid increase of target gene levels after 4 h. But while at 24 h of aldosterone exposure oxidant levels remained high, a decrease in Nrf2 activation, glutathione, and target gene levels was observed. Both, *in vitro* and *in vivo*, Nrf2 induction depended on MR-triggered activation of NADPH oxidase and nitric oxide synthase. Nrf2 activation could not protect cells against oxidative DNA damage, since aldosterone-induced double strand breaks and 8-oxodG lesions steadily rose. The Nrf2 activator sulforaphane enhanced the Nrf2 response *in vitro* and *in vivo* and thereby prevented aldosterone-induced DNA damage in the comet assay. Conclusion: Aldosterone-induced Nrf2 adaptive antioxidant response cannot neutralize oxidative actions of chronically increased aldosterone, which therefore could be causally involved in the increased cancer incidence of hypertensive individuals. Supplements elevating the Nrf2 response might exhibit beneficial effects.

Procyanidins from grape seed extract modifies fecal endogenous metabolome

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In recent years, the role of dietary procyanidins as health protective agents has become an important area of nutrition research. Procyanidins may regulate anti-inflammatory responses by modulating arachidonic acid pathway and nuclear factor NF-κβ expression, inhibiting gene transcription, protein expression, and activity of eicosanoid-generating enzymes. However, the low bioavailability of dietary procyanidins has called into question their possible protective role. Most procyanidins remain in the digestive tract until reaching the colon where the host microbiota partly metabolize them into small phenolic acids and related compounds. These metabolites may be more easily absorbed, and the source of biological activity. This work aims to evaluate changes in the fecal metabolomic profile due to grape seed extract intake by analysis using LC-LTQ-Orbitrap in conjunction with multivariate statistics in a pig model.

Six crossbred female pigs were fed with a diet containing 1% (w/w) of MegaNatural® Gold grape seed extract daily for 6 days. A run-in and a postfeeding period of 3 days were applied before and after the intervention period. Fresh pig fecal samples were collected during the daily morning feeding time.

A combination of chemometric analysis such as PLS-DA, data-dependent MS/MS scan and accurate mass database matching was used to measure the effect of the treatment on fecal composition. Of the 30,000 ions detected, 4502 showed a fold-change greater than 1.5 and were statistically different (p<0.01). These results

demonstrate that the procyanidin rich GSE supplement modifies the fecal endogenous metabolome. Tentatively, the results suggest that oligomeric procyanidins from grape seed extract may modify bile acid and steroid excretion which could exert a hypocholesterolemic effect. This outcome may represent a new nutritional property of grape seed extract.

Wine grape pomace extract and wine grape pomace attenuate high-fat-fructose diet-induce metabolic syndrome through modulation of inflammation and redox signaling

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Polyphenols from grape has been found to exert beneficial effect on metabolic syndrome (MS), in part attributed to their capacity to regulate oxidant production and pro-inflammatory signals. The aim of this study was to evaluate the effect of wine grape pomace extract (GPE) and wine grape pomace (GP) on weight gain and metabolic alteration related with high fat-fructose diet-induce MS. The total phenolic content, the main phenolic compounds, and ORAC were determined in GPE and GP. Thirty male Wistar rats were fed for 6 wk: control diet (C), high fat-fructose diet (20 % each w/w) (HFFD), HFF supplemented with GPE (100 and 300 mg/kg BW/d respectively), and HFF plus GP in a dose of 1 g/Kg BW/d. At the end of the study HFF diet increased weight gain, systolic blood pressure, plasma and liver tryglicerides and decreased HDL-cholesterol that were ameliorated/prevented by high doses of GPE and GP. In liver tissue HFF rats increased NOX activity and p67 translocation to the cell membrane that were totally and partially prevented by GPE and GP supplementation, respectively. Visceral adipose tissue weight and the area of adipocytes were increased in rats receiving HFFD and had an alteration in the insulin signaling pathway (IRS-1, Akt and ERK1/2), and inflammation (JNK, resistin) that were attenuated by high doses of GPE and GP. Consumption of food or food-rich in polyphenols may be useful in the prevention and/or amelioration of MS and MS-associated diseases.

ADME of cocoa flavanols in healthy young and old individuals

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Dietary flavanols are well reported to induce beneficial vascular effects. Despite this, variations in flavanol absorption, distribution, metabolism and excretion (ADME) are yet to be fully established and are a prerequisite for the establishment of reference daily intake values. The aim of this study was to characterize ADME of cocoa flavanols in healthy young and elderly individuals. A 3-armed randomized, cross-over intervention study was performed in 40 healthy, caucasian men aged 18-35 or 60-80 years old. The subjects received either 5.3 or 10.7 mg CF/Kg body weight (BW); 1 gram of acetaminophen was used as a control to compare differences in metabolism. Plasma and urine samples were collected over 24 hours post-consumption, and were analysed by HPLC with UV, fluorescence and electrochemical detection. Our data demonstrate that the intra- and inter-individual variability of flavanol absorption is relatively low, and while small differences in the metabolism and catabolism of flavanols were identified as a function of age, in general, flavanols were absorbed, metabolised and excreted in a similar fashion, thus supporting future efforts to establish population-level cocoa flavanol intake recommendations.

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Drug Screening for Friedreich's Ataxia based on redox deficiency identifies protective drugs

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The rare disease Friedreich's ataxia (FA) is caused by an inherited deficiency in the mitochondrial protein frataxin. Frataxin deficiency results in a decrease in iron-sulfur cluster biogenesis and antioxidant defense. We identified a thiol-based redox deficiency in FA cells and animal models and screened a 1600-compound library which identified the topical anesthetic dyclonine as protective. Dyclonine increased frataxin transcript and protein dose-dependently in FA cells and brains of animal models. Further, dyclonine rescued frataxin-dependent enzyme deficiencies in the iron-sulfur enzymes aconitase and succinate dehydrogenase. Additionally, dyclonine induces the Nrf2 transcription factor, which we show binds an upstream response element in the frataxin locus. Dyclonine also inhibited the activity of histone methyltransferase G9a, known to methylate histone H3K9 to silence Friedreich's chromatin. The pharmacokinetics, systemic anesthesia, and sodium channel pharmacology of dyclonine were also examined in mice and cells. Chronic dosing in Friedreich's model mice prevented a performance decline in balance beam studies. A human clinical proof-of-concept study was completed in eight Friedreich's ataxia patients dosed twice daily using a 1% dyclonine rinse for one week. Six of eight patients showed an increase in buccal cell frataxin levels, and fold induction was significantly correlated with disease severity. Dyclonine represents a novel therapeutic strategy that can potentially be repurposed for the treatment of FA.

Effect of lipoic acid on brain glucose uptake, insulin signaling, and synaptic plasticity in a mouse model of Alzheimer's disease

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Alzheimer's disease is a progressive neurodegenerative disease that entails impairments of memory, thinking and behavior and culminates into brain atrophy. Impaired glucose uptake (accumulating into energy deficits) and synaptic plasticity have been shown to be affected in the early stages of Alzheimer's disease. This study examines the ability of lipoic acid to increase brain glucose uptake and lead to improvements in synaptic plasticity on a triple transgenic mouse model of Alzheimer's disease (3xTg-AD) that shows progression of pathology as a function of age; two age groups: 6 months (young) and 12 months (old) were used in this study. 3xTg-AD mice fed 0.23% w/v lipoic acid in drinking water for 4 weeks showed an insulin mimetic effect that consisted of increased brain glucose uptake, activation of the insulin receptor substrate and of the PI3K/Akt signaling pathway. Lipoic acid supplementation led to important changes in synaptic function as shown by increased input/output (I/O) and long term potentiation (LTP) (measured by electrophysiology). Lipoic acid was more effective in stimulating an insulin-like effect and reversing the impaired synaptic plasticity in the old mice, wherein the impairment of insulin signaling and synaptic plasticity was more pronounced than those in young mice.

Brain glucose/acetate metabolism in a triple transgenic mouse model of Alzheimer's disease and the effect of lipoic acid

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Alzheimer's disease is characterized by age-dependent biochemical, metabolic, and physiological changes. These age-dependent changes ultimately converge to impair cognitive functions. This study was carried out to examine the metabolic changes by probing glucose/acetate and TCA cycle metabolism in 7-month (young) and 13-month (old) old triple transgenic mouse model of Alzheimer's disease (3xTg-AD). The effect of lipoic acid, an insulin mimetic agent, was also investigated to examine its ability in modulating age-dependent metabolic changes.

Young and old 3xTg-AD mice were given intravenous infusion of [1-^{13}C]glucose followed by an *ex vivo* ^{13}C NMR to determine the concentrations of ^{13}C labeled isotopomers of glutamate, glutamine, aspartate, GABA, and N-acetylaspartate. Enrichments of glutamate, glutamine, and aspartate were calculated after quantifying the total (^{12}C + ^{13}C) concentrations by HPLC in both young and old mice. Additionally, an intravenous infusion of [1-^{13}C]glucose + [1,2-^{13}C]acetate was given for different periods of time to distinguish neuronal and astrocytic metabolism in the young mice.

A hypermetabolic state was clearly evident in young 3xTg-AD mice in contrast to the hypometabolic state found in the old 3xTg-AD mice. Hypermetabolism in the young 3xTg-AD mice was evidenced by prominent increase of ^{13}C labeling and enrichment in

the 3xTg-AD mice. Lipoic acid feeding to the hypermetabolic 3xTg-AD mice brought the metabolic parameters to the levels of nonTg mice. In the old mice however, total ^{13}C labeling and percent enrichment decreased by ~50% in the 3xTg-AD mice; this hypometabolism was partially or completely restored by lipoic acid feeding. The ability of lipoic acid to restore glucose metabolism and subsequent TCA cycle-related metabolites further substantiates its role in overcoming metabolic changes inherent in early stages of Alzheimer's disease.

Excessive α-tocopherol exacerbates microglial activation and brain injury caused by acute ischemic stroke

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The vitamin E family includes both tocopherols and tocotrienols, where a-tocopherol (aTOC) is the most bioavailable form. Clinical trials testing the therapeutic efficacy of high dose aTOC against stroke have largely failed or reported negative outcomes when a "more is better" approach to supplementation (>400 I.U. per day) was employed. This work addresses mechanisms by which supra physiological aTOC may contribute to stroke-induced brain injury. Ischemic stroke injury and the neuroinflammatory response were studied in tocopherol transfer protein deficient mice maintained on diet containing aTOC vitamin E at the equivalent human dose of 1680 IU/day. Ischemic stroke-induced brain injury was exacerbated in the presence of supraphysiological brain aTOC levels. At 48h post-stroke, S100B and RAGE expression were increased in stroke-affected cortex of mice with elevated brain aTOC levels. Such increases were concomitant with aggravated microglial activation and neuroinflammatory signaling. A post-stroke increase in markers of oxidative injury and neurodegeneration in the presence of elevated brain aTOC establish that at supraphysiological levels aTOC potentiate neuroinflammatory responses to acute ischemic stroke. Against the background of clinical failure for high-dose aTOC, outcomes of this work identify risk for exacerbating stroke-induced brain injury as a result of supplementing diet with excessive levels of aTOC.

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Effect of oral administration of *Pereskia aculeata* leaves on fecal bile acids levels in rats

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The *Pereskia aculeata*, ora-pro-nobis (OPN), is a Cactaceae plant commonly consumed in Brazil that is a good source of protein, vitamins and minerals. To date, there are no studies about OPN effects on metabolism, specifically Bile acids (BA). BA are synthesized in liver through oxidative metabolism of cholesterol by cytochrome P450. Previous studies have shown BA increased in response to a high diet fat and elevated fecal BA concentration are associated with higher incidences of colon cancer. The high exposure of BA increases the formation of reactive oxygen species and DNA damage. To test the effect of OPN on the metabolism of BA, we administered to 24 female, Wistar, rats a single oral dose of 0 (Control group), 1250 (Group1), 2500 (Group2) and 5000 mg/kg (Group3) of plant ethanolic extract. Feces from individual rats were collected 24 hours after the administration and analyzed by Liquid choromatography tandem mass spectrometry for BA. Our results indicate the fecal excretion of total BA are inversely associated with the dose of OPN extract (Control Group – 156.73$\pm$27.98 μg/g; Group 1 – 137.11$\pm$14.00 μg/g; Group 2 – 119.21$\pm$39.74 μg/g; Group 3 – 76.92$\pm$24.07 μg/g; $p<0.05$). Also deoxycholic acid, chenodeoxycholic acid and cholesterol sulphate decreases after the plant administration. Interestingly, fecal excretion, body weight and food intake did not change between the groups, besides no animals presented signs of toxicity. Our preliminary findings suggest that OPN decreases total BA excretion when compared to the control group. Further investigations are underway to determine the potential mechanism behind OPN effect on BA synthesis.

Early stage development for metabolic profiling of iPSC-derived neural cells from the Jalisco & Columbian presenilin-1 familial Alzheimer's disease cohorts

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Alzheimer's disease (AD) is a national and global epidemic with complex pathoetiology including compromised brain metabolic activity and decreased regenerative capacity. Allopregnanolone (Allo) is an investigational neuroregenerative therapeutic, which has been demonstrated to promote neural stem cell (NSC) proliferation and neural differentiation. In the current study, we sought to investigate the impact of Allo on mitochondrial function. We first determined that Allo improves basal and maximal capacity in cultures of health rat NSCs by 20% and 60% respectively and in health rat neurons by 20% and 35%. To develop a biomarker of regenerative capacity in humans, we investigated the impact of Allo on human induced pluripotent stem cells (iPSCs) and iPS-derived neural cells. An initial proof-of-concept bioenergetic assay was conducted using NSCs derived from a healthy donor, demonstrating project feasibility and allowed for the identification of 40,000 NSCs/well as the optimal cell density for this cell type in future metabolic assays. Currently we are reprograming presenilin-1 mutations (A431E: Jalisco cohort, and E280A: Columbia cohort) containing cells from familial AD patients. Once iPSC lines containing the mutation lineage are established, they will be differentiated into NSCs, neurons, astrocytes, and oligodendrocytes. We will then evaluate the efficacy and mitochondrial mechanisms of Allo in rescuing the compromised phenotypes. Outcomes of these analyses will form the foundation for establishing a

biomarker of regenerative capacity phenotype, to enrich clinical trials of therapeutics targeting the endogenous regenerative capacity of the brain in persons with AD, regardless of etiology, to determine and monitor responses to therapeutics.

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Mitochondrial complex I is indispensable for normal heart function

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Complex I (CI) deficiency is the most common disorder of mitochondrial oxidative phosphorylation system in humans, the patients with clinical phenotypes have often cardiomyopathy and die from heart failure with a rapid progression. Despite our wealth of knowledge regarding its activities at the molecular level, the roles of CI and its potential contributions to heart failure remain elusive. Mitochondria are one of the main regulators of cellular oxidative stress. More severe oxidative stress can cause damaging cells and contribute to many diseases including heart failure. Oxidative phosphorylation in respiratory chain is an important source of ROS. Ndufs4 is an important subunit of complex I, and its mutations cause deficiencies in complex I activity. Using a mouse model with Ndufs4 deletion, we observed that Ndufs4$^{-/-}$ mice develop cardiac dysfunction and evidence of pathological cardiac hypertrophy at around p45. Of note, Ndufs4$^{-/-}$ mice have greater levels of oxidative stress in heart compared to littermate wild type mice at p30-p45. Mitochondrial copy number is dramatically reduced in heart of Ndufs4$^{-/-}$ mice at p45. Collectively, our in vivo data demonstrate that complex I activity is crucial for postnatal myocardial development, through its role in maintaining mitochondrial function, and redox homeostasis in cardiomyocytes.

Zinc and the modulation of transcription factors STAT1 and STAT3: consequences on brain development

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During development, the brain is highly susceptible to fluctuations in zinc availability. Transcription factors STAT1 and STAT3 play central roles in brain progenitor cells proliferation and in their differentiation into astroglial cells. This work investigated the role of zinc on STAT1/3 modulation in the developing rat brain and in human neuroblastoma IMR-32 cells. In IMR-32 cells, a decrease in cellular zinc impaired the activation of STAT1/3, as indicated by: i) lower levels of phosphorylation at Tyr 701 and 705 for STAT1 and STAT3, respectively, ii) decreased STAT1/3 nuclear translocation and DNA binding and iii) decreased transactivation of STAT1/3-driven reporter genes. Gestational marginal zinc deficiency caused similar alterations in the STAT1/3 pathway in the brain of day 19 embryos (E19) as those observed in IMR-32 cells. According to the role of STAT3 in the differentiation of progenitor cells into astroglial cells, gestational marginal zinc deficiency resulted in a decrease in the astrocyte markers GFAP and S100b in the rat brain cortex at E14, E19 and postnatal day 2. Thus, a suboptimal fetal zinc availability can affect normal brain development in part through a disregulation of STAT1/3 and the associated impairment of astrogliogenesis.

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Intervention of *Spirulina platensis* and phycocyanin in ferric nitrilotriacetate-induced renal oxidative damage and inflammatory alteration in mice

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Oxidative injuries are known to be involved in age-related renal dysfunction. It is known oxidative stress (OS) aggravation results in inflammatory signal transduction. The to-and-fros of OS and inflammation amplify each other and verges to chronic inflammation that would be involved in many diseases. Therefore, daily dietary intake of antioxidative food may decrease the burden of oxidative stress and be beneficial for the promotion of renal function. Our previous study showed *Spirulina platensis* (SP) worked so well in suppressing the cross talk of OS and inflammation. To study the effects of SP and its functional dye, phycocyanin against oxidative related renal injuries, we employed the mouse model of ferric nitrilotriacetate (Fe-NTA)-induced renal tubular oxidative injury. Animal experiment was performed in 8 groups; Control, Fe-NTA (iron 7.5mg/kg/day, i.p.), Fe-NTA+0.10, 0.25, 0.75 SP (0.10, 0.25, 0.75g/kg/day, respectively), Fe-NTA+0.02, 0.05, 0.15 PC (0.02, 0.05, 0.15g/kg/day, respectively). For a mouse, SP or PC was administrated daily for 2 weeks, and thereafter a peritoneal Fe-NTA injection was performed. The pretreatments of SP or PC suppressed elevation of serum creatinine and blood urea nitrogen 24 hr after the 7.5 mg iron/kg Fe-NTA administration. Protective effects against renal oxidative tubular damage and maintenance of antioxidant enzyme activities were observed in the SP and PC-pretreated group. Their pretreatments were demonstrated to prevent renal iron deposit and neutrophil infiltration into kidney 24 hr after the Fe-NTA injection. These results suggest SP and PC intakes are useful for the prevention of renal tubular oxidative damage and inflammatory alteration mediated by iron.

Quantitation of glutathione and related thiols in acid-preserved samples by hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-MS)

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Glutathione is the most abundant non-protein thiol in the body. Reduced glutathione (GSH) is important for protection from oxidative damage. Accurate determination of GSH, and its oxidized form (GSSG), provides an important indicator of the redox environment of cells, tissues and organisms.

LC-UV and LC-fluorescence methods for quantifying GSH, GSSG, and other thiols require lengthy sample preparation and chromatography. Mass spectrometry (MS) could provide faster and more specific determination of non-protein thiols, but samples prepared using perchloric acid (PCA) must be adapted. While free thiols must be protected for accurate measurement of the redox state, no chromophore or fluorophore is needed for MS detection.

PCA- preserved plasma and tissue homogenates were mixed with N-ethyl maleimide (NEM), then ammonium bicarbonate. The latter neutralized PCA in an MS-friendly manner, yielding a pH optimal for NEM derivatization, protecting free thiols. NEM-reacted samples were separated using a HILIC column. Analytes were detected by MS using positive mode electrospray.

Good separation of analytes was achieved on 2 different HILIC columns in 20 minutes, including equilibration. Current optical methods take 50-70 min/sample. Linear calibration curves were obtained for cysteine-NEM and GSH-NEM, but not for the oxidized forms, cystine and GSSG. Cystine response increased linearly, then dropped at high levels due to poor solubility during sample preparation. Changing the range of the calibration standards eliminated the problem. The exponential response of GSSG was more problematic, requiring changes to both chromatography and electrospray source conditions to correct.

Alternatives to experimental testing: the use of QSAR models for the assessment of antioxidative activity of compounds

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Historically most drugs in clinical use today had been discovered by serendipitous findings, but nowadays the trend in drug discovery is turning towards rational drug design. Important tools in such approach are *in silico* methods, which include studies of quantitative relationships between the structure of compounds and their activity, also known as QSAR modeling. The most appealing thing with QSAR models is that they can be developed from relatively small datasets, providing the sufficient diversity of chemical structures and availability of their experimentally determined properties. Once the QSAR models are properly built, they can be used to predict the investigated feature for all the compounds in the chemical space that the model is applicable to. These predictions can be used to make a prioritization list of compounds for *in vitro*/*in vivo* testing and can hence reduce the scope of time-consuming and expensive experimental work [1]. Here we present QSAR models for the prediction of the antioxidative activity, developed on the basis of *in vitro* data collected by measuring the antioxidative potential of several pulvinic acid and coumarine derivatives [2]. The models have been developed in accordance with the OECD principles, including the validation and the determination of the domain of applicability. They exhibit satisfactory predictive performances and can be used for the prediction of the antioxidative activity of compounds that have not been yet experimentally tested.

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Early changes in malondialdehyde and lipid profile after the consumption of tomato products suggest an improvement on oxidative stress

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Malondialdehyde (MDA) is a biomarker of lipid peroxidation and progression of atherosclerosis that correlates with oxidative stress, one of the main mechanisms responsible of cardiovascular lesions. Between different foods, epidemiological studies have pointed out that tomato intake may reduce cardiovascular risk due to its *functional components such as* carotenoids, vitamin C and polyphenols. Some researchers have suggested that cooking and addition of a fatty matrix may increase the bioavailability of bioactive compounds. However, to our knowledge, no previous studies have analyzed the effects of a single dose of raw and processed tomato products on oxidative status. The aim of this study was to examine the postprandial effects of a single dose of raw tomato (RT), tomato sauce (TS) and tomato sauce with refined olive oil (TSOO) on oxidative stress parameters. We performed an open, prospective, randomized, cross-over feeding trial in 40 healthy volunteers who received these three interventions in random order. Participants were instructed to abstain from tomato and polyphenols-rich foods 48h before each intervention. Plasma lipid parameters and MDA *concentration* were assessed at baseline and 6h after tomato intake. The results indicate that a single tomato intake decreased plasma MDA, triglycerides, total cholesterol and LDL concentrations, and increased HDL concentrations. However, these effects were significantly greater with TSOO than with RT and TS. We concluded that tomato intake have beneficial effects on oxidative status, especially when it is consumed cooked and/or enriched with olive oil, possibly due to the better bioavailability of its functional compounds in a fatty matrix.

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Antioxidant activity, total phenolic contents, antiproliferative and antimutagenic potential of pupunha fruit peel (*Bactris gasipae*) of Brazilian Amazon Forest

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Bactris gasipaes, known as pupunha in Brazil and peach-palm in English-speaking countries, is a tropical palm, native to the American tropical rainforests, that has economic potential related to its fruits and heart-of-palm production. Pupunha's fruit is a promising source of antioxidant compounds such as carotenoids and polyphenols and has attracted much attention due to their potention health benefits to humans. Therefore, in an attempt to contribute to valorize *Bactris gasipaes*, this study was conducted to quantify total phenols using Folin Ciocaulteau method and to evaluate the antioxidant activity of *Bactris gasipaes* peel in undigested and digested extracts through the methods 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and Oxygen Radical Absorption Capacity (ORAC). In addition, antiproliferative and antimutagenic activities of *B. gasipaes* ethanolic extract were investigated. Total phenolic content were 2396.76±298.89 and 512.46±16.16mg of gallic acid equivalents/100g of pupunha's peel undigested and digested, respectively. DPPH and ORAC values were respectively 729.38 ± 23.44 µmol Trolox equivalents (TE)/100g and 14130.58 ± 726.10 µmol TE/100g for undigested and not detected and 9084.11 ± 702.33 µmol TE/100g for digested. The extract was evaluated for its ability to inhibit the growth of tumor cell lines and great part of the cell lines was sensitive to pupunha's ethanolic extract. Antimutagenic activity was investigated by micronucleus test in mice. Ethanolic extract of *B. gasipaes* showed higher antimutagenic property at the lowest concentration tested (30 mg/kg of body weight). As a result, the potentially valuable compound could be used as a preventive agent against cancer.

Leptin induces a oxidative response in human mammary epithelial cells dependent of neoplasic status: approach *in vitro*

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Obesity, frequently associated with hyperleptinemia, promotes the recurrence of breast cancer and increases mortality risk. Accumulation of fat in adipose tissue results in adipokines secretion such as leptin which induce several signalling pathways. Some of them involve reactive oxygen species (ROS) as intracellular messengers linked to the onset of oxidative stress well known as a risk factor of chronic diseases such as cancer.

Our aim was to elucidate if leptin (10/100 ng/ml) modulates *in vitro* the oxidative status of healthy (HMEC) or neoplasic cells (MCF-7, MDAMB-231). The oxidative balance was measured by ROS production (cytometry) and enzymes (enz) mRNA expression (NADPH oxidases, NO synthase); mRNA expression and activity of anti-oxidant enz (heme oxygenase (HO), glutathione reductase & S-transferase); signalling pathways by phospho STAT3 and SOCS-3 expression. Whatever the cell line and the leptin concentration, a light increase of ROS production was observed (from 3.0 ± 0.1 up to 4.2 ± 0.1, 3.5 ± 0.2 RFU, $p<0.05$, respectively for HMEC, MCF-7). This production was dependent of cytosolic superoxyde anion synthesis through NADPH oxidase 5 and induced different antioxidative response in cells. In HMEC, leptin activated anti-oxidative enz (*i.e*. HO: mRNA expression x 10; activity 2.6 ± 0.6 *vs* 0.47 ± 0.05 UI/g; $p<0.05$) whereas no change was observed in neoplasic cells despite over-expression of STAT3 ($p<0.05$). So, leptin induces a different anti-oxydant response in healthy and neoplasic cell models, which leads to various oxidative stress intensity. This highlights a direct contribution of leptin in adverse effects of obesity in the breast carcinogenesis.

High environmental stress yields greater tocotrienol concentration while altering vitamin E profiles within wild emmer wheat seeds

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Vitamin E functions as a potent lipid soluble antioxidant essential for human nutrition. Only produced by photosynthetic organisms, vitamin E is found in grains such as emmer wheat, precursor to durum wheat and the cereal of Old World Agriculture that still grows wild in the Fertile Crescent today. Evolution Canyon, Israel, provides a microsite that models effects of contrasting environments. The north-facing and south-facing slopes exhibit low and high stress environments, respectively. Abiotic stressors impact plant oxidative stress levels and are hypothesized to alter vitamin E (four tocopherols and four tocotrienols) content of emmer seeds collected from the canyon. Seeds from high stress areas contained more total vitamin E (108 ± 15 nmol/g) than seeds from low stress environments (80 ± 17 nmol/g, $P = 0.0004$). Vitamin E profiles within seeds from these different environments revealed significant differences in isoform concentrations. Within each region, β- plus γ-tocotrienols represented the highest concentration of wheat tocotrienols (high stress, $P < 0.0001$; low stress, $P < 0.0001$), while α-tocopherol represented the highest concentration of the tocopherols (high stress, $P = 0.0002$; low stress, $P < 0.0001$). Percentages of both δ-tocotrienol and δ-tocopherol increased in high stress conditions. Changes under higher stress apparently are due to increased pathway flux toward more tocotrienol production. The production of more δ-isoforms suggests increased flow through a divergent path controlled by the VTE1 gene. Hence, stress conditions alter plant responses such that vitamin E profiles are changed, likely an attempt to provide additional antioxidant activity to promote seed viability and longevity.

Altered expression of peroxiredoxins in a mouse model of spinal muscular atrophy: Peroxiredoxin 6 as new therapeutic candidate

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Spinal muscular atrophy is a genetic disorder which usually is not investigated with regard to oxidative stress. Our previous work showed that vacuolar mitochondria are typical for degenerative motor neurons in a mouse model of spinal muscular atrophy, "legs at odd angles" Loa mutant mice. This vacuolar type of mitochondria is considered a sign of mitochondrial oxidative stress in a mouse model of amyotrophic lateral sclerosis (ALS). In the present study, we investigated expression of mitochondrial peroxiredoxins (Prx) 3 and 5 as well as Prx 6 in spinal motor and sensory motor neurons, surrounding glial cells, and quadriceps muscle of newborn $Loa^{+/-}$ mice. Quantitative immunohistochemical analysis demonstrates statistically significant increased expression of mitochondrial Prx in sensory and motor neurons and surrounding glial cells, and also increased expression of secretory Prx 6 in sensory neurons, surrounding glial cells and quadriceps muscle. However, Prx 6 was significantly decreased in glial cells and extracellular space surrounding motor neurons, which is typical pathology for chronic oxidative stress. Thus, our data indicate that oxidative stress occurs in early stages of the disease model. In addition, quantitative morphological analysis showed features of neuroinflammation of sensory and especially motor neurons, and muscular inflammation with atrophic and denervated myofibers. Our data indicate that Prx 6 may be a new therapeutic candidate for prevention and treatment of spinal muscular atrophy. Literature review shows a similar mechanism of neurodegeneration in other diseases such as ALS and Alzheimer's through glial-neuronal interactions with decreased expression of Prx 6 around degenerating motor neurons.

The cardioprotectant 3',4'-dihydroxyflavonol inhibits the mitochondrial permeability transition pore (mPTP).

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3',4'-Dihydroxyflavonol (DiOHF) reduces myocardial ischaemia and reperfusion (IR) injury associated with reduced oxidative stress. Oxidative stress contributes to the opening of the mPTP, a key event in myocardial IR injury. We investigated the effect of DiOHF on mPTP opening and the generation of reactive oxygen species (ROS) by cardiac mitochondria after IR in rats. Male rats were anaesthetised with pentobarbitone (70 mg/kg iv) and ventilated. The left coronary artery was occluded for 30 min and reperfused for 15 min. The heart was rapidly removed and mitochondria isolated from the area at risk of ischaemia. DiOHF (10 mg/kg iv) or vehicle (DMSO) was administered 5 min before reperfusion. mPTP opening was measured by mitochondrial Ca^{2+} retention capacity. Mitochondrial ROS generation was measured as the rate of H_2O_2 production using Amplex red. Treatment of sham rats with DiOHF significantly increased the concentration of Ca^{2+} required to stimulate mPTP opening (sham 87±6; sham+DiOHF 120±9 μM) and decreased H_2O_2 release (sham 0.028±0.002; sham+DiOHF 0.019±0.002 nmol/min/mg protein). IR decreased the concentration of Ca^{2+} required to stimulate mPTP opening (IR 44±5 μM), and increased H_2O_2 release (IR 0.034±0.001 nmol/min/mg protein) compared to sham. Treatment with DiOHF prevented IR-induced changes in mPTP opening (IR+DiOHF 78±7 μM) and H_2O_2 release (IR 0.028±0.002 nmol/min/mg protein). In normal rats DiOHF inhibits mPTP opening and decreases mitochondrial ROS production. Importantly, DiOHF administration before reperfusion prevents IR-induced mPTP opening and increases in ROS production. Beneficial actions of DiOHF on mitochondria are likely to make a major contribution to its cardioprotective actions.

Impact of perimenopausal transition on bioenergetic and synaptic function in hippocampus of female rat model of human perimenopause

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The aim of current study is to discover the biological transformations that occur in the aging female brain during the perimenopausal transition that can result in phenotypes predictive of risk for development of Alzheimer's disease (AD) pathology. We investigated changes in hippocampal bioenergetic and synaptic function in a rodent model of human perimenopause using rats at different age and different stages of ovarian senescence (as exemplified by regular cycler, irregular cycler and constant estrus). Our customized gene arrays firstly identified the transition from regular cycler to irregular cycler (9-10month old) as the stage where the major age-independent decline in brain bioenergetic gene expression occurs during perimenopause. Functional group analyses revealed decreased expression of genes involved in glucose metabolism, mitochondrial biogenesis/dynamics, molecule transport, and redox homeostasis during this transition period. Bioinformatic analyses further indicated that differences in expression patterns are regulated by upstream insulin/IGF1 and PGC1α signaling pathways. Consistently, biochemical analyses manifested that the regular-irregular transition is associated with compromised mitochondrial function in terms of decreased respiratory capacity, increased production of H_2O_2, and decreased activity of key bioenergetic enzymes. Finally, the above-mentioned changes during the transition were accompanied by a decline in functional outcomes, including decreased brain glucose uptake (FDG-microPET) and decreased synaptic plasticity (long-term potentiation). Together, these data from a rodent model of perimenopause provide novel mechanistic insights into the effects of reproductive aging on bioenergetic and synaptic function, which is critical for understanding the perimenopausal transition in both normal brain aging and the development of phenotype of AD risk.

Spirulina platensis and its ingredient prevent NASH to attenuate oxidative stress from mitochondrial energy metabolism and chronic inflammation followed by metabolic turnover alteration

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Non-alcoholic steatohepatitis (NASH) is recognized as a precursor to more severe liver disease and sometimes evolves into cryptogenic cirrhosis. So, it is desired the agents to manage NASH. Our previous study exhibited spirulina (SP) worked so well in preventing fibrosis and ameliorating fatty liver of an animal NASH model. The crosstalk of oxidative stress (OS) and inflammation amplify each other and verges to chronic inflammation that would be involved in NASH. Our study to focus on phycocyanin indicated other effective ingredient. So this study aimed to find an active ingredient to focus on biopterin, which is a constituent as its glycoside (BPG) in SP, and is metabolized *in vivo* to tetrahydrobiopterin involving in cell turnover, and clarify action mechanisms. For NASH [Patent No. JP 5109134] rats, SP and BPG were administrated perorally during the 2nd hit period following fatty liver formation. The 2nd hit is the recurrent and intermittent hypoxemia. The efficacies of SP and BPG on NASH were evaluated by blood biochemical tests and histopathological examinations. The mechanisms of actions were investigated to focus on OS injuries, inflammatory reactions and lipid metabolism regulations. Histopathological examination and blood biochemical tests demonstrated significant efficacies of SP and BPG to prevent fibrosis and to alleviate steatosis. Their efficacies were resulted from intervening between OS and inflammation. The protein expression concerning with lipid metabolism and vasodilation, revealed SP and BPG could redress the lipid metabolism disorder, furthermore could increase the oxygen supply.

Microglial and astrocyte activation in a mouse model of Leber's Hereditary Optic Neuropathy

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Leber's Hereditary Optic Neuropathy (LHON) is a retinal neurodegenerative disorder resulting from inheritance of mutations in mitochondrial complex I (NADH dehydrogenase). The mechanism of LHON pathogenesis is unknown, however, multiple mechanisms are possible, one of which is the over production of reactive oxygen species (ROS). The recent development of ndufs4 knockout mice as a model of complex I deficiency has allowed for more rigorous testing. We analyzed global gene expression using RNA sequencing (RNA-seq) in eyes of ndufs4 knockout and wild type littermate mice during a critical period of disease onset. It has been reported that ndufs4 mice lose visual function at postnatal days 30 (P30). Therefore, we examined the pattern of gene expression before, during, and after this period. RNA-seq data, which was confirmed with RT-qPCR and immunostaining, from P30 retinas revealed an increase in immune and inflammatory genes in ndufs4 knockout mice. Further analysis suggests that microglia and astrocytes are highly activated at onset of pathogenesis as seen from increases in genetic markers of Aif1 and Cxcl10, respectively. RT-qPCR at P20 also suggests that activation of microglia and astrocytes may begin at an earlier time point. Glial activation may then cause an increase in inflammation and neurodegeneration, resulting in cell loss of retinal ganglion cells (RGCs), which we have shown through decreased DAPI and Brn3a cell count in the RGC layer. These data support the hypothesis that loss of mitochondrial function induces retinal inflammatory genes in ndufs4 mice, and suggests a potential route of therapeutic treatment in LHON.

Dietary (–)-epicatechin effects on oxidative metabolism and nitric oxide (NO) steady state in LPS-treated rats

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Diets enriched in flavonoids have shown protective effects on inflammatory conditions in humans and experimental models in animals. In this work we studied the effect of a particular flavonoid, the flavan-3-ol (–)-epicatechin on the oxidative manifestations present in acute endotoxemia. Male Sprague-Dawley rats were injected i.p. with lipopolysaccharide of *Escherichia coli* (LPS, 4 mg/kg) as a model of acute endotoxemia with or without previous administration of a diet enriched with (–)-epicatechin (E) (100 mg/kg bw) during 4 d. Animals were euthanized 6 h after LPS injection. Experimental groups were: Control (C), Control+E (CE), LPS (L) and LPS + E (LE). Steady state level of NO in blood was studied through the determination of the NO-Hemoglobin adduct by electronic paramagnetic resonance. NO-Hemoglobin was not detectable in C and CE groups, and increased significantly after LPS-challenge. The NO increase triggered by LPS in (–)-epicatechin-supplemented animals was significantly higher respect to non-supplemented animals (LE= 17 ± 2 arbitrary units, L= 12 ± 2 arbitrary units; $p < 0.05$). At tisular level (kidney, heart, adipose tissue), dietary administration of (–)-epicatechin attenuated the increase in NADPH-dependent SOD inhibitable superoxide anion induced by LPS treatment. These results suggest that dietary (–)-epicatechin should be able to mitigate the alteration in oxidative metabolism during the onset of acute endotoxemia through a common mechanism. Further experiments should be necessary to understand the effects on NO production.

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Nitric oxide bioavailability in hypertensive animal models: effect of (-)-epicatechin supplementation

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This study evaluates the effects of dietary (-)-epicatechin (EC) supplementation on nitric oxide (NO) bioavailability in heart tissue, in two models of hypertension: i) rats deficient in NO by treatment with L-NAME, and ii) rats with metabolic syndrome (MetS) induced by consumption of a high fructose diet. In both models, EC prevented the hypertension-associated increases in: systolic blood pressure (SBP), $GSSG/GSH^2$ and superoxide anion production and expression of $p47^{phox}$ NOX subunit ($p<0,05$ EC treated rats vs. L-NAME or fructose-overload rats). Regarding nitric oxide synthase activity (NOS) in the L-NAME model, EC administration prevented the decrease in both, NOS activity and the expression of phosphorylated eNOS (p-eNOS), observed in hypertensive rats ($p< 0.05$). In the MetS model, a significant increase in NOS activity and p-eNOS expression was observed in rats receiving EC in their diet compared to hypertensive and control rats ($p<0,01$). Dietary EC also prevented the augmented superoxide dismutase activity found in L-NAME treated rats and the decreased activity observed in fructose-fed rats. In conclusion, EC supplementation prevented the elevation of SBP and the oxidative alterations observed in L-NAME and MetS rat models, suggesting that EC modulates NO bioavailability by diminishing the expression and inhibiting the activity of NOX, and increasing expression and activity of NOS.

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