OXIDANTS AND ANTIOXIDANTS IN BIOLOGY

REDOX MEDICINE AND NUTRITION

4-6 May 2016
UNIVERSITY OF CALIFORNIA DAVIS CONFERENCE CENTER
DAVIS, CALIFORNIA
OXIDANTS AND ANTIOXIDANTS IN BIOLOGY
REDOX MEDICINE AND NUTRITION
4-6 MAY 2016
UNIVERSITY OF CALIFORNIA DAVIS CONFERENCE CENTER

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Hagen Schroeter
MARS INC., MCLEAN, VIRGINIA, USA
SCIENTIFIC PROGRAM
The remarkable effects of altering copper metabolism in familial SOD-linked amyotrophic lateral sclerosis

Joseph S. Beckman
Linus Pauling Institute, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA
SESSION I  REDOX REGULATION OF CELL SIGNALING

CHAIRPERSONS  
Junji Yodoi  
DEPARTMENT OF BIOLOGICAL RESPONSES, INSTITUTE FOR VIRUS RESEARCH, GRADUATE SCHOOL OF MEDICINE, KYOTO UNIVERSITY, KYOTO, JAPAN

Fawaz Haj  
DEPARTMENT OF NUTRITION, UNIVERSITY OF CALIFORNIA, DAVIS, USA

02:00 – 02:30  
Sites of mitochondrial ROS production  
Martin D. Brand  
BUCK INSTITUTE FOR RESEARCH ON AGING, NOVATO, CA, USA

02:30 – 03:00  
Role of redox-sensitive p38a in the regulation of cytokinesis in the liver  
Juan Sastre  
DEPARTMENT OF PHYSIOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF VALENCIA, VALENCIA, SPAIN

03:00 – 03:30  
Finding the right balance: how exquisite interrelationships of conformation and chemistry govern peroxiredoxin catalysis  
P. Andrew Karplus  
DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS, OREGON STATE UNIVERSITY, CORVALLIS, OREGON, USA

03:30 – 04:00  
Coffee break

04:00 – 04:30  
Translational bioenergetics and oxidative stress in human patients  
Victor Darley-Usmar  
DEPARTMENT OF PATHOLOGY, UNIVERSITY OF ALABAMA AT BIRMINGHAM, BIRMINGHAM, ALABAMA, USA
04:30 – 05:00  Crosstalk between the aryl hydrocarbon receptor (Ahr) and oxidative stress: Insights from studies of AhR ligand promiscuity
_Michael S. Denison_

**DEPARTMENT OF ENVIRONMENTAL TOXICOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA**

05:00 – 05:15  Nitrite-mediated S-nitrosylation of caspase-6 prevents hypoxia-induced neural injury
_Tzu-Ching Meng_

**INSTITUTE OF BIOLOGICAL CHEMISTRY, ACADEMIA SINICA, TAIPEI, TAIWAN**

05:15 – 05:30  Nrf2 signaling as potential therapy for mitochondrial diseases
_Genki Hayashi_

**MOLECULAR BIOSCIENCES, SCHOOL OF VETERINARY MEDICINE, UNIVERSITY OF CALIFORNIA, DAVIS, USA**
### ROUND TABLE DISCUSSION

**05:30 – 06:30**  
**REDOX CHEMISTRY IN CELL FUNCTIONS**  
*Dean P. Jones, Discussion Leader*

**Division of Pulmonary, Allergy, and Critical Care Medicine, Emory University, Atlanta, GA, USA**

*Martin D. Brand*

**Buck Institute for Research on Aging, Novato, CA, USA**

*Bob Buchanan*

**Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA**

*P. Andrew Karplus*

**Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon, USA**

*Santiago Lamas*

**Centro de Biología Molecular “Severo Ochoa” (CSIC-UAM), Madrid, Spain**

**06:30 – 08:00**  
**Poster Viewing and Welcome Reception**
SESSION II  NUTRITION AND THE GENOME-EPIGENOME

CHAIRPERSONS  Maret G. Traber
                LINUS PAULING INSTITUTE, DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS, OREGON STATE UNIVERSITY, CORVALLIS, OR, USA

Tilman Grune
                GERMAN INSTITUTE OF HUMAN NUTRITION POTS DAM-REHB RÜKE, NUTHETAL, GERMANY

09:00 – 09:30  Lactoferrin and its receptor: Effects on gene transcription
                Bo Lönnerdal
                DEPARTMENT OF NUTRITION, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA

09:30 – 10:00  Iron overload and genome alterations
                Shinya Toyokuni
                DEPARTMENT OF PATHOLOGY AND BIOLOGICAL RESPONSES, NAGOYA UNIVERSITY
                GRADUATE SCHOOL OF MEDICINE, NAGOYA, JAPAN

10:00 – 10:30  Proteomic profiles in humans in response to diet:
                Inflammation and oxidative stress biomarkers
                Johanna W. Lampe
                PUBLIC HEALTH SCIENCES DIVISION, FRED HUTCHINSON CANCER RESEARCH CENTER, SEATTLE, WA, USA

10:30 – 11:00  Coffee break
11:00 – 11:30  A role for nutrition and metabolism in epigenetic control of gene expression
  
  *Frederick Domann*
  
  Department of Radiation Oncology and Holden Comprehensive Cancer Center, The University of Iowa, Iowa City, IA, USA

11:30 – 12:00  Nutritional regulation of cathelicidin antimicrobial peptide gene expression
  
  *Adrian F. Gombart*
  
  Linus Pauling Institute, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA

12:00 – 12:15  Tocotrienols induce apoptosis via endoplasmic reticulum stress in HeLa cells
  
  *Fabio Virgili*
  
  Council Agricultural Res and Economics - Food and Nutrition Research Centre, Rome, Italy

12:15 – 12:30  Soluble epoxide hydrolase podocyte deficiency protects against hyperglycemia-induced renal injury and improves systemic glucose homeostasis
  
  *Ming-Fo Hsu*
  
  Department of Nutrition, University of California, Davis, CA, USA

12:30 – 01:00  Group Photo

01:00 – 02:00  Lunch and Poster Viewing
**SESSION III**  
**REGENERATIVE MEDICINE**

**CHAIRPERSONS**  
*Giuseppe Poli*  
Department of Clinical and Biological Sciences, University of Torino, San Luigi Hospital, Torino, Italy

*Chandan K. Sen*  
Center for Regenerative Medicine and Cell Based Therapies, The Ohio State University Wexner Medical Center, Columbus, OH, USA

02:00 – 02:30  
Non-selenocysteine containing phospholipid hydroperoxide glutathione peroxidase: A critical role in regenerative wound healing  
*Chandan K. Sen*  
Center for Regenerative Medicine and Cell Based Therapies, The Ohio State University Wexner Medical Center, Columbus, OH, USA

02:30 – 03:00  
Diabetes irreversibly depletes progenitor cell subpopulations critical for tissue repair  
*Geoffrey C. Gurtner*  
Stanford University School of Medicine, Stanford, CA, USA

03:00 – 03:30  
miRNA in resolution of inflammation  
*Sashwati Roy*  
Center for Regenerative Medicine and Cell Based Therapies, The Ohio State University Wexner Medical Center, Columbus, OH, USA

03:30 – 04:00  
Coffee break

04:00 – 04:30  
Redox regulation of airway epithelial stem cell maintenance and repair  
*Brigitte N. Gomperts*  
Department of Pediatrics/Hematology-Oncology, University of California, Los Angeles, CA, USA
4:30 – 05:00  RedoximiRs involved in organ fibrosis
_Santiago Lamas_
_CENTER DE BIOLOGÍA MOLECULAR “SEVERO OCHOA” (CSIC-UAM), MADRID, SPAIN_

05:00 – 05:15  Scavenger receptor B1 as a possible player in cutaneous tissue proliferation and differentiation
_Giuseppe Valacchi_
_DEPARTMENT OF LIFE SCIENCES AND BIOTECHNOLOGY, UNIVERSITY OF FERRARA, FERRARA, ITALY_

05:15 – 05:30  Nanotechnology-enabled non-viral tissue reprogramming
_Daniel Gallego-Perez_
_DEPARTMENTS OF SURGERY AND BIOMEDICAL ENGINEERING, CENTER FOR AFFORDABLE NANOENGINEERING OF POLYMERIC BIOMEDICAL DEVICES, CENTER FOR REGENERATIVE MEDICINE AND CELL-BASED THERAPIES, THE OHIO STATE UNIVERSITY, COLUMBUS, OH_

05:30 – 05:45  Oral supplementation of fermented papaya preparation to type 2 diabetes mellitus patients improves the respiratory burst function in chronic wound-site macrophages
_Ryan Dickerson_
_DEPARTMENT OF SURGERY, THE OHIO STATE UNIVERSITY WEXNER MEDICAL CENTER, COLUMBUS, OH, USA_

05:45 – 07:00  Poster Viewing
<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Speaker</th>
<th>Institution</th>
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<tbody>
<tr>
<td>09:00</td>
<td>Enhancing dentate gyrus function with dietary flavanols improves cognition in older adults</td>
<td>Scott A. Small</td>
<td>Columbia University, New York, NY, USA</td>
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<td>09:30</td>
<td>Potential mechanisms by which polyphenols influence memory and cognition</td>
<td>Jeremy P.E. Spencer</td>
<td>Food and Nutritional Sciences, University of Reading, Reading, UK</td>
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<td>10:00</td>
<td>Neurovascular coupling mediated by neuronal nitric oxide in hippocampus: Functional communication with ascorbate and nitrite</td>
<td>João Laranjinha</td>
<td>Center for Neurosciences and Cell Biology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal</td>
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<tr>
<td>10:30</td>
<td>Coffee break</td>
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11:00 – 11:30  Nutrient biomarker patterns, cognition, and Alzheimer’s pathology in older adults
Gene L. Bowman
NESTLÉ INSTITUTE OF HEALTH SCIENCES, EPFL CAMPUS, LAUSANNE, SWITZERLAND

11:30 – 12:00 The role of lutein in cognitive function
Elizabeth J. Johnson
ANTIOXIDANTS RESEARCH LABORATORY, TUFTS UNIVERSITY, BOSTON, MA, USA

12:00 – 12:15 Zinc and lipoic acid in the modulation of the STAT3 pathway in the developing brain
Suangsuda Supasai
DEPARTMENTS OF NUTRITION AND ENVIRONMENTAL TOXICOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA

12:15 – 12:30 Nrf2-mediated antioxidant protection in the retinal pigment epithelium
Jiyang Cai
DEPARTMENT OF OPHTHALMOLOGY, UNIVERSITY OF TEXAS MEDICAL BRANCH, GALVESTON, TX, USA
USANA HEALTH SCIENCES INC., SALT LAKE CITY, UT, USA

12:30 – 12:45 Fast food decreases the anti-inflammatory capacity of HDL particles in stimulated peripheral blood mononuclear cells
Angela M. Zivkovic
DEPARTMENT OF NUTRITION, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA

12:45 – 02:00 Lunch and Poster Viewing
SESSION V  MICROBIOME AND GUT INFLAMMATION

CHAIRPERSONS  Mulchand S. Patel
DEPARTMENT OF BIOCHEMISTRY, SCHOOL OF MEDICINE AND BIOMEDICAL SCIENCES, UNIVERSITY AT BUFFALO, STATE UNIVERSITY OF NEW YORK, BUFFALO, NY, USA

Carl L. Keen
DEPARTMENT OF NUTRITION, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA

02:00 – 02:30  Redox-dependent signaling in the gut: A mechanism of host-microbiota crosstalk
Andrew S. Neish
PATHOLOGY, EMMORY UNIVERSITY SCHOOL OF MEDICINE, ATLANTA, GA, USA

02:30 – 03:00  The gut microbiome and chronic inflammatory disease
Susan V. Lynch
DIVISION OF GASTROENTEROLOGY, DEPARTMENT OF MEDICINE, UNIVERSITY OF CALIFORNIA, SAN FRANCISCO, CA, USA

03:00 – 03:30  Plant bioactives for alleviating metabolic diseases: Effects on the gut microbiota and beyond
André Marette
SCHOOL OF MEDICINE, LAVAL UNIVERSITY, QUEBEC, CANADA

03:30 – 04:00  Coffee break

04:00 – 04:30  Nutritional impact of pomegranate polyphenol antioxidants on microbiome metabolism and proliferation in vitro and in vivo
David Heber
UCLA CENTER FOR HUMAN NUTRITION, UNIVERSITY OF CALIFORNIA, LOS ANGELES, CA, USA

04:30 – 05:00  The role of the milk glycome in the development of the infant gut microbiota: Why babies cry over spilled milk
David A. Mills
FOODS FOR HEALTH INSTITUTE, DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY AND DEPARTMENT OF VITICULTURE AND ENOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA
ROUND TABLE DISCUSSION

05:00 – 05:45  THE MICROBIOME: CAUSES AND SOLUTIONS FOR STRESS

*J. Bruce German*, discussion leader
FOODS FOR HEALTH INSTITUTE, DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA

_Bethany Henrick_
FOODS FOR HEALTH INSTITUTE, DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA

_Steven A. Frese_
FOODS FOR HEALTH INSTITUTE, DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA

_David A. Mills_
FOODS FOR HEALTH INSTITUTE, DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY AND DEPARTMENT OF VITICULTURE AND ENOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA

_David Heber_
UCLA CENTER FOR HUMAN NUTRITION, UNIVERSITY OF CALIFORNIA, LOS ANGELES, CA, USA
07:00 – 08:00  
*Piano concert*

**STUDIO THEATER, MONDAVI CENTER, UNIVERSITY OF CALIFORNIA, DAVIS, CA, USA**

*Yoshihiro Ota & Moeko Ota*

Pianists

08:00  
*Gala Dinner — Prizes and Awards*

**ALUMNI CENTER, UNIVERSITY OF CALIFORNIA, DAVIS**

*Health Sciences Prize*

**SELECTION COMMITTEE**

Lester Packer, Jarrow Rogovin, Enrique Cadenas

*Science and Humanity Award*

**SELECTION COMMITTEE**

Helmut Sies, Maret G. Traber, Yuki Hayashi

*Young Investigator Awards*

- Oxygen Club of California
- Linus Pauling Institute
- Archives of Biochemistry & Biophysics – Elsevier

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PLENARY LECTURE
The remarkable effects of altering copper metabolism in familial SOD-linked amyotrophic lateral sclerosis

Joseph Beckman

Linus Pauling Institute, Department of Biochemistry and Biophysics,
Oregon State University, Corvallis, OR, USA

Mutant SODs confer a toxic gain of function that leads to motor neuron degeneration in humans, dogs, mice, rats, zebrafish, and Drosophila. This gain-of-function involves partially unfolded intermediates of SOD that lack at least one of the two metal atoms critical for stabilizing SOD. Overexpression of mutant human superoxide dismutase (SODG93A) in transgenic mice induces many features of ALS and has become the most widely used model for any neurodegenerative disease. However, after 20 years of searching, no pharmaceutical treatment has reproducibly extended the 130-day lifespan of high-expressing SODG93A mice by more than 10-20%. We instead began exploring what processes accelerate the development of ALS in SOD mice. The most rapid acceleration of ALS results when the human Copper-Chaperone-for-SOD (CCS) is coexpressed with human SOD, which results from an acute copper deficiency in the CNS. Remarkably, CuATSM given to SODG93A mice coexpressing the human Copper Chaperone for SOD (CCS) protected against ALS progression for over 20 months with many of the mice still alive. If treatment is removed, the mice develop ALS symptoms in a few months. The mechanism by which SOD kills motor neurons in ALS is far subtler than protein aggregation. While still quite controversial, we believe that the role of SOD in causing ALS is beginning to be revealed and that can be controlled therapeutically.
SESSION I

REDOX REGULATION OF CELL SIGNALING
Mitochondria are potent sources and potential sinks for reactive oxygen species (ROS) in mammalian cells. Different sites of production may have different roles in redox signaling and pathology. Eleven distinct sites associated with substrate oxidation and oxidative phosphorylation leak one electron to oxygen to produce superoxide or two electrons to oxygen to produce hydrogen peroxide. They are in the oxoacid dehydrogenase complexes that feed electrons to NAD$^+$; respiratory complexes I and III, and dehydrogenases, including complex II, that use ubiquinone as acceptor. The topologies, capacities, and substrate dependences of each site have recently clarified. Complex III and mitochondrial glycerol 3-phosphate dehydrogenase generate superoxide to the intermembrane space as well as the matrix, the other sites generate superoxide and/or hydrogen peroxide exclusively in the matrix. The sites have very different maximum capacities, but the net rate of superoxide or hydrogen peroxide generation from each site depends on the substrates present and the energy demand. The rate at each site can now be measured in complex substrate mixtures. In skeletal muscle mitochondria in media mimicking muscle cytosol at rest, four sites dominate, two in complex I (sites I$_Q$ and I$_F$) and one each in complexes II (site II$_F$) and III (site III$_Qo$). Specific suppressors of sites I$_Q$ and III$_Qo$ have been identified. They prevent superoxide/hydrogen peroxide production from the site without affecting oxidative phosphorylation, making them excellent tools to investigate the status of the sites in redox signaling, and to suppress the sites to prevent pathologies.
Role of redox-sensitive p38 alpha in the regulation of cytokinesis in the liver

Juan Sastre

Department of Physiology, Faculty of Pharmacy, University of Valencia, Valencia, Spain

Cytokinesis is the last step in mitosis and consists in the cleavage of the mother cell into two daughter cells, which implies remodelling of the actin cytoskeleton. A failure in cytokinesis is one of the major mechanisms responsible for the formation of polyploid cells in mammals and may lead to genetic instability. Our results show that cytokinesis is regulated by redox sensitive MAP kinase p38α. MNK-1 activation by phosphorylation is dependent on p38α and MK2 and it is required for the abscission of the intercellular bridge during mitosis. Mice with liver-specific deficiency in p38α exhibit markedly less MNK-1 phosphorylation in the liver. Accordingly, increased binucleation occurs in the liver of p38α deficient mice under basal conditions, after partial hepatectomy and during biliary cirrhosis. In addition, the relative liver mass was reduced in all these conditions, and p38α deficiency eventually leads to increased mortality in biliary cirrhosis. Furthermore, in old mice the absence p38α also causes a decrease in the phosphorylation of HSP27, which is required for actin polymerization. Hence, a marked deficit in actin polymerization was found in the liver of old mice with deficiency in p38α. Consequently, our results show that hepatocytes enter into the S phase but do not complete cell division when there is deficiency in p38α, causing failure in cytokinesis and binucleation. Additionally, the long-term deficiency in p38α causes a severe impairment in actin assembly during aging.
Finding the right balance: how exquisite interrelationships of conformation and chemistry govern peroxiredoxin catalysis

Andy Karplus, Arden Perkins, Derek Parsonage, Kim Nelson and Leslie Poole

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331
Department of Biochemistry, Wake Forest School of Medicine, Winston-Salem, NC 27157

Peroxiredoxins (Prx), the dominant peroxidases in most organisms, require for catalysis a key cysteine in a substrate-ready fully folded (FF) conformation. This peroxidatic Cys is oxidized to sulfenic acid by the peroxide substrate, and then further reacts, after a local unfolding (LU) of the active site, to form a disulfide bond with a second ‘resolving’ Cys. If oxidation by a second peroxide occurs the Prx becomes hyperoxidatively inactivated. For certain “sensitive” Prxs facile hyperoxidation appears to have been promoted during evolution to enable them to function in redox signaling. I will summarize some key structural studies that advanced our understanding of Prx catalysis, including recognizing the existence of sensitive Prxs in eukaryotes, proposing the origins of the remarkably efficiency of Prx catalysis, and using crystals that accommodate both the FF and LU conformations of *Salmonella typhimurium* AhpC, to learn how subtle changes can influence the thermodynamics of the FF / LU equilibrium and the susceptibility to hyperoxidation. This latter observation provides insight into how posttranslational modifications could regulate Prx function, and implies that caution must be exercised in interpreting the behavior of peroxidatic or resolving Cys mutants. Also, in recent work using active crystals of *Xanthomonas campestris* PrxQ that diffract to atomic resolution, we have visualized the catalytic pathway in unprecedented detail, and find evidence that a further conformational influence can come from destabilization of the FF active site upon SOH formation. These crystals also provide an unanticipated proof-of-principle for conformation-targeting inhibitors as an effective strategy for selectively inhibiting Prxs.
Translational bioenergetics and oxidative stress in human patients

Victor M. Darley-Usmar

Mitochondrial Medicine Laboratory, Department of Pathology, University of Alabama at Birmingham, USA

Bioenergetics is now at the forefront of our understanding of pathological mechanisms, new therapies and as a biomarker for the susceptibility of the progression of diseases associated with increased oxidative stress. A key concept is that the mitochondrion can act as the “canary in the coal mine” by serving as a sensor of systemic oxidative stress and an early warning of bioenergetic crisis in patient populations. Furthermore, cellular mitochondrial function is known to vary between populations due to differences in genetic background and in response to lifestyle changes including diet and exercise. We have developed a high-throughput assay to measure cellular energetic function in the small numbers of cells that can be isolated from human blood. The sequential addition of well-characterized inhibitors of oxidative phosphorylation allows a bioenergetic profile to be measured in cells isolated from normal or pathological samples. This profile can define the extent to which these cells utilize mitochondrial oxygen consumption to produce ATP, are using protons for other processes or leak and the maximal respiration and respond to changes in oxidative stress. Taken together we propose these parameters are a measure of bioenergetic health of a cell population. We therefore propose the development of the Bioenergetic Health Index (BHI), which is a single value that defines bioenergetic health based upon the analysis of cellular mitochondrial profiles in cells isolated from human subjects. Ultimately, BHI has the potential to be a new biomarker for assessing patient health of (or for) both prognostic and diagnostic value.
Crosstalk between the aryl hydrocarbon receptor (AhR) and oxidative stress: Insights from studies of AhR ligand promiscuity

Michael S. Denison

Department of Environmental Toxicology, University of California, Davis, CA 95616, USA

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that mediates the toxic and biological effects of a wide variety of structurally diverse chemicals. Ligand-dependent activation of the AhR has been shown to directly stimulate expression of genes encoding phase I (CYP1A1/2 and other cytochrome P450s) and phase II (NQO1, GSTA1, UGT1A6) metabolic enzymes, as well as numerous other genes (e.g. Nrf2 and others) via direct interaction of the ligand-activated AhR complex with its DNA binding site (e.g., the dioxin responsive element) present upstream of these genes. The production reactive oxygen species (H$_2$O$_2$, O$_2^-$) by increased CYP1A1 enzymatic activity, or by oxidative stress, can directly activate NRF2, which then stimulates expression of these same phase II genes via an interaction with its specific DNA binding site (the antioxidant response element). The AhR and AhR-dependent gene expression reportedly can be activated by oxidative stress (in the absence of added exogenous ligand), however the responsible mechanism is unknown. Studies into the structural diversity of AhR ligands suggests that oxidative stress conditions can produce a variety of AhR active chemicals that could directly stimulate the expression of AhR-responsive genes, concomitant with Nrf2-dependent induction, resulting in an enhanced gene expression response to oxidative stress. Activation of the AhR by structurally diverse ligands indicates that a dramatically wider range of chemicals could affect the expression of oxidative stress-associated genes than previously thought. Together, the AhR and Nrf2 can coordinately regulate the adaptive response to oxidative stress by multiple mechanisms.
Nitrite-mediated S-nitrosylation of caspase-6 prevents hypoxia-induced neuronal injury

Yen-Jung Chen\textsuperscript{1,2} and Tzu-Ching Meng\textsuperscript{1,2}

\textsuperscript{1}Institute of Biochemical Sciences, National Taiwan University,
\textsuperscript{2}Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

Coordination of neuronal function is an absolutely oxygen-demanding process. About 20\% of inhaled oxygen is supplied to the brain. It is therefore not surprising that systematic hypoxia caused by cardiovascular diseases, lung diseases or obstructive sleep apnea leads to damage of neurons. To date the underlying mechanism of hypoxia-induced neuronal injury remains elusive. It is also not known what strategy might be used to prevent neurons from hypoxic damage. In this study, we demonstrate that exposure of differentiated Neuro-2a (N2a) cells and primary rat cortical neurons to hypoxia resulted in significant neurite/axon retraction, a hallmark of neuronal injury. We show that collapse of neurite/axon in hypoxic neurons was promoted by proteolysis of Tau, a protein stabilizing microtubule structure. Interestingly, the detrimental effect of hypoxia on neurons was completely prevented by nitrite treatment, suggesting a protective role of nitrite in neuronal function. We then found that caspase-6, which was activated in the neurites of hypoxic neurons, cleaves Tau. Importantly, with the supply of nitrite, caspase-6 was inactivated via S-nitrosylation of its active-site Cys146 residue, thus incapable of proteolysing Tau in neurons exposed to hypoxia. Using C57BL/6 mouse as an \textit{in vivo} model, we confirmed that nitrite inhibits hypoxia-induced anxiety and protects the cortex from Tau degradation by caspase-6. These results are the first to demonstrate a critical role of nitrite in neuronal protection against hypoxic insult. Our finding also shows that nitrite holds a great potential for the treatment of diseases associated with hypoxia-induced neuronal injury.
Nrf2 signaling as potential therapy for mitochondrial diseases

Genki Hayashi and Gino Cortopassi

Molecular Biosciences, School of Veterinary Medicine,
University of California, Davis, CA, USA

There is no FDA-approved therapy for mitochondrial disease, which is frequently lethal. We have carried out screens of repurposed drugs in cell models of mitochondrial disease. In a screen of 1600 drugs, multiple Nrf2 inducers were protective, including the drug dimethyl fumarate, DMF. In cells, DMF dose-dependently increased mitochondrial copy number, mitochondrial gene expression, and mitochondrial function in the Seahorse. DMF dose-dependently increased the protein frataxin, whose deficiency is the only cause of Friedreich's ataxia. Mice dosed with DMF experienced an increased mitochondrial copy number and gene expression in multiple tissues. DMF has been proposed to have two targets, the Keap1/Nrf2 complex, and HCAR2. Our studies demonstrated that the mitobiogenic effect of DMF depended on Nrf2 and not HCAR2. Because DMF has already gone through extensive safety testing and is used in treatment of psoriasis and MS, this drug should be considered for mitochondrial disease indications.
SESSION II

NUTRITION AND THE GENOME-EPIGENOME
Lactoferrin and its receptor: Effects on gene transcription

Bo Lönnerdal, Rulan Jiang, and Eric Nonnecke

Department of Nutrition, University of California, Davis, CA, USA

Lactoferrin (Lf) is a major constituent of breast milk, constituting about 15-20% of total protein. It can bind two ferric ions with very high affinity (1023). Breast-fed infants consume large quantities of Lf, but as there is much less Fe in breast milk on a molar basis, it is primarily in its Fe-unsaturated (apo-Lf) form. Lf is unusually stable against proteolysis and is found intact in the stool of breast-fed infants. Orally ingested Lf can exert bacteriostatic, bactericidal and anti-viral activities within the gut lumen; however, Lf also exerts a multitude of cellular functions by binding to its mucosal receptor. We isolated and characterized this Lf receptor and found that it is a homo-trimer with a subunit of 35 kD. We subsequently cloned the gene coding for this receptor and studied its tissue expression in the human fetus/infant. It is highly expressed in the small intestine but also present in other tissues. We further found that it takes up Lf by clathrin-mediated endocytosis. By transfecting human intestinal cells with the Lf receptor gene, we found enhanced uptake of Fe. Upon binding and internalization, Lf translocates to the nucleus thereby acting as a transcription factor and modifier of cellular signaling events. We have shown that apo-Lf stimulates cell proliferation and differentiation, thus stimulating mucosal growth and development. Further, by binding to the promoters of several cytokines, Lf can affect immune function and exert anti-inflammatory activities. These findings may explain the pluripotent activities of Lf, which are beyond those in the gut lumen.
Iron overload and genome alterations

Shinya Toyokuni

Department of Pathology and Biological Responses, Nagoya University Graduate School of Medicine, Nagoya, Japan

Iron is abundant universally. However, iron works as a double-edged sword, and its excess can be a risk for cancer, presumably via generation of reactive oxygen species. Thus far, diseases such as hemochromatosis, chronic viral hepatitis B/C, exposure to asbestos as well as endometriosis have been recognized as iron overload-associated risks for human cancer. Indeed, iron is carcinogenic in animal experiments. We used a rat renal carcinogenesis model with repeated intraperitoneal ctions of ferric nitrilotriacetate (NTA) and rat mesothelial carcinogenesis models by intraperitoneal administration of asbestos fibers or multiwalled carbon nanotubes (MWCNT). The obtained tumors were analyzed with array-based comparative genome hybridization. During the carcinogenesis, iron accumulation in each target cell were evident. Furthermore, these studies unexpectedly revealed that there are common target genes in these iron-induced carcinogenesis (e.g. homozygous deletion of CDKN2A/2B, etc.) with massive genomic amplifications and deletions. MWCNT also induced malignant mesothelioma, with 50 nm diameter MWCNT most carcinogenic. The fact that massive genomic alterations were observed for the first time in the iron overload-associated animal models of WILD-type animals suggests that iron overload may be a major mechanism in various human carcinogenesis. These genetic changes would be helpful for diagnosing early stage of cancer in pathology specimens. Recent epidemiological studies reported that iron reduction by phlebotomy decreased cancer risk in the apparently normal population. These results warrant reconsideration of the role of iron in carcinogenesis and suggest that fine control of body iron stores would be a wise strategy for cancer prevention. The association of iron with L-ascorbate or epigenome would be also discussed.

Proteomic profiles in humans in response to diet: Inflammation and oxidative stress biomarkers

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Chronic low-grade systemic inflammation is a hypothesized underlying pathophysiologic mechanism linking obesity and lifestyle factors to cancer and other chronic diseases. Multi-dimensional “omics” approaches lend themselves towards characterizing response to changes in diet and lifestyle, providing insight into complex biologic systems. We have applied omics approaches in controlled interventions to test hypotheses related to diet and cancer and to identify novel pathways responding to dietary treatments. In a randomized, placebo-controlled, crossover study, we found that glucosamine and chondroitin (G&C) supplementation, compared to placebo, differentially altered 30 pathways as measured by plasma proteome analysis (Navarro et al, PLOS ONE, 2015). We found that G&C significantly reduced circulating C-reactive protein concentrations and gene set enrichment analyses indicated that cytokine activity and other inflammation-related pathways were significantly decreased. Currently, we are using proteomics to evaluate the effects of two dietary patterns in a randomized crossover feeding trial on plasma protein biomarkers of regulatory pathways important in cancer susceptibility. Eighty participants [40 normal BMI (18.5 –25.0 kg/m²) and 40 overweight/obese (BMI 28.0-40.0 kg/m²)] consumed two controlled diets for 28 days each. One diet was high in whole grains, legumes, and fresh fruits and vegetables. The other was a dietary pattern that substituted refined grains for the whole grains, and provided other carbohydrates from high-glycemic index food sources. Taken together, these studies and others support the utility of combining robust study designs and proteomics approaches in experimental human studies to better understand the impact of the totality of diet on the totality of health.
A role for nutrition and metabolism in epigenetic control of gene expression

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Not all genes are expressed in all cells at all times, but rather expression of the genetic code is influenced by developmental, hormonal, metabolic and environmental factors. Chromatin structure and topology regionally affect the accessibility of the DNA for efficient transcription. There is an emerging realization that all or nearly all enzymes that participate in chromatin remodeling require biochemical intermediary metabolites as co-factors for efficient activity. These enzymes include both the so-called chromatin “writer” and “eraser” enzymes that respectively add and remove chemical modifications to histones and DNA. Examples of writer enzymes include DNA and histone methyltransferases, both families of which require Sadenosylmethionine (SAM) as the methyl-donating cofactor. SAM is linked both to nutritional methyl donors such as choline and folate and to cellular redox metabolism through the glutathione (GSH) system via the transulfuration pathway. Histone acetyltransferases are also writer enzymes; their obligate cofactor is acetyl-CoA, a direct product of beta oxidation of dietary fats. Meanwhile, examples of eraser enzymes include histone and DNA demethylases, both families of which require an interesting combination of co-factors indicative of their connection to intermediary metabolism and therefore diet: O₂, Fe²⁺, α-ketoglutarate (2-oxoglutarate, a TCA cycle intermediate), and ascorbate (vitamin C), which in humans can only be obtained via diet. Another interesting example is the sirtuin family of NAD⁺ dependent histone deacetylases that link NAD⁺ biochemistry to histone acetylation. Taken together, the dependence of chromatin remodeling enzymes on nutrients and their derived intermediary metabolites demonstrates a clear link between nutrition and epigenetic control of gene expression.
Nutritional regulation of cathelicidin antimicrobial peptide
gene expression

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The human cathelicidin antimicrobial peptide (CAMP) gene encodes the human cathelicidin hCAP18 that is cleaved to release the antimicrobial peptide LL-37. It is synthesized and secreted by immune and epithelial cells and is critical for barrier defense. Deficiency in LL-37 increases susceptibility to infection. In addition to killing microbes, LL-37 binds lipopolysaccharide, chemoattracts immune cells and modulates the expression of cytokines and chemokines. Vitamin D, short chain fatty acids and primary and secondary bile acids induce human CAMP expression. The induction of CAMP by vitamin D and primary bile acids occurs through the vitamin D receptor (VDR) and the farnesoid X receptor (FXR), respectively. Also, our group and others have demonstrated that resveratrol, curcumin and lactose induce CAMP expression. Further, we recently discovered that the plant polyphenol xanthohumol induces CAMP gene expression through FXR. These findings suggest that diet and/or consumption of nutritional supplements may improve and/or modulate immune function in the gut via the nuclear receptor family of transcription factors. In addition, by-products of gut microbe metabolism could be important for communicating with intestinal epithelial and immune cells, thus affecting the expression of CAMP. We hypothesize that this interaction between nutrients and CAMP expression functions to establish a mucosal barrier that protects against invasion of the intestinal epithelium by microorganisms and possibly shapes the composition of the gut microbiota. Vitamin D deficiency correlates with increased susceptibility to infection, and adequate levels may improve defense against infection. Regulating the endogenous expression of CAMP offers potential therapeutic treatments for infection and disease.
Tocotrienols induce apoptosis via endoplasmic reticulum stress in HeLa cells

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We have previously demonstrated that the ability of γ- and δ-tocotrienol (γ- and δ-T3) in inducing gene expression and apoptosis in human breast cancer cells (MDA-MB-231 and MCF-7) is, at least in part, due to the binding and activation of the estrogen receptor-β (ERβ). The interrogation of a set of transcriptomic data obtained within these studies, suggested the existence of an alternative pathway, activated by specific T3 forms and leading to apoptosis, independently of ER activation. Further experiments conducted in HeLa cells, a line of human cervical cancer cells void of any canonical ER, also utilizing transcriptomic platforms and further confirmed by RT-PCR, demonstrate that the administration of γ- and δ-T3 is associated to Ca2+ release. This event is eventually followed by the induction of specific Ca-dependent signals leading to the expression and activation of IRE1-α and, in turn, to the alternative splicing of the pro-apoptotic protein sXBP-1 and other molecules involved in the Unfolded Protein Response, the core pathway coping with endoplasmic reticulum stress in eukaryotic cells. Finally, a separate set of experiments indicates that treatment of HeLa cells with γ- and δ-T3 indeed induces the expression of miR190b, involved in the alternative splicing of the pro-apoptotic protein XBP-1. Overall, our study demonstrates that γ- and δ-T3 activate endoplasmic reticulum stress pathways, inducing ERβ-independent apoptosis. On the basis of our observations we suggest that tocotrienols could have significant therapeutic potential in the clinical treatment of tumors.
Soluble epoxide hydrolase podocyte deficiency protects against hyperglycemia-induced renal injury and improves systemic glucose homeostasis

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Diabetic nephropathy (DN) is the leading cause of renal failure and podocyte dysfunction plays a significant role in DN pathogenesis. Soluble epoxide hydrolase (sEH) is a cytosolic enzyme whose inhibition has beneficial effects but its role in podocytes remains unexplored. To that end, we generated mice with podocyte sEH deletion (pod-sEHKO) and determined alterations under normoglycemia and streptozotocin-induced hyperglycemia. Pod-sEHKO mice exhibited mild improvement in renal function and glucose homeostasis at normoglycemia. However, under hyperglycemia pod-sEHKO mice displayed remarkable improvement in renal function, glucose homeostasis and attenuation of hyperglycemia-induced increase in blood pressure. In addition, sEH deficiency protected podocyte structure against hyperglycemia-induced injury. Consistent with improved glucose homeostasis, pod-sEHKO mice displayed decreased gluconeogenesis and increased glucosuria under hyperglycemia. Moreover, sEH deficiency was associated with decreased renal endoplasmic reticulum (ER) stress and enhanced autophagy with corresponding decrease in inflammation and fibrosis. Notably, these effects were recapitulated in differentiated podocytes treated with sEH pharmacological inhibitor, consistent with being cell autonomous. Furthermore, elevation of ER stress and attenuation of autophagy mitigated the protective effects of sEH inhibition demonstrating causal association. Importantly, sEH pharmacological inhibition in vivo attenuated hyperglycemia-induced renal injury and improved glucose homeostasis. Collectively, these findings identify a novel role for sEH in podocytes with potential therapeutic implications.
SESSION III
REGENERATIVE MEDICINE
Non-selenocysteine containing phospholipid hydroperoxide glutathione peroxidase: A critical role in regenerative wound healing

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Unlike that in adults, fetal skin tissue is widely recognized for its perfect execution of the epidermal regeneration. This observation leads to two seminal queries. First, do drivers of the fetal repair process exist in the adult tissue either in part or in its entirety? Second, if the critical elements are present in the adult tissue, can they be engaged to improve adult tissue repair outcomes? One key contrast between fetal and adult tissue that remain unaccounted for is the observation that post transcriptional gene silencing (PTGS) by microRNAs (miRNAs) is for the most part, muted in the fetal tissue to enable development. Following injury in adults, the skin miRNAs are transiently silenced showing similar expression pattern as evident during fetal development. In this work we demonstrate that this transient and selective muting of miRNA dependent PTGS in the fetal skin tissue contributes to regenerative epidermal healing. We report GPx7 co-operates with exoribonuclease XRN2 to silence mature miRNA and drive regenerative wound healing. Interestingly, GPx7 and Xrn2 are regulated by miR-29c and miR-203. We observe, Mcpip1, an exoribonuclease demonstrated as an adult factor that is rapidly turned on by blood clot derived lipids to sub-optimally induce NPGPx and XRN2 in the adult skin wound tissue. In adults, induced MCIPIP1 degraded pre-miRNAs suppressing mature miR-29c and miR-203 thereby de-silencing their targets NPGPx and XRN2. Overexpression of MCIPIP1, either experimentally or triggered by injury, induced the expression of skin development hallmark gene such as p63 and all the “Yamanaka factors” such as OCT-4, SOX-2, c-MYC and KLF-4 in vitro. MCIPIP1→NPGPx-XRN2 pathway induced regenerative healing by suppression of miRNA action thereby inducing the expression of numerous developmentally active coding genes and fetal proteins in adult wound tissue relevant to tissue growth. This favorable response was blunted in non-healing wounds such as in diabetic mice. Interestingly, gene delivery of Npgpx and Xrn2 markedly improved non-healing diabetic wound healing in adults.
Diabetes irreversibly depletes progenitor cell subpopulations critical for tissue repair

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Diabetes induces pathologic changes in a wide variety of tissues and organs, especially in response to injury. A number of alterations in stem/progenitor cell populations have been described in both experimental and clinical systems, raising the question of whether diabetes fundamentally alters the organismal response to injury, leading to protean downstream manifestations clinically observed as diabetic complications.

In order to better understand progenitor cell impairments, we have developed a single cell analytical approach to interrogate rare cell populations (such as stem cells) in complex in vivo systems. We have been able to demonstrate that in human and animal models, diabetes irreversibly depletes a subset of progenitor cells important for vascular and tissue regeneration in multiple contexts, including wound healing. Selectively replacing these cells appears to correct many of the downstream functional deficits associated with diabetic wound healing.

Perturbations in specific progenitor cell subpopulations, visible only on a single cell level, represent a previously unreported mechanism for the myriad dysfunctions observed in diabetes. These findings support efforts to develop cell-based therapies to reverse these defects and impact diabetic complications.

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miRNA in resolution of inflammation

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Inflammation is a protective response of the body to infection or injury. Timely resolution of inflammation is essential to restore tissue equilibrium and to circumvent chronic inflammation. Given that miRNAs are fundamental to the post-transcriptional control of gene expression, it is not surprising that role of specific miRNAs in the immune and inflammatory response has been discovered. The best-characterized miRNA for inflammation are miR-146, miR-21 and miR-155, all of these have been shown to be strongly induced in multiple cell types by proinflammatory stimuli following tissue injury. The transcription factor nuclear factor kappaB (NF-κB) and AP-1 are known to be redox-sensitive factors that play critical roles in immune responses and inflammation. We first reported that miRNA-21 negatively regulates LPS induced NFκB and AP-1 trans-activation in human macrophages. Such regulation of miRNA on redox sensitive transcription factors was associated with transitioning the macrophages to a pro-regenerative/resolving phenotype. The molecular pathway of such regulation by miRNA will be presented. Furthermore, a novel macrophage targeted miRNA-based therapeutic approach will be discussed.

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Redox regulation of airway epithelial stem cell maintenance and repair

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Airway epithelial stem cells play a vital role in repairing the epithelial barrier after injury through a tightly regulated process that prevents excessive proliferation. The airways are exposed to reactive oxygen species (ROS), which also have physiological roles as signaling molecules that regulate stem cell function. However, the functional significance of both steady and dynamically changing ROS levels in different stem cell populations, as well as downstream mechanisms that integrate ROS sensing into decisions regarding stem cell homeostasis, are unclear. Here, we show in mouse and human airway basal stem cells (ABSCs) that intracellular flux from low to moderate ROS levels is required for stem cell self-renewal and proliferation. We also found that quiescent stem cells are predominantly low in ROS and that ROS increases during the G1 to S transition and then decreases during mitosis to basal levels. Changing ROS levels activates Nrf2, which activates the Notch pathway to stimulate ABSC self-renewal as well an antioxidant program that scavenges intracellular ROS, returning overall ROS levels to a low state to maintain homeostatic balance. We used EMSAs to demonstrate that Nrf2 binds to an ARE binding site in the Notch promoter in ABSCs. Notch reporter studies demonstrated that flux of ROS levels from low to a moderate level in ABSCs increased Notch expression. We have identified a ROS-Nrf2-Notch-dependent mechanism that tightly controls ABSC proliferation in airway epithelial repair. This redox-mediated regulation of lung stem cell function has implications for stem cell biology, repair of lung injuries, and diseases like cancer.
RedoximiRs involved in organ fibrosis

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Recently miRNAs have emerged as powerful regulators of fibrotic processes and have been termed “fibromiRs”. MiRNAs also influence the expression of genes involved in ROS generation and antioxidant defense and are termed “redoximiRs”. We investigated the regulation of γ-glutamate-cysteine-ligase (GCL) by miRNAs. MiR-433 was identified as a strong candidate for targeting GCL. Overexpression of miR-433 in HUVEC showed downregulation of both GCLc and GCLm subunits in an Nrf2-independent manner. Treatment of HUVEC with miR-433 resulted in reduced antioxidant state, increased S-glutathionylation and reduced eNOS activation. Mice models of renal and hepatic fibrosis were associated with TGF-β1-related reduction of GCLc and GCLm levels that were miR-433-dependent. In miRNA arrays performed in human lung fibroblasts incubated or not with 100µM hydrogen peroxide for 8 h, we spotted miR-9-5p, whose targets represent genes related to the TGF-β-signaling pathway. MiR-9-5p overexpression significantly reduced TGFBR2 and TGF-β1-NOX4 expression and abrogated the TGF-β-dependent transformation of lung fibroblasts into myofibroblasts as reflected in the changes of α-SMA and extracellular matrix (ECM) proteins. To study the role of miR-9 in vivo, we employed a mouse model of bleomycin-induced pulmonary fibrosis. Pre-treatment with lenti-miR-9 decreased the expression of ECM and α-SMA protein levels. In keeping, lungs with up-regulated miR-9-5p showed decreased TGFBR2 and TGF-β1-NOX4 induced mRNA levels. Histological analysis demonstrated attenuation of fibrosis in mice pre-treated with lenti-miR-9-5p. We propose that miR-9-5p plays a protective role in lung fibrosis and that both miR-433 and miR-9-5p belong to the fibromiRs and redoximiRs categories.
Scavenger Receptor B1 as a possible player in cutaneous tissue proliferation and differentiation

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Scavenger receptor B1 (SR-B1) is a transmembrane protein, involved in tissue reverse cholesterol transport and known as HDLs main receptor. Several studies have demonstrated that SR-B1 is also implicated in several other processes, such as bacteria and apoptotic cells recognition and regulation of intracellular antioxidant levels. Although this receptor is mainly localized in the liver and steroidogenic tissues, it has been shown that it is significantly expressed also in human skin, especially in the epidermis. Our purpose, by using 2D and 3D human keratinocytes cultures, was to investigate the role of SR-B1 in cutaneous tissue proliferation and differentiation under exogenous oxidative stress conditions. We have demonstrated that environmental stressors, such as ozone, air particles and cigarette smoke, down-regulate SR-B1 expression affecting also keratinocytes ability to recover from wound scratch. In fact, SR-B1 knockdown reduced cell proliferation capacity and wound scratched knockdown keratinocytes presented lower nuclear Cyclin D1 protein levels. SR-B1 knockdown in tridimensional skin equivalents showed changes in stratum corneum thickness and this correlated with changes in its lipid distribution. Our findings suggest that SR-B1 might play an important role in keratinocytes proliferation and recovery from external insults, as well as in epidermal differentiation and lipid composition.
Nanotechnology-enabled non-viral tissue reprogramming

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In vivo cell reprogramming has the potential to enable more effective autologous cell therapies. We developed a powerful yet simple to implement non-viral approach to controllably transfect and reprogram tissues in vivo. Cleanroom- and non-cleanroom-based methods were used to nanofabricate chips to deliver reprogramming genes into exposed tissue surfaces via nanochannel-based electroporation. Transfection experiments were carried out using a murine model. Labeled DNA and reprogramming factors were used as model cargo. Transfection and reprogramming were evaluated by immunohistochemistry and qRT-PCR. Our results confirmed successful and effective delivery of labeled DNA and reprogramming factors into mouse skin in a rapid (~milliseconds) and non-invasive manner. Reprogramming factor activity post-delivery was confirmed by positive reporter fluorescence protein expression (e.g., GFP and RFP) and qRT-PCR (at the mRNA level). Long-term (> 10 weeks) studies indicated that terminally differentiated skin tissue successfully reprogrammed into other types of tissue (e.g., neuronal, endothelial) depending on the specific combination of reprogramming factors used during transfection. We then demonstrated that skin tissue reprogramming towards endothelium can be a viable approach for rescuing traumatized ischemic tissues and limbs from necrosis. Our results indicate that terminally differentiated tissues can be successfully reprogrammed in vivo into other types of tissue via nonviral nanotechnology-based methods. This technological breakthrough has an exponential number of potential biomedical applications.
Oral supplementation of fermented papaya preparation to type 2 diabetes mellitus patients improves the respiratory burst function in chronic wound-site macrophages

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Respiratory burst (RB) at the wound site helps fight infection and supports the healing process. Impaired healing is a common diabetic complication often leading to amputation. Type 2 diabetes mellitus (T2D) patients exhibit compromised RB compared to non-T2D. The objective of this work was to test whether a natural dietary supplement may correct wound-site macrophage RB in T2D patients. Fermented Papaya Preparation (FPP) is a carbohydrate-rich antioxidant nutritional supplement. We have reported that wound macrophages from diabetic (db/db) mice orally supplemented with FPP (0.2g/kg body wt) exhibited an increased RB when stimulated by PMA. Peripheral blood monocytes (PBM), the circulating precursor of wound macrophages, isolated from T2D patients orally supplemented with FPP (3g, 3 times/day) also demonstrated enhanced ROS production when stimulated with PMA (40nM, 30min). The NADPH oxidase enzyme complex is the primary generator of $\text{O}_2^-$, and derivative ROS in leukocytes, requiring both activation and co-localization on the cell membrane to facilitate a proper RB. We observed that ex vivo FPP supplemented (3mg/mL culture media) PBM increased expression of Rac2 and phosphorylation of p47phox. Thus, FPP supports PKC phosphorylation of p47phox and increases Rac2 expression via a phosphatidylinositol signaling pathway dependent mechanism, leading to improved NADPH oxidase function during RB. We hypothesize that oral supplementation of FPP to T2D patients with chronic wounds will improve wound macrophage RB function. Chronic wound T2D patients currently undergoing negative-pressure wound therapy (NPWT) are being recruited. Thirty patients have been randomized into 2 groups; standard of care (SoC) and SoC with FPP oral supplementation (3g, 3 times/day). Fresh NPWT dressings are being collected and lavaged to yield live wound macrophages prior to supplementation, and again 2 and 3 weeks post start of supplementation. Inducible ROS production in isolated wound macrophages was measured using 2',7'-dichlorofluorescin diacetate (5µM), using flow cytometry, before and after stimulation with PMA. Preliminary data from study in progress: wound macrophages isolated from T2D patients who received FPP supplementation exhibited increased RB compared to SOC counterparts (n=3, p=0.005). This study is supported by NIDDK R01 DK076566 (SR) and in part by Osato Research Institute (Gifu, Japan).
SESSION IV
BIOACTIVES: COGNITION AND AGING
Enhancing dentate gyrus function with dietary flavanols improves cognition in older adults

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The dentate gyrus (DG) is a region in the hippocampal formation whose function declines in association with human aging and is therefore considered to be a possible source of age-related memory decline. Causal evidence is needed, however, to show that DG-associated memory decline in otherwise healthy elders can be improved by interventions that enhance DG function. We addressed this issue by first using a high-resolution variant of functional magnetic resonance imaging (fMRI) to map the precise site of age-related DG dysfunction and to develop a cognitive task whose function localized to this anatomical site. Then, in a controlled randomized trial, we applied these tools to study healthy 50–69-year-old subjects who consumed either a high or low cocoa flavanol–containing diet for 3 months. A high-flavanol intervention was found to enhance DG function, as measured by fMRI and by cognitive testing. Our findings establish that DG dysfunction is a driver of age-related cognitive decline and suggest non-pharmacological means for its amelioration.
Potential mechanisms by which polyphenols influence memory and cognition

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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 neuro-inflammation 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 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.
Neurovascular coupling mediated by neuronal nitric oxide in hippocampus: Functional communication with ascorbate and nitrite

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The functional connection of glutamate receptors with neuronal nitric oxide synthase in neurons accounts for the coupling between neuronal activation and changes in local cerebral blood flow, i.e., the neurovascular coupling. Upon glutamatergic stimulation, nitric oxide (NO) is synthesized and, by diffusing a few hundreds of microns towards neighboring vessels, induces vasodilation via the canonic pathway involving soluble guanylate cyclase. Failure in neurovascular coupling, either during aging and disease (Alzheimer’s disease, AD) or following acute hypoxic conditions, compromises brain integrity and functionality. The regulation of neuro-vascular coupling is under the concerted cooperation of the cells comprising the neurovascular unit. However, the complementary task of identifying modulators of NO activity on neurovascular coupling has remained largely under-appreciated. We have come to conjecture that the redox and functional interplay of nitric oxide with ascorbate and nitrite would modulate the functionality of glutamatergic synapses in terms of neurovascular coupling. By using a multimodal approach to probe the dynamics of NO, ascorbate and cerebral blood flow in vivo in hippocampus of Wistar and Fisher 344 rats and of a triple transgenic mice model of AD we support that (1) neuronal-derived NO acts as a direct mediator of neurovascular coupling, (2) upon glutamatergic stimulation, volume signaling by NO is an intrinsically controlled mechanism due to increased blood flow, (3) neurovascular coupling is impaired in AD and aging due to vascular dysfunction, (4) under acidic/hypoxic conditions, nitrite is reduced by ascorbate to NO and (5) the redox interaction of nitrite/ascorbate/NO contributes to neurovascular coupling. Given that nitrite increases NO bioavailability and augments cerebral blood flow in hippocampus one may envisage that dietary nitrate via the nitrate:nitrite:NO pathway may help sustaining neurovascular coupling in aging and disease.
Nutrient biomarker patterns, cognition, and Alzheimer’s pathology in older adults

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The role of lutein in cognitive function

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Epidemiological studies suggest that dietary lutein may be of benefit in maintaining cognitive health. Among the carotenoids, lutein and its isomer zeaxanthin, are the only two that cross the blood-retina barrier to form macular pigment (MP) in the eye. Lutein also preferentially accumulates in human brain. Lutein and zeaxanthin in macula were found to be significantly correlated with their levels in matched brain tissue. Therefore, MP can be used as a biomarker of lutein and zeaxanthin in human brain tissue. This is of interest given that a significant correlation was found between MP density and global cognitive function in healthy adults. Examination of a relationship between cognition and lutein levels in brain tissue of decedents from a population-based study of adults found that among the carotenoids, only lutein was consistently associated with a wide range of cognitive measures to include executive function, language, learning and memory. Furthermore, lutein concentrations in the brain were significantly lower in individuals with mild cognitive impairment compared to those with normal cognitive function. Lastly, in a controlled trials increased lutein intakes through supplements or food sources resulted in increased MP density that was associated with an improved cognitive function. Taking all of these observations into consideration, the idea that lutein can influence cognitive function warrants further study.
Zinc and lipoic acid in the modulation of the STAT3 pathway in the developing brain

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Previous evidence showed that a deficit of Zn causes NADPH oxidase and NO synthase activation via the NMDA receptor in the developing brain. The Zn deficiency-associated oxidative stress impaired microtubule (MT) dynamics with evidence of altered MT-dependent cellular transport. This study investigated the impact of Zn deficiency on the activation and potential MT-mediated nuclear shuttling of transcription factor STAT3, and the protective actions of α-lipoic acid, both in IMR-32 human neuroblastoma cells and in fetal brain from dams fed marginal Zn deficient (MZD) diets throughout pregnancy. Gestational MZD decreased STAT3 Tyr705 phosphorylation, impaired binding of STAT3 to MTs, and affected cytosol-to-nucleus transport of STAT3 in fetal brain. Similarly, Zn deficient IMR-32 cells showed low levels of STAT3 Tyr705 phosphorylation, decreased cytosolic to nuclear STAT3 transport, and decreased STAT3-dependent transcriptional activity. Chemical cytoskeletal disruption in IMR-32 cells impaired STAT3 nuclear import, indicating the requirement of a functional cytoskeleton in this process. Lipoic acid prevents MT oxidation and restores MT polymerization in Zn deficient IMR-32 cells. Accordingly, although low phosphotyrosine705 STAT3 levels were not recovered by lipoic acid, it did restored STAT3 nuclear import. The association of altered STAT3 signaling and astrogliogenesis will be discussed. In summary, this work presents in vivo and in vitro evidence that Zn deficiency affects STAT3, at least in part due to oxidative stress and MT oxidation. It also shows that MTs are required for the nuclear shuttling of STAT3. These findings may in part explain the deleterious effects of maternal MZD on fetal brain development.

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Nrf2-mediated antioxidant protection in the retinal pigment epithelium

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The retinal pigment epithelium (RPE) provides essential support to the outer retina and choroidal vasculature. RPE degeneration is an early initiating event in age-related macular degeneration (AMD), a leading cause of blindness in elderly people. Lifelong oxidative stress and cumulative oxidative damage to the RPE likely play causative roles in the development and progression of AMD. To prove the concept, we developed an animal based on knockout mice that are deficient of nuclear factor erythroid 2-related factor 2 (Nrf2). With histopathological examination and in vivo imaging, we found that eyes of Nrf2-deficient mice developed moderate RPE degeneration after 10 months. When fed with high fat, cholesterol-rich diet, the time course of progressive retinal and RPE degeneration was markedly accelerated. Inflammation and gamma delta T cell-mediated immune responses were observed in the outer retina and choroid. The ocular phenotype of Nrf2 knockout mice had high similarities to pathological features of human AMD eyes. We further examined a number of naturally occurring nutritional supplements on the induction of Nrf2 activity in cultured RPE cells. Results showed that quercetin was a potent Nrf2 activator in the RPE and its effects were further potentiated by resveratrol. The latter compound has been reported as protective in various animal models and clinical studies related to AMD. Taken together, our data suggest that Nrf2 is important in maintaining the functions of the RPE, particularly at advanced age. Activating Nrf2-mediated antioxidant protection by nutritional supplements can be an effective preventive strategy for AMD.
Fast food decreases the anti-inflammatory capacity of HDL particles in stimulated peripheral blood mononuclear cells

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High-density lipoproteins (HDL) are well known for their role in reverse cholesterol transport. However, HDL also performs an array of other functions associated with protection from heart disease including antioxidant function and anti-inflammatory function. Despite the fact that fast food represents as much as 20% of the American diet, very few studies have examined the effect of fast food on cardiometabolic health. In this pilot study we examined the effect of short-term fast food consumption on the antiinflammatory capacity of HDL. Five healthy individuals were recruited and enrolled in the study after written consent was obtained. Participants consumed no fast food (healthy diet phase) for one week, followed by fast food for five days. Blood was drawn at baseline, following the healthy diet phase, and at the end of the fast food phase. HDL particles were separated by sequential density ultracentrifugation and incubated with peripheral blood mononuclear cells (PBMCs). Subsequently, the PBMCs were stimulated with citrullinated fibrinogen to simulate an auto-immune stimulus, and incubated overnight at 37°C. Tumor necrosis factor (TNF)-α was measured in the supernatant using an ELISA kit. The addition of HDL decreased the production of TNF-α at all three time points compared to positive control. However, TNF-α levels were higher following the fast food diet phase compared to the healthy diet phase. These results suggest that short-term fast food consumption decreases the anti-inflammatory capacity of HDL particles in the context of an auto-immune stimulus.
SESSION V
MICROBIOME AND GUT INFLAMMATION
Redox-dependent signaling in the gut: A mechanism of host-microbiota crosstalk

Andrew Neish

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The prokaryotic communities –the microbiota- resident in the metazoan gut can influence a range of physiological functions, including induction of epithelial growth and differentiation, regulation of immune responses, and augmenting restitution from injury. While prokaryotic organisms are well known to stimulate inflammatory signaling networks, less is known how commensals can influence physiological and homeostatic pathways. Recent findings have shown that enteric commensal bacteria can stimulate gut epithelia to rapidly generate reactive oxygen species (ROS). While the induced production of ROS in professional phagocytes via activation of NADPH oxidase 2 (Nox2) is a well-studied process, ROS are also similarly elicited in other cell types, including intestinal epithelia, in response to microbial signals and paralogous epithelial NADPH oxidases (Nox’es). ROS generated by Nox enzymes function as critical second messengers in multiple signal transduction pathways via the rapid and transient oxidative modification of a redox sensor proteins bearing oxidant-sensitive thiol group, as well as direct redox dependent signaling pathways such as the Nrf2 system. These events control a network of redox dependent effector molecules that include regulators of innate immunity, cellular motility and proliferation/differentiation. ROS-dependent mechanisms may mediate some of the established effects of the normal microbiota on intestinal physiology.
Asthma and atopy, classically associated with hyper-activation of the T helper 2 (Th2) arm of adaptive immunity, are among the most common chronic illnesses worldwide. Emerging evidence relates atopy and asthma to the composition and function of the human microbiome, the collection of microbes that reside in and on and interact with the human body. The ability to interrogate microbial ecology of the human host is due in large part to recent technological developments that permit identification of microbes and their products using culture-independent molecular detection techniques. Here we explore the role of the gut, and environmental microbiomes in asthma and allergic disease development, manifestation, and attenuation. Though still a relatively nascent field of research, evidence to date suggests that the gut microbiome may represent a fertile target for prevention or management of allergic asthma and other chronic inflammatory diseases in which adaptive immune dysfunction is a prominent feature.
Plant bioactives for alleviating metabolic diseases: 
Effects on the gut microbiota, and beyond

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The worldwide epidemic rise in obesity and type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD) calls for novel therapeutic strategies for these inflammatory conditions. Current pharmacological treatments for T2D and NAFLD have limited efficacy or unwanted side effects. I will present growing evidence that the gut microbiota is a key determinant of diet-induced obesity and T2D and NAFLD. I will show that new polyphenol-rich extracts from various plant sources can protect against obesity-linked inflammation and alleviate T2D and NAFLD in high fat-fed mice, at least in part by reshaping the gut microbiota and favoring the abundance of specific bacterial populations such as the mucin-degrading bacterium Akkermansia muciniphila. I will also present recent data showing that other plant bioactives may also help mitigate T2D through actions on key metabolic and inflammatory pathways in relevant cell types.
Nutritional impact of pomegranate polyphenol antioxidants on microbiome metabolism and proliferation in vitro and in vivo

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The health benefits of pomegranate (POM) consumption are attributed to ellagitannins and their metabolites, formed and absorbed in the intestine by the microbiota. In this study twenty healthy participants consumed 1000 mg of POM extract daily for four weeks. Based on urinary and fecal content of the POM metabolite urolithin A (UA), we observed three distinct groups: (1) individuals with no baseline UA presence but induction of UA formation by POM extract consumption (n = 9); (2) baseline UA formation which was enhanced by POM extract consumption (N = 5) and (3) no baseline UA production, which was not inducible (N = 6). Compared to baseline the phylum Actinobacteria was increased and Firmicutes decreased significantly in individuals forming UA (producers). Verrucomicrobia (Akkermansia muciniphila) was 33 and 47-fold higher in stool samples of UA producers compared to non-producers at baseline and after 4 weeks, respectively. In UA producers, the genera Butyrivibrio, Enterobacter, Escherichia, Lactobacillus, Prevotella, Serratia and Veillonella were increased and Collinsella decreased significantly at week 4 compared to baseline. The consumption of pomegranate resulted in the formation of its metabolites in some but not all participants. POM extract consumption may induce health benefits secondary to changes in the microbiota.
The role of the milk glycome in the development of the infant gut microbiota: Why babies cry over spilled milk

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Human milk contains numerous components that shape the microbial content of the developing infant gastrointestinal tract. A prominent feature of milk is an array of complex glycans and glycoconjugates that serve a passive immune function by sequestering and deflecting pathogens while simultaneously enriching a protective, milk-oriented microbiota (MOM) often dominated by bifidobacteria. Recent research suggests the timing of establishment, and proper function of a MOM is critical for infant development. An infant’s MOM is initially established through environmental transfer to the gut and subsequently shaped by diet (milk) and host genetics. Once established, MOMs dominated by bifidobacteria exhibit low residual milk glycans and higher levels of short chain fatty acids in the feces, suggesting a strongly saccharolytic colonic microbiota. The mechanistic basis for milk glycan consumption by bifidobacteria has been the subject of active research. Different infant-borne bifidobacteria contain specific glycosidases and transport systems required to utilize free glycans or glycoconjugates. Consumption of milk glycans enhances specific bifidobacterial interaction with the infant host through both direct and indirect routes. Growth on free milk glycans results in increased bifidobacterial binding to epithelial cells and beneficially modulates intestinal function. In addition, metabolites generated during growth on milk glycans dampen inflammation and strengthen gut barrier function. In aggregate, these studies suggest a co-evolutionary relationship between mammalian milk glycans, infant-borne bifidobacteria and the infant host resulting in a programmed enrichment of a protective bifidobacterial-dominant MOM during a critical stage of infant development. Importantly, disruption of this programmed enrichment, by poor environmental transfer, antibiotic use, or infection, can lead to a “poorly functioning” MOM that may pose a risk for negative health outcomes. Further analysis of this naturally evolved system will shed light on effective pre- and probiotic tools that support and ensure a protective MOM for all at risk infants.
The transcription factor Nrf2 mediates endogenous antioxidant upregulation in response to hypericin-photodynamic therapy in melanoma cells

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Metastatic melanoma is a highly aggressive, treatment refractory form of skin cancer which although represents less than 5% of all type of skin cancers, accounts for 80% of skin cancer-related deaths. Photodynamic therapy (PDT) is a novel therapeutic modality for treatment of cancers, which requires a photosensitizer drug and molecular oxygen in concert with a harmless visible light source to facilitate selective destruction of cancer cells. Our group had previously shown that hypericin-based PDT (HYP-PDT) is effective in killing melanoma cells in vitro, with cells able to induce an autophagic response after an initial necrotic response. In this study we investigated the molecular mechanisms responsible for such autophagic response. In particular we evaluated the endogenous antioxidant response mechanism of A375 (unpigmented) and UCT-MEL 1 (pigmented) melanoma cells to HYP-PDT in an in vitro model and the possible involvement of the transcription factor Nrf2. Our results showed that basally, both melanoma cells exist at a higher oxidative stress state compared to normal human melanocytes (NHM) as indicated by a significantly higher intracellular ROS and MDA levels. HYP-PDT treatment elicited a time-dependent increase in intracellular ROS generation in A375 cells up to 4 hour post-irradiation. In the UCT-MEL 1 cells, ROS generation was also increased up to 1 hour after irradiation. Immuno-fluorescence analysis showed that HYP-PDT stimulate the translocation of Nrf2 into the nucleus in the UCT-MEL 1 cells at all time points. Photoactivation of hypericin induced the mRNA expression of Nrf2 at 30 min, 1 hr and 4 hr in UCT-MEL 1 and at 1 hr, 2 hr and 4 hr in A375 cells. This Nrf2 mRNA induction correlate with increased transcription of GCLc, SOD 1, SOD 2, GPx, CAT and thioredoxin reductase which are all downstream target of Nrf2. These data support the relevant role of Nrf2 in modulating the upregulation of endogenous antioxidants in response to HYP-PDT in melanoma cells.
Soluble epoxide hydrolase pharmacological inhibition ameliorates experimental acute pancreatitis in mice

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Acute pancreatitis (AP) is an inflammatory disease, and is one of the most common gastrointestinal disorders worldwide. Soluble epoxide hydrolase (sEH; encoded by Ephx2) deficiency and pharmacological inhibition have beneficial effects in inflammatory diseases. Ephx2 whole-body deficiency mitigates experimental AP in mice but the suitability of sEH pharmacological inhibition for treating AP remains to be determined. We investigated the effects of sEH pharmacological inhibition on cerulein- and arginine-induced AP using the selective sEH inhibitor 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU) which was administered before and after induction of pancreatitis. Serum amylase and lipase levels were lower in TPPU-treated mice compared with controls. In addition, circulating levels and pancreatic mRNA of the inflammatory cytokines TNFα, IL-1β and IL-6 was reduced in TPPU-treated mice. Moreover, sEH pharmacological inhibition before and after induction of pancreatitis was associated with decreased cerulein- and arginine-induced NF-κB inflammatory response, endoplasmic reticulum stress and cell death. sEH pharmacological inhibition before and after induction of pancreatitis mitigated cerulein- and arginine-induced AP. This work suggests that sEH pharmacological inhibition may be of therapeutic value in acute pancreatitis.
Global trends in micronutrient deficiencies

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Assuring everybody has access to sufficient and healthy food remains one of the world’s most pressing challenges. Undernourishment has been declining but remains very high at about 11% of the global population. Moreover, poor quality diets can lead to micronutrient deficiencies, particularly in populations with low total food intake. Such ‘hidden hunger’ can cause pregnancy complications and child growth failure, increase susceptibility to disease, and reduce cognitive development. An increasing part of the world population consumes excessive amounts of food, increasing the incidence of obesity and risk of noncommunicable diseases such as diabetes and heart disease. The ideal diet is adequately dense in micronutrients to meet requirements without facilitating overconsumption. We provide an overview of global trends in micronutrient deficiencies and dietary nutrient density. We combine national level food consumption data, population data, and food composition tables to estimate per capita intake of nutrients by sex and age group. Nutrient intake distributions are estimated from published studies and estimated average requirement thresholds are used to estimate the deficiency prevalence for 14 micronutrients. National dietary nutrient densities are calculated to demonstrate the spatial and temporal patterns of diet quality.
Variation of fasting plasma inflammatory markers in overweight and obese individuals

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\textsuperscript{1}This project was made possible by support from the National Dairy Council, Rosemont, IL and the USDA, Agricultural Research Service, Western Human Nutrition Research Center

\textsuperscript{2}This study is registered on ClinicalTrials.gov Identifier: NCT01811329

Metabolic dysregulation and chronic inflammation are associated with the presence of obesity as defined by BMI. Responsiveness to a high-fat meal was expected to elicit an inflammatory response in a group of overweight and obese individuals, but instead yielded unexpected variability in baseline and postprandial markers of inflammation. The objective of the current analyses was to identify baseline markers that are associated with inflammatory response, and to define responsiveness using baseline variation in inflammatory markers. The present study measured baseline and 1, 3, and 6-hour post-prandial inflammatory markers, including serum amyloid A (SAA), C-reactive protein (CRP), various cytokines, and adhesion markers. Postprandial responsiveness was determined using each participant’s variability for each marker across four baseline timepoints. Data was analyzed in Microsoft Excel and JMP Pro 12. The coefficient of variation for SAA at baseline was 304.8% across all participants. Participants were either classified as a responder (1) or a nonresponder (0) at each timepoint for each marker. Baseline SAA concentration was significantly different between responders and nonresponders for sVCAM-1 at one hour, MCP-1 at three hours, and IL-10 at six hours postprandially. These results suggest that variation in inflammatory phenotypes exist, even between individuals of similar BMI classification. The amount of fluctuation within each participant offers insight to better understand which biomarkers may be useful clinically. Future work includes better characterization of different inflammatory phenotypes using metabolomics and gene expression data. Overall, this work contributes to the development of personalized nutrition, medicine, and disease prevention.
Soluble epoxide hydrolase deficiency in the glomerular podocytes attenuates lipopolysaccharide-induced proteinuria

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Chronic kidney disease is a serious health problem worldwide and its two main causes are diabetes and high blood pressure. Proteinuria is an early marker of renal injury and may accelerate disease progression to renal failure. Podocytes are specialized epithelial cells that play a significant role in preventing urinary protein leakage and in maintaining glomerular capillary loop integrity. Soluble epoxide hydrolase (sEH) is a cytosolic enzyme whose inhibition has beneficial effects in inflammatory diseases and in renal function, but its role in podocytes remains unexplored. We report that sEH expression is increased in murine podocytes upon lipopolysaccharide (LPS) treatment. To investigate the in vivo role of sEH in podocytes we generated mice with podocyte sEH deficiency (pod-sEHKO) then examined the effects of sEH deficiency on LPS-induced proteinuria. Pod-sEHKO mice exhibited significant improvement in LPS-induced proteinuria compared with controls. In addition, renal mRNA and serum concentrations of the inflammatory cytokines IL-1β, IL-6 and TNFα were significantly lower in LPS-treated pod-sEHKO mice compared with controls. Moreover, pod-sEHKO mice exhibited decreased renal LPS-induced NF-κB inflammatory response, MAPKs activation and decreased endoplasmic reticulum stress. Importantly, these effects were recapitulated in differentiated E11 podocytes treated with sEH pharmacological inhibitor consistent with being cell autonomous. Together, these findings establish a novel role for sEH in LPS-induced proteinuria and identify sEH in podocytes as a contributor to glomerular function.
Antioxidant activity, inhibitory effect on nitric oxide production and total phenolic content of *Avicennia marina* wood extracts

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Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are normally produced by phagocytic cell in order to help maintain homeostasis at the cellular level in the normal healthy issues. Nevertheless, the imbalance between cellular production of ROS/ RNS and the counteracting antioxidant mechanisms leads to various diseases. In the present study, the *Avicennia marina* wood was extracted using maceration and decoction methods. In chemical assays, antioxidant activity was assessed by DPPH and FRAP assays. In cell-based assay, nitric oxide scavenging activity was assessed using macrophage cell line RAW 264.7 cells. The content of total phenolic was determined by Folin-Ciocalteu assay. The results demonstrated that the ethanolic extract showed strong antioxidant activities using DPPH and FRAP assays (EC$_{50}$ value of 7.92 $\mu$g/mL and FRAP value of 332.92 mg Fe$^{2+}$/g, respectively). The extract exhibited stronger antioxidant activity than standardize butylhydroxytoluene (BHT). Similar to chemical assays, this extract exhibited the highest nitric oxide inhibitory activity with an IC$_{50}$ value of 6.74 $\mu$g/mL. In contrast, the water extract showed the lowest activity with an IC$_{50}$ value of 21.03 $\mu$g/mL. The total contents of phenolics in water and ethanolic extracts were 95.85 and 67.16 mg GAE/g, respectively. Consequently, the wood extracts may lead to implement-tation of natural products for health.
The bergamot essential oil exerts anti-inflammatory and anti-nociceptive effects in *in vivo* models

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Renewed interest in natural products as potential source of drugs led us to investigate on the anti-inflammatory and analgesic activities of bergamot essential oil (BEO). *Citrus bergamia* Risso et Poiteau (bergamot), is a small tree belonging to the Rutaceae family growing in the southern of Calabria Region (Italy). Bergamot fruit is used mostly for the extraction of its essential oil from the peel, widely used in perfume industries. BEO chemical composition are known\(^1\,^2\). Since the bergapten toxicity, our study has been performed using the BEO fraction deprived of bergapten (BEO-BF).

Treatment with BEO-BF (i.p.) before the subplantar injection of carrageenan led to a significant inhibition of paw oedema associated with reduction of IL-6, IL-1β and TNF-α production as well as PGE2 and nitrite/nitrate levels. The latter could be related to the antioxidant properties of BEO-BF. Moreover, histological examination of paw biopsies showed a reduction of pathological changes typically of oedema in BEO-BF treated rats.

The analgesic activity of BEO-BF showed in the writhing test strengthen its anti-inflammatory capability, while the results of hot-plate test suggest that the supra-spinal analgesia participates to the antinociceptive effect of BEO-BF.

In summary, our study indicate that BEO-BF exerts protective effects in carrageenan-induced paw oedema accompanied by a pronounced analgesic response, suggesting its potential role as anti-inflammatory and analgesic drug.

(2) Navarra et al., J Pharm Pharmacol. 2015, 67(8): 1042-53
A flavonoid-rich extract of orange juice reduces obesity in an overfed zebrafish

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Obesity is a pathological condition due to an imbalance between energy intake and consumption that now reached epidemic proportions. Therefore, worldwide scientists are making considerable effort to find both novel strategies to prevent and new effective treatments to reduce obesity. In recent years there has been increasing interest in plant derivatives because of their potential health-promoting properties, including the anti-obesity activity. It is known that Citrus sinensis (orange) juice (OJ) can exert many beneficial effects on human as well as has been reported that OJ may play a role in weight management and obesity. The present study was designed to evaluate the effect of a flavonoid-rich extract of OJ (OJe) on a diet-induced obese zebrafish. Adult zebrafish were allocated to four diet groups and over 4 weeks were fed a diet containing 20 mg (control group) or 60 mg (overfeeding) Artemia plus or not OJe (5ml/l in fish water). The zebrafish overfed with Artemia exhibited weekly increased body mass index (BMI) accompanied to a modulation of gene expression (leptin, ghrelin, orexin, POMC, NPY) in both gut and brain. Notably, the daily addition of OJe to the feeding of overfed zebrafish significantly reduced BMI compared to fish fed with the same diet without the extract, and restore the expression of genes regulating appetite and food intake. This study adds new insights into the anti-obesity property of the pool of flavonoids present in OJ and confirm zebrafish as a useful model to study obesity.
Anthocyanins inhibit tumor necrosis alpha-induced loss of Caco-2 cell barrier integrity

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An increased permeability of the intestinal barrier is a major event in the pathophysiology of inflammatory bowel diseases, and obesity-associated pathologies. Chronic inflammation is involved in conditions with increased intestinal permeability. In this scenario, tumor necrosis factor alpha (TNFα) plays a central role promoting tight junction barrier dysfunction. This study investigated the capacity of anthocyanins (AC) and AC-rich extracts to inhibit TNFα-induced permeabilization of Caco-2 cell monolayers, characterizing the relationship between AC chemical structure/conformation to their protective capacity. AC composition of the extracts was measured by reversed phase liquid chromatography coupled with triple quadropole mass spectrometry. Cell monolayers were incubated in the absence/presence of TNFα, with or without the addition of extracts or pure ACs. Extracts inhibited TNFα-induced loss of monolayer permeability, assessed as changes in transepithelial electrical resistance (TEER) and paracellular transport of FITC-dextran. Within the range of concentrations studied, the protective actions of the extracts on TNFα-induced TEER decrease was not correlated to the extract’s total AC, malvidin, petunidin, or peonidin content. However, a positive and significant correlation was found for TEER and cyanidin (r²=0.73) and delphinidin (r²=0.73) extract content. Similarly, when the actions of pure compounds (0.1-1 µM) were assessed, the 3-O-glucosides of cyanidin and delphinidin, but not malvidin protected the monolayer from TNFα-induced decrease of TEER and increased FITC-dextran permeability. The AC protective capacity was related to the substitutions in the B ring. These results suggest that consumption of cyanidins and delphinidins could mitigate the adverse effects of inflammatory conditions affecting the gastrointestinal tract.
(-)-Epicatechin inhibits ER Stress in adipocytes and in adipose tissue from high fat-fed obese mice

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Obesity and overweight are a major health concern worldwide. Obesity is associated with the development of insulin resistance and endoplasmic reticulum (ER) stress. Our previous studies showed that the flavan-3-ol (-)-epicatechin (EC) mitigates obesity-induced insulin resistance in mice. Given the proposed role of ER stress in the development of insulin resistance as a consequence of overnutrition, this study investigated the hypothesis that EC and its metabolites (ECM) can improve insulin sensitivity by preventing high fat-induced ER stress both \textit{in vivo} and \textit{in vitro}. \textit{In vivo}: mice were fed for 15w: a control diet (C), high fat diets without (HF) or with supplementation of EC (20mgEC/kg BW). \textit{In vitro}: 3T3-L1 adipocytes were treated with 0.25mM Pal, and with or without EC (0.1-1\,\mu M) or ECM (1\,\mu M). The activation of the three ER stress branch components (PERK, IRE1\textalpha, ATF6, XBP-1, JNK, eIF2\alpha) was assessed by Western blot. In adipose tissue and in 3T3 cells EC mitigated/decreased high fat- and palmitate-induced activation of IRE\textalpha, XBP-1 and JNK. While EC did not affect PERK activation both in adipocytes and HF adipose tissue, it inhibited eIF2\alpha activation in adipocytes. Cleaved ATF-6 was increased by Pal and HF diet but was not affected by EC. In 3T3-L1 cells ECM had similar effects to those of EC. Overall results suggest that the inhibition of select ER stress branches (particularly IRE\textalpha), can in part explain the capacity of dietary EC to mitigate insulin resistance occurring as a consequence of overnutrition.

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Effects of NADPH oxidase inhibitors on palmitate-induced insulin resistance in HepG2 cells

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Obesity is associated to insulin resistance (IR) and type 2 diabetes in humans, and consumption of high fat diets leads to obesity and insulin resistance in rodents. Previous evidence suggests that excess liver oxidant production may lead to IR, being chronic NAPDH oxidase activation a major contributor. We have recently shown that the flavanol-3ol (-)-epicatechin (EC) improves insulin sensitivity in high fat-fed mice. The aim of this study was to evaluate if the inhibition of NADPH oxidase could improve insulin sensitivity in hepatocytes (HepG2 cells) treated with palmitate (Pal). Cells were incubated for 24 h with 0.25mM Pal and with or without the following NADPH oxidase inhibitors: EC (0.25-1 µM), EC metabolites (ECM) (1µM), apocyanin (Apo) (1 µM) and VAS2790 (1 µM). NADPH oxidase activity, cell oxidants production (evaluated with DCF), mRNA and protein levels of the NADPH oxidase subunits, protein carbonylation, and the insulin-triggered signaling cascade were assessed. Results showed that Pal caused an impaired response to insulin, in association with increased NADPH oxidase activity and subunit (NOX3, p22phox, p47phox) expression, an increased production of oxidants, and high protein carbonylation. EC and ECM attenuated Pal-induced expression of NOX3, but not that of p47phox and p22phox, NADPH oxidase activity, and protein carbonylation. Accordingly, EC and ECM improved Pal-mediated impairment of the insulin pathway in the presence of Pal. Apo and VAS inhibited NADPH oxidase and showed a trend of improvement of the insulin response. Our data suggest that NADPH oxidase inhibition, particularly by EC, could be an important strategy to mitigate lipotoxicity-induced insulin resistance. Supported by NIFA-USDA (CA-D*-xxx-7244-H)
MAPK and AP-1 pathways are involved in protective effect of a
Citrus bergamia juice extract against β-amyloid-induced
pro-inflammatory activation of THP-1 cells

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Flavonoids have been shown to be effective in protecting against age-
related cognitive and motor decline in both in vitro and in vivo models. Recently, a flavonoid-rich extract of Citrus bergamia juice (BJe) has been shown to display anti-oxidant and anti-inflammatory properties against LPS-
induced activation of human THP-1 monocytes. In the light of these observations, we wondered whether BJe may be beneficial against neuro-
inflammatory processes, such as those observed in Alzheimer’s disease. To this aim we used THP-1 monocytes to investigate the mechanisms underlying the beneficial potential of BJe against amyloid-beta₁₋₄₂ (Aβ₁₋₄₂)-mediated inflammation. Exposure of THP-1 cells to Aβ₁₋₄₂ significantly induced the expression and secretion of IL-6 and IL-1β in THP-1 cells and increased the phosphorylation of ERK 1/2 as well as p46 and p54 members of JNK family. Moreover, Aβ₁₋₄₂ raises AP-1 DNA binding activity in THP-
1-treated cells. Interestingly, all these effects were reduced in the presence of BJe. Our data indicate that BJe may effectively counteract the pro-
inflammatory activation of monocytes/microglial cells exposed to amyloid fibrils, suggesting a promising role as a natural drug against neuro-
inflammatory processes.
Hexameric procyanidins inhibits the EGFR signaling cascade at different levels

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Dietary proanthocyanidins (PACs) consumption has been associated with gastrointestinal (GI) health-promoting activities, including a decreased risk of colorectal cancer (CRC). High molecular weight PACs are poorly absorbed and they could exert local GI effects in part through their interactions with lipid rafts domains in epithelial intestinal cell membranes. Based on this evidence, we aimed to investigate if the capacity of large PACs to inhibit CRC cell growth and promote apoptosis could be due to the modulation by hexameric PACs (Hex) of the lipid raft-located epidermal growth factor receptor (EGFR) and of its downstream signaling. Hex inhibited the activation of the EGFR in EGF-stimulated Caco-2 cells. The inhibition of EGFR by Hex led downstream to the inhibition of the two major proliferative and antiapoptotic signaling pathways controlled by the receptor, ERK1/2 and PI3K/Akt. Hex did not affect the location of the EGFR at lipid rafts, but partially prevented the formation of the receptor dimer upon ligand binding. Measurement of the EGFR remaining at the cell membrane, by ELISA and immunofluorescence, showed that Hex promoted EGFR internalization both in the absence and presence of EGF. Hex also inhibited the EGFR activating phosphorylation at Tyr1068, but increased Tyr 1045 phosphorylation. The latter would promote EGFR degradation by the proteasome. In summary, this study provides evidences that the anti-CRC actions of Hex could be in part due to the modulation of the EGFR signaling pathways at multiple levels.

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Antimicrobial and antioxidant activities of the extracts from
*Entada rheedii* Spreng

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*Entada rheedii* Spreng. (Leguminosae - Mimosoideae) has been commonly used as drugs in Thai traditional medicine. Seed of *E. rheedii* is used as herbal drug for the treatment of skin diseases. The present study was carried out to evaluate the extract obtained from maceration and decoction methods of *E. rheedii* seed. The antimicrobial activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) *Pseudomonas aeruginosa* and *Candida albicans* was determined by disc diffusion, MIC, and MBC methods. In additional, their antioxidant activities were measured by chemical methods: DPPH and ABTS scavenging assays, and cell-based methods: nitric oxide (NO) scavenging assay. The results showed that ethanolic extract exhibited antimicrobial activity against *S. aureus* (inhibition zone=11.33 mm, MIC and MBC = 1.25 mg/ml) and MRSA (inhibition zone = 8 mm, MIC and MBC = 5 mg/ml). Moreover, this extract also displayed high antioxidant activity using DPPH and ABTS scavenging assays (EC$_{50}$ = 8.95 and 9.91 µg/ml, respectively), and it showed moderate nitric oxide scavenging activity (IC$_{50}$ = 62.58 µg/ml). This study also revealed that the extract from maceration method demonstrated promising antimicrobial and antioxidant activities. The ethanolic extract could be further developed as a potential source for antibacterial drugs. Thus, this study provides the first science data that support the ethnotherapeutic claim of *E. rheedii* seed.
Dietary supplementation with (-)-epicatechin inhibits reticulum endoplasmic stress in the pancreas of high fat diet-fed mice

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In the pancreas, endoplasmic reticulum (ER) stress can compromise β cell function and survival, ultimately leading to apoptotic cell death. Pancreatic ER stress has been observed in individuals with type 2 diabetes (T2D), obesity, and in association to high fat diet consumption. The flavan-3-ol (-)-epicatechin (EC) is present in large amounts in fruits and vegetables (e.g. cocoa, tea, berries). EC consumption has been linked to improved insulin sensitivity in humans and in rodent models of T2D. The aim of this study was to investigate the impact of a high fat diet on the pancreatic unfolded protein response (UPR), and the potential capacity of EC to mitigate ER stress. Mice were fed for 15 w: a control diet (C), and a high fat diet without (HF) or with EC (20 mg EC/kg body weight). Mice fed the HF diet had abnormal glucose and insulin tolerance test responses, which were prevented by EC supplementation. We measured the activation of: i) the insulin pathway, and ii) components of the three UPR branches (PERK, ATF6, XBP-1, JNK). The response to insulin was impaired in the HF pancreas, which was mitigated by EC supplementation. The three UPR branches were activated in the HF-fed animals. EC supplementation decreased the phosphorylation of PERK and JNK, and the levels of spliced XBP-1 and ATF-6. Results suggest that dietary EC could protect the pancreas from the deleterious effects of high fat diet consumption by protecting the beta cells from ER stress-induced apoptosis. Supported by NIFA-USDA (CA-D*-XXX-7244-H).
Protective role of a flavonoid-rich extracts of *Citrus* Juices in A549 Cells stressed by Fe$^{3+}$

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Exogenous iron in particulate matter and imbalanced iron homeostasis cause deleterious effects on health. Natural and synthetic iron chelators may be of therapeutic benefit, therefore we evaluated the protective effect of two *Citrus* flavonoids-rich extracts from bergamot and orange juices in iron overloaded human lung epithelial cells.

Cytofluorimetric, biochemical and genotoxic analyses were performed in Fe$_2$(SO$_4$)$_3$ exposed A549, pretreated with each extract whose chemical composition was previously detected. Chelating activity was assessed in cells by a calcein ester.

Both extracts reduced the generation of reactive oxygen species and membrane lipid peroxidation, improved mitochondrial functionality, and prevented DNA-oxidative damage in iron-exposed cells. Antioxidant effects were attributed to the chelating property, blocking upstream the redox activity of iron. Flavonoid-rich extracts also induced antioxidant catalase.

Both bergamot and orange juice extracts prevent iron oxidative injury, suggesting that these natural iron chelators could be used as preventive or therapeutic drugs against environmental stressors.
Reactive oxygen species regulate bioelectric responses during tail regeneration of Xenopus laevis tadpoles

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Multiple signaling mechanisms have been shown to modulate regeneration. Biochemical signals such as reactive oxygen species (ROS) and biophysical signals such as electric currents were, independently, shown to participate in this process. However, the interplays between biochemical and biophysical signals during regeneration remain poorly understood. Here, we take advantage of the fluctuating regenerative abilities of Xenopus laevis tadpole tail, that fail to regenerate only at specific developmental stages (refractory period), to investigate the interactions between redox and bioelectric activities during regeneration. We show that inhibition of NADPH oxidases-mediated production of ROS, scavenging or blocking their diffusion into cells impair regeneration and consistently regulate the dynamics of membrane potential (Vm), transepithelial potential (TEP) and electric current densities (JI) during regeneration. Depletion of ROS mimics the refractory period altered TEP and JI. Short-term application of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) rescues (from depleted ROS) and gains (from refractory period) regeneration, TEP increase and JI reversal. H\textsubscript{2}O\textsubscript{2} is thereby necessary for and sufficient to induce regeneration and to regulate TEP and JI. The unique early JI reversal in regeneration bud is therefore switched on by H\textsubscript{2}O\textsubscript{2} and robustly correlates with regeneration efficiency. In conclusion, this study unveils interplays between biochemical and biophysical signals during regeneration, suggesting a novel mechanism mediating regeneration: early H\textsubscript{2}O\textsubscript{2}-switched JI reversal. It also highlights the opportunities of interdisciplinary integration of apparently disparate states that may reserve promising advances for translational medicine.
LPS-induced TLR4-NFκB activation in rat renal cortex is inhibited by (−)-epicatechin

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This study was designed to evaluate the effect of dietary (−)-epicatechin on renal alterations induced by LPS challenge in rats. Sprague-Dawley rats were divided into two groups and treated during 4 d with control diet (C) or (−)-epicatechin supplemented diet (80 mg (−)-epicatechin /kg body weight/day) (E). On the fourth day, animals from C were administered ip with saline solution (Sal) or LPS (LPS) (4 mg/kg body weight) and animals from E were administered ip with LPS obtaining 3 experimental groups: C-Sal; C-LPS and E-LPS. After 6 h animals were euthanized. C-LPS showed renal injury, measured as increased levels of plasma creatinine and urea, and augmented kidney expression of iNOS, IL-6 and TNF-α, respect to C-Sal. In E-LPS, the functional and inflammatory manifestations determined were attenuated or absent. Activation of TLR4-NFκB pathway in renal cortex was studied evaluating TLR4 expression, phosphorylation and degradation of the NFκB inhibitory peptide IκBα, p65 phosphorylation in total homogenates and the ratio nuclear/cytosolic p65 protein. TLR4 expression was increased in C-LPS compared to C-Sal (48%, p<0.05), while no differences were found in E-LPS respect to C-Sal. C-LPS showed activation of the NFκB cascade and E-LPS exhibited significantly lower activation of this pathway. In parallel, renal cortex from C-LPS showed higher ex vivo superoxide anion production respect to C-Sal in association with increased expression of NOX2 subunit p47phox, respect to C-Sal. As a conclusion, (−)-epicatechin administration was associated with an inhibition of the TLR4-NFκB pathway in renal cortex, mediated by down-regulation of TLR4 expression and/or decreased oxidant production that results in kidney protection.

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Cyanidin-3-O-glucoside ameliorates palmitate-induced insulin resistance by modulating IKK and JNK induced serine IRS-1 phosphorylation

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Cyanidin-3-O-glucoside (C3G) is an anthocyanin (AC) commonly present in dark red colored fruits and vegetables from Mediterranean Diet. Although several in vitro and in vivo studies suggest that AC exert significant cardiovascular health-promoting effects and reduce endothelial dysfunction, the effect of C3G on insulin resistance has not yet been reported. We have investigated C3G activity in countering palmitic acid (PA)-induced insulin resistance in the endothelium. PA is known to impair PI3K signaling and to increase ET-1 and PAI-1 mRNA expression, generating insulin resistance and impaired insulin hemodynamic effects.

Our results indicate that C3G suppresses insulin-dependent expression of the ET-1 and PAI-1 genes and effectively inhibits PA induced impairment and NO-dependent vasodilator release, by modulating IRS1/Akt/eNOS pathway. Interesting this effect was eradicated by transfection with Nrf2 siRNA, suggesting that C3G protective effect on insulin pathway requires the activation of Nrf2. These results suggest that C3G modulates the expression and the activity of hemodynamic mediators in endothelial cells. Moreover, we observed that C3G reduces PA-induced IRS-1 phosphorylation at serine residues mediated by JNK and IKK, and that restores the phosphorylation reduction at the tyrosine of IRS-1 in response to insulin.

Overall, our findings indicate that C3G ameliorates endothelial dysfunction associated to insulin resistance by modulating IRS-1 phosphorylation. This effect might have potential to be applied for the management of cardiovascular diseases associated to diabetes and insulin resistance.
This network diagram is presented to aid in conceptualizing the many processes of aging, the causal chains of events, and the interactions among them. Contemplation of this network suggests promising intervention points for therapy development. This diagram is maintained on the Web as a reference for researchers and students. Content is updated as new information comes to light. [www.LegendaryPharma.com/chartbg.html]

At first glance the network looks like a complicated web. However, as a conceptual summary, in one view, we can see how various biogerontological processes relate to each other. Importantly, examination of these relationships allows us to pick out reasonably plausible causal chains of events. Within these chains, we can see age-related changes or accumulations that appear to be promising targets for future therapy development. The many observable signs of human senescence have been hypothesized by various researchers to result from several primary causes. Inspection of the biochemical and physiological pathways associated with age-related changes and with the hypothesized causes reveals several parallel cascades of events that involve several important interactions and feedback loops. This network model includes both intracellular and extracellular processes. It ranges in scale from the molecular to the whole-body level. Effects due to externalities, lifestyle, environment, and proposed interventions are highlighted around the margins of the network.
The roles of transition metal ions, manganese, selenium, and cadmium in redox proteome and health

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Multiple transition metal ions are present in the human diet, with some being required as essential nutrients and all causing toxicity at high intakes. Impacts on the redox proteome are complex because they are present as mixtures and at variable concentrations. In this study, we examined redox proteome responses of three metal ions including manganese (Mn), selenium (Se) and cadmium (Cd) using cells and mice. Mn is transition metal and essential nutrient for growth, and occurs in food at low doses. Excessive Mn disrupts redox control system and causes neurotoxicity. We found dynamic mitochondrial redox responses to Mn level, e.g., stimulation and inhibition of oxygen consumption rate by Mn concentration. Se, essential micronutrient for human and a component of seleno-redox enzymes plays a key role in regulating redox system. Se in high doses disrupts endocrine regulation and causes selenosis. Our results show that mice treated with Se accumulate Cd in organs higher than control mice, suggesting that excess Se elevates Cd toxicity. Toxic effects of Cd from cigarette smoke and occupational sources have been extensively studied, yet low-level Cd occurs in human diet. Cd accumulates in vivo due to a twenty-year biologic half-life. Our studies on fibroblasts and mouse lung tissue show that Cd at low levels stimulates inflammation and fibrosis by activation of NF-κB and Smad3/4 transcription factors, respectively, by altering redox proteome, metabolome and transcriptome. Together, the results suggest that presence of multiple transition metal ions in diet may affect human health by potentiating metal toxicity.
Alterations in erythrocyte fractions of different life span after single exhausting exercise in young untrained man

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In this study we show changes in properties of erythrocyte fractions of young untrained men after exhausting exercise. Eight healthy, untrained males (age, 21±3.0 years; BMI, 24±3.5 kg/m²) volunteered to participate in this study. The volunteers were healthy according to the medical examination based on the exclusion criteria: resting blood pressure, resting heart rate, smoking, antioxidant supplementation and medicaments usage. The approval of the ethical committee of the Medical University of Lodz was obtained prior to the study. Erythrocytes were fractionated into young (YF), middle-aged (MEF) and old cells (OF) in Percoll density gradient. The studies were conducted on all three fractions before exercise, immediately after exercise and 1 h of rest. Flow cytometry showed that the most homogeneous was fraction of MEF, while the OF fraction was the most heterogenic. Light microscopy indicated higher level of changed cells in OF. In OF the level of thiols was 2-fold lower than in YF and MEF. After exercise and 1 h of rest the thiols significantly increased to the level observed in YF and MEF. There were no differences in thiol concentrations in YF and MEF following exercise and after 1h of rest. There was no difference in lipid membrane fluidity in all fractions measured with TMA-DPH in 3 time points of study. A tendency to decrease fluidity in the hydrophobic region of lipid bilayer was found after exercise in YF and MEF measured using DAUDA. These findings show that short acute exercise of untrained man may change properties of erythrocytes.
Mass spectrometry evidence for the formation of estrogen-homocysteine thiolactone conjugates indicates potential involvement of estrogens in CVD and other diseases

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Homocysteine thiolactone (HCTL) is the heterocyclic intramolecular thioester of the amino acid homocysteine (HCsy), which is formed via nucleophilic attack by the side chain sulfhydryl with its carboxyl group. Dysregulation of methionine metabolism leading to hyperhomocysteinemia can lead to toxic buildup of HCTL. HCTL-mediated cardiovascular toxicity has been shown in vitro and in vivo through several pathways. Estrogens, in contrast, have been suggested to lower cardiovascular disease risk. Estrogen quinones, E1(E2)-2,3-Q and E1(E2)-3,4-Q, generated in situ by oxidative enzymes, metal ions, or molecular oxygen, can alkylate DNA as well as form conjugates with glutathione. Our previous studies have demonstrated the ability of estrogen quinones to interact with HCsy to form conjugates, which may offer a pathway for increased elimination of toxic levels of HCys while simultaneously quenching the reactive estrogen quinones. We hypothesize a new pathway for the elimination of toxic HCTL via conjugate formation with estrogen quinones, which may lead to cardiovascular protective effects. To test this hypothesis, we treated estrogen quinones with HCTL. Tandem mass spectrometry analysis of the assay mixture confirmed the formation of E1(E2)-HCTL conjugates. In addition, catechol estrogens incubated with myeloperoxidase in the presence of HCTL resulted in the formation of E1(E2)-HCTL conjugates. Conformation of the identities of the E1(E2)-HCTL conjugates were made by comparing them to pure-synthesized E1(E2)-HCTL standards. It is feasible that estrogens could chemically regulate HCTL levels through conjugate formation. In addition, these conjugates could be used as potential biomarkers in determining health and disease risk.
Citizen science: the development of a novel study design for dietary intervention research

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Controlled clinical trials are the gold standard for nutritional intervention research. Yet the time, cost, and effort required for recruitment and execution of such studies limits sample size and research populations to those who reside near traditional research centers. Emerging social media technologies have the potential to revolutionize research. Citizen science is an exciting new approach to conduct studies in broader populations, as illustrated by the success of projects such as the American Gut Project. However, this approach has never been validated. The goal of this project is to develop, test, and validate the use of citizen science to determine whether it can provide reliable data for dietary intervention research. A controlled clinical study and a citizen science study are being conducted simultaneously, investigating the effects of egg consumption on the gut microbiota and production of microbial metabolites known to have cardiometabolic effects on the host. The design of the citizen science project includes elements similar to the controlled trial (inclusion/exclusion criteria, collection of dietary and health data, analytical methods). However, the intervention (i.e. egg consumption and collection of samples) is administered by the subjects themselves and is unsupervised. Several approaches for ensuring data and sample quality are being adapted to the citizen science platform. Given the powerful potential of citizen science approaches for both observational and intervention research in nutrition, it is critical that it be optimized and validated to provide the most reliable data possible.
Protein tyrosine phosphatase 1b is a potential therapeutic target for nonalcoholic steatohepatitis

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As obesity rates increase, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are becoming the most common liver diseases. Progression of NAFLD to NASH is characterized by a necroinflammatory process with hepatocyte ballooning, lipoapoptosis, and progressive fibrosis. Type 2 diabetes mellitus and obesity increase the risk of developing NASH, but the mechanisms are not well understood. Protein tyrosine phosphatase 1B (PTP1B) is a physiological regulator of glucose homeostasis and energy balance. The metabolic role of PTP1B in insulin-responsive tissues has been reported. In addition, a recent study demonstrates multiple roles of PTP1B in liver diseases including non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma. In the current study, we assessed the physiological role of hepatic PTP1B using the Cre-loxP system to achieve liver-specific deletion. In vivo metabolic, biochemical and metabolomics studies were performed to investigate the role of PTP1B in NASH. Hepatic PTP1B protein expression was evaluated in mice fed regular chow, choline-sufficient (CSAA) and L-amino acid defined (CDAA) diets. In addition, we generated liver-specific PTP1B knockout mice (KO) using the Cre-loxP approach. Control and KO mice were fed regular chow and fast food diet (FFD) supplemented with high-fructose corn syrup in drinking water for 20 weeks to induce NASH. Insulin sensitivity and glucose tolerance of mice were evaluated using insulin tolerance tests (ITTs) and glucose tolerance tests (GTTs). Immunoblot analyses of total liver lysates from mice fed regular chow, CSAA and CDAA diets revealed increased hepatic PTP1B expression upon CDAA feeding (NASH model). In addition, liver-specific PTP1B KO mice fed FFD (FFD-KO) exhibited improved insulin sensitivity compared with CT fed FFD (FFD-CT) mice in the absence of body weight differences. Moreover, FFD-KO mice displayed significantly enhanced glucose tolerance compared with FFD-CT as measured by GTTs. These data reveal dynamic regulation of hepatic PTP1B expression in NASH model and suggest that dysregulation of PTP1B signaling in liver may be relevant to glucose homeostasis in liver and NASH pathogenesis.
Pharmacologic inhibition of NADPH Oxidase4/Nox4 provides renoprotection in contrast induced nephropathy

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Contrast induced nephropathy (CIN) may occur, in part, as a result of intrarenal oxidative stress. NADPH oxidases are important sources of reactive oxygen species (ROS). Among various type of NADPH oxidases, Nox4 is expressed predominantly in rodent kidney. The aim of the present study was to assess the effect of Nox4 inhibition on the prevention of CIN.

Using HK-2 cells, Nox4 mRNA and protein were determined after exposure to iohexol with/without pretreatment of the most specific Nox1/4 inhibitor, GKT137831. Caspase3/7 activity, DHE stain and amplex red activity were also measured. Proinflammatory and apoptotic markers (pNFkB/NFkB, pp38/p38, pJNK/JNK, pERK/ERK and pcleaved caspase/caspase) were measured for investigation of intracellular pathway associated with Nox4. In addition, the effects of Nox4 inhibition were evaluated in mice model of CIN. Our results demonstrated that the expression of Nox4 in HK-2 cells significantly increased by iohexol exposure. Pretreatment of GKT137831 resulted in reduced production of ROS, down regulation of proinflammatory marker (p38), that are implicated in CIN, reduced caspase 3/7 activity and increased cellular survival in iohexol exposed HK-2 cells. Silencing of the Nox4 gene replicated these effects by down regulation of proinflammatory markers. In CIN mice model, pretreatment with GKT137831 resulted in an attenuated vacuolar degeneration, tubular epithelial cell shedding, cellular cast formation and tubular dilatation. Collectively, these results identify Nox4 as a key source of ROS responsible for kidney injury in CIN and provide proof of principle for an innovative small molecule approach to prevent contrast induced nephropathy.
The modulation of steroid metabolism in Chinese students after their migration to the United States

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There are major differences between the diets, life-style, and environment in China and the United States. Especially China has been suffering from serious environment pollution in recent years. The incidence of diseases in these two countries varies widely. Steroids are involved in various important physiological functions; such as neural activities, gene expression, secondary sexual characteristics, maturation, reproduction, digestion, cardiovascular health, stress response and immune response. We hypothesize that the above factors can have an effect on steroid metabolic pathways. So the relationship between steroid metabolome profile and environment deserves a comprehensive investigation in order to develop biomarkers for early detection of diseases related to life-style. To study this, thirty five freshmen students were recruited from UC Davis. All participants were Chinese students from major cities, who had never travelled to other countries for more than one month before their participation in this study. Saliva samples were collected the morning after they arrived in the U.S. and a second sample 4 months later. The steroid metabolic profiling was done using a liquid–liquid extraction (LLE) followed by ultra-performance liquid chromatography (UPLC)-tandem mass spectrometry (MS/MS) for simultaneous measurement of over 100 endogenous steroids. A total of 78 different steroids were detected in the saliva samples. Of the 78 detected steroids, 5 changed significantly after subjects lived in the U.S. for 4 months. The levels of Androstenedione and 3-Methoxy estriol increased, whereas 3b-Hydroxy-5-pregnen-20-one-3-SO$_4$, Cholesterol-3-SO$_4$, and Cholesterol-3-Glu decreased. In conclusion, the migration to the U.S. changes the steroid metabolism leading to effects on physiology.
Montmorency cherry concentrate supplementation does not enhance myofibrillar protein synthesis response to exercise and protein in healthy older males

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Oxidative stress and inflammation are suggested to contribute to muscle anabolic resistance to protein and exercise in older adults. We investigated whether consumption of montmorency cherry concentrate (MCC) with antioxidant and anti-inflammatory properties increased anabolic sensitivity to protein ingestion and resistance exercise in healthy older men. Sixteen healthy older men were randomised to receive MCC (60ml.d⁻¹) or placebo (PLA) for two weeks, after baseline measures in week 1. During week 3, participants consumed 10 g whey protein.d⁻¹ and completed three bouts of unilateral leg resistance exercise (4 x 8-10 repetitions at 80% 1RM). Participants consumed a bolus (150 ml) and weekly (50 ml) doses of deuterated water. Saliva samples were taken to measure ²H enrichment of body water and vastus lateralis biopsies were taken from non-exercised leg after weeks 1, 2 and 3, and exercised leg after week 3, to measure tracer incorporation at rest, in response to protein and protein + exercise. Myofibrillar protein synthesis (MPS) increased in response to exercise + protein compared to rest (p = 0.003), but there was no effect of supplement on this MPS response (MCC: 1.50 ± 0.30 vs 1.32 ± 0.23; PLA: 1.52 ± 0.22 vs 1.27 ± 0.15; all %.d⁻¹). Muscle total NFκB (17.8 ± 31.3%) and COX1 (10.7 ± 38.4%) protein were increased by exercise + protein in PLA, but decreased in MCC (NFκB: -20.7 ± 17.5%; COX1: -20.9 ± 18.7%; main interaction effect: p = 0.027, NFκB; p = 0.024, COX1). Short-term MCC ingestion did not affect the anabolic response to protein and exercise in healthy, relatively active, older men. However MCC ingestion attenuated expression of proteins involved in the muscle inflammatory response to exercise, which may influence the chronic training response.
Anti-diabetes effects of Melanian snail protein hydrolysates in high fat diet-fed mice

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Control of postprandial hyperglycemia and inhibition of oxidative stress have been suggested to be important in the treatment of diabetes. Therefore, a great deal of research effort has been directed toward the search for effective and safe functional foods or compounds derived from natural materials for treating diabetes. In this study, the hypoglycemic, hepatoprotective, and nephroprotective effects of Melanian snail (Semisulcospira libertina) protein hydrolysates (MPh) were analyzed in mice adapted to a 45% kcal high-fat diet (HFD) after 12 weeks of continuous oral administration of MPh at 125, 250, and 500 mg/kg. HFD-fed mice exhibited increase in body weight, blood glucose, and insulin levels, with decrease in serum high-density lipoprotein (HDL) levels. In addition, increases in steatohepatitis regions, hepatocyte hypertrophy, and lipid droplet deposit-related renal tubular vacuolation degenerative lesions were detected, with marked expansion and hyperplasia of pancreatic islets, increases in insulin- and glucagon-producing cells, as well as decreased zymogen contents. However, glucose intolerance and all diabetic complications were dose-dependently and significantly inhibited by oral treatment with MPh. In addition, marked dose-dependent inhibition of hepatic lipid peroxidation, depletion of the liver endogenous antioxidant defense system, and changes in hepatic glucose-regulating enzyme activities were observed. These results suggest that MPh exhibits potent antidiabetic effects, along with amelioration of related complications in HFD-fed mice.
Metabolomic investigation of tamoxifen induced 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 antiestrogenic 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-desmethyl-tamoxifen 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 chromatography 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.
Effect of milk fat globule membranes as a complementary food on the serum metabolome of 6-12 months old Peruvian infants

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Growing evidence shows that breast milk offers significant advantages over infant formula for the development of the neonatal immune system, which provides protection against infections. This indicates that important components in breast milk are absent in formulas. One of those components is the milk fat globule membrane (MFGM), a lipid-protein complex surrounding milk triglycerides that is lost during formula manufacture. In a 6-month double-blind randomized controlled trial, Peruvian infants (n = 499; starting at > 6 months of age) who were fed a bovine MFGM fraction twice daily as a complementary food had significantly fewer episodes of bloody diarrhea. Notably, diarrhea is one of the main causes of mortality and stunting in Peruvian children younger than 5 years old. Although attempts have been made at identifying the structural components of MFGM, the contributions of the identified components to the metabolic fate and biological functions upon ingestion have been poorly elucidated. Building on the observational study, serum samples, which were collected before and after the intervention, were selected by stratified random sampling (n = 50) and 75 low-molecular weight soluble metabolites were analyzed by ¹H nuclear magnetic resonance (NMR) based metabolomics. Significant metabolites that differentiate the infants who received MFGM from the infants who did not include branched-chain amino acids, essential and/or non-essential amino acids involved in nitric oxide (NO) synthesis, the glutathione pathway and choline metabolism, and also several metabolites from host-microbial co-metabolism. Our findings suggest that infants fed MFGM show metabolic shifts that may lead towards enhanced immune function.
Developmental marginal zinc deficiency affects redox status, redox-regulated signals and cellularity in the adult brain cortex

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Zinc (Zn) plays a key role in the modulation of brain redox status. The present work investigated if marginal zinc deficiency (MZD) throughout development can cause oxidative stress, affect microtubule-modulated signals, and promote changes in cellularity in the adult brain cortex. Rat dams were fed control (25 µg zinc/g diet) or marginal (10 µg zinc/g diet) zinc diets from gestation day 0 until postnatal (P) day 21, offspring continued in the maternal diet until P56. MZD caused high levels of tubulin thiol oxidation in the brain cortex leading to impaired in vitro tubulin polymerization. MZD was associated to a decreased expression of the glutamate-cysteine ligase modifier subunit, suggesting a compromised glutathione synthesis. In the P56 MZD brain cortex, the altered tubulin assembly caused an impaired nuclear transport of transcription factors that we had previously shown are transported to the nucleus by microtubules: NF-κB, NFATc2, and STAT3. These signalling cascades play key roles in progenitor cell proliferation and/or differentiation into the different brain cell types. In MZD brain cortex, we observed significantly lower levels of the neuronal marker NeuN, of the astroglial marker S100β, and a trend for low levels of the myelin small basic protein and proteolipid protein. The above results further support the concept that zinc is essential for normal brain development. The deleterious effects of developmental MZD on the brain can be in part due to an oxidation-mediated impairment of microtubule assembly and the associated deregulation of signalling pathways central to cell proliferation/differentiation. Supported by NIFA-USDA (CA-D*-XXX-7244-H) and PackerWentz Endowment.
HDL Particles modulate cell surface morphology and membrane structure in LPS-stimulated monocytes

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HDL are increasingly being recognized for their immunomodulatory effects in monocytes but the mechanisms by which HDL exert these effects are not well defined. HDL can exert their immunomodulatory effects via three potential mechanisms: 1) deplete cholesterol from lipid rafts, inhibiting inflammatory signaling associated with lipid raft proteins, 2) bind toxins like lipopolysaccharide (LPS) and thus neutralize the stimulatory effects of LPS on monocyte activation, and 3) have direct priming effects that attenuate monocyte response to inflammatory stimuli. In vitro experiments in LPS-stimulated monocytes were conducted to determine whether the immunomodulatory effects of HDL are modified by direct LPS binding vs. priming. Monocytes were isolated from peripheral blood mononuclear cells and either pre-incubated with HDL followed by LPS stimulation, or stimulated directly with LPS that had been pre-mixed with HDL. Cells were fixed and stained with fluorescent probes prior to imaging with confocal microscopy. Monocytes that were pre-incubated (i.e. priming) with HDL prior to LPS stimulation had lower cell surface area (i.e. less cell membrane protrusions) than monocytes treated with pre-mixed HDL+LPS. The pre-incubated monocytes also had less lipid raft internalization but increased lipid raft clustering at the cell surface compared with cells treated with pre-mixed HDL+LPS, as well as decreased scavenger receptor SR-B1 staining compared with cells treated with either HDL alone, or pre-mixed HDL+LPS. These results suggest that priming monocytes with HDL attenuates the morphological changes induced by LPS stimulation. These results also suggest that LPS-bound HDL are internalized via a lipid-raft-mediated mechanism, which may not involve SR-B1.
A novel nutrient blend mimics calorie restriction transcriptomics in brain of mice

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The identification of caloric restriction mimetics (CRMs), compounds that mimic the beneficial effects of caloric restriction (CR) without restriction of dietary energy would be a scientific advancement. The present study investigated whether the transcriptomic profile of a putative CRM nutrient blend could mimic that of CR following long-term feeding. B6C3F1 male mice were studied; n=7 per group. Young Controls (YC; 5 months) and three groups treated from 14-30 months of age: Old Controls (OC), Old CR (OCR; 25% CR) and Old Supplemented (OS)( fish oil, resveratrol, quercetin, purple corn extract, rosemary leaf extract, citrus bioflavonoids, Coenzyme Q₁₀, alpha lipoic acid, carotenoids, vitamins D₃ and K₂ and d-limonene).

Gene expression profiling in cortex was performed using Affymetrix Mouse 2.0ST arrays. Principal component analysis revealed that gene expression profiles of YC and OC were distinct from one another and from the OCR and OS groups; patterns for OCR and OS were similar. Using differential analysis, 3,468 genes commonly expressed in OCR and OS groups compared to the OC group were identified. Compared to OC, OCR and OS modulated nuclear hormone receptor, proto-oncogene B-Raf, and miR-24-1 subnetworks and gene ontology/biological processes including: cell cycle regulation, respiratory chain, oxidative phosphorylation, signal transduction, metabolic processes and DNA-dependent transcription. A major finding of the present study is that the nutrient blend effectively mimicked the general profile of the transcriptomics of CR, modulating ~3,500 cortex genes in common with CR. These CRM effects, elicited by a mid-life intervention, could have positive implications for healthy human aging or ‘youthspan’ and warrants further investigation.
PUFA diet effects on neurobehavior and ROS in a Friedreich’s ataxia mouse model

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Friedreich’s ataxia (FA) is a neurodegenerative disease caused by a GAA repeat expansion of the FXN gene. The resulting decrease in Frataxin protein results in a decreased iron-sulfur cluster biogenesis, and therefore decreased capacity of cells to protect against reactive oxygen species (ROS). Mouse models of FA have traditionally resulted in a mild and late onset neurobehavioral phenotype. By creating novel tools for phenotypic assessment, our lab was able to establish a more robust and translatable neurobehavioral suite of tests for important hallmarks of the clinical pathology. The Inverted Screen Test assessed muscle strength and endurance, Treadscan Automated Gait Analysis assessed ataxia and motor coordination parameters, and Von Frey assessed peripheral sensitivity. KIKO and wild type littermate control mice were kept on either regular mouse chow of a 15% PUFA diet for 9 months from the time of weaning. Behavioral assessments of mice showed that the FA mice given a PUFA diet had a significantly decreased peripheral sensitivity and muscle strength, with increased ataxic gait parameters. Additionally the 8-oxo-guanosine assay was used to assess the cerebellar and DRG tissue of the mice for oxidative damage to DNA.
Effects of CuATSM derivatives on SOD1 maturation in an ALS drug-screening mouse model

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Diacetyl-bis(4-methylthiosemicarbazone) copper II – “CuATSM” – is remarkably protective in transgenic mouse models of amyotrophic lateral sclerosis (ALS) and a promising candidate drug for the treatment of human ALS. In the present study, we explore the effects of CuATSM derivatives on human SOD1WT maturation in transgenic mice coexpressing human SOD1WT and the human copper-chaperone-for-SOD (CCS). We synthesized more than a dozen different CuATSM derivatives and tested five in SOD1WT x CCS mice, subsequently measuring copper incorporation into SOD1WT by mass spectrometry of intact SOD1 isolated from mouse brains. Mass spectra of SOD1WT from brains of 6-day-old mice treated once daily starting at 4-days revealed a variety of treatment effects. CuATSM was the most effective for restoring copper to SOD, increasing Cu,Zn SOD1WT from 44% of total SOD1WT in controls to 72% with CuATSM treatment. In contrast, the derivative CuGTSM was toxic, and also the least effective for restoring copper to SOD. CuGTSM lacks two methyl groups on the diacetyl backbone of ATSM. This increases the coordinated copper’s reduction potential and expedites copper release. In animals treated with CuGTSM, Cu,Zn SOD1WT decreased from 44% in controls to 30% of total SOD1WT. These results suggest that the therapeutic mechanism of CuATSM may depend upon its selective delivery of copper in the CNS to CCS, rather than simply increasing copper availability in the CNS.
In extremely preterm infants, does exposure to early postnatal oxidants affect their neurodevelopmental outcome at 18 months corrected age?

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**Background:** Oxygen (O₂) and peroxides contaminating parenteral nutrition (PN) represent the two main oxidants to which extremely preterm infants are exposed.

**Objective:** To examine the relation between early oxidants, oxidative stress markers at 36 weeks post menstrual age (PMA) and neurodevelopmental (ND) outcome at 18 months corrected age.

**Design/Methods:** A prospective cohort study included 51 infants <29 weeks of gestation. After consent, blood sample was obtained at 36 weeks PMA. Whole blood glutathione (GSH and GSSG) were measured by capillary electrophoresis and the redox potential was calculated using Nernst equation. Baseline clinical characteristics and ND outcome data (Bayley-III) were collected. Early oxidants were defined as FiO2 ≥25% on day 7 of life and PN duration >14 days. t-student test and logistic regression were used as appropriate. P<0.05 was significant.

**Results:** At 36 weeks PMA, both FiO2 ≥25% on day 7 of life and PN duration>14 days were associated with higher GSSG (P= 0.04 and 0.02 respectively) and more oxidized redox potential (P= 0.02, 0.03 respectively). Both oxidant exposure (P= 0.03) and more oxidized redox potential (P=0.01) were associated with poorer ND outcome. In a logistic regression model including gestational age, illness severity score and maternal education, the redox potential at 36 weeks PMA remained a significant independent factor for motor Bayley score <85 (P=0.045).

**Conclusions:** In extremely preterm infants, early postnatal exposure to oxidants is associated with prolonged oxidative stress and worse ND outcome at 18 months corrected age.
Safety and tolerability in consuming *Bifidobacterium longum* subspecies *infantis* in exclusively breastfed term infants

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*Bifidobacterium longum* subspecies *infantis* (*B. infantis*) is the dominant strain of intestinal bacteria in breast-fed infants that were delivered vaginally. In developed nations, infants delivered by cesarean section (C-section) or vaginally have lower levels of intestinal *B. infantis* than infants born in developing nations. Previous studies have found that *B. infantis* was well-tolerated when provided to premature infants, but such data has not yet been reported. One objective of the ongoing Infant Microbiota and Probiotic Intake (IMPRINT) Study was to determine if the daily consumption of *B. infantis* by exclusively breastfed infants for 21 days would be safe and well-tolerated given its implications towards reducing inflammation. Before Day 6 postnatal, women and their infants delivered either vaginally or by section-section, were randomized into a lactation support (LS) group or lactation support plus *B. infantis* (Bi+LS) group. C-section births were matched for the time when membranes ruptured before birth (≤ 6 hr or > 6 hr). Starting with Day 7 postnatal, and for 21 consecutive days thereafter, infants in the Bi+LS group were given daily doses of 1x10^10 cfu of *B. infantis* (sealed individual sachets provided by Evolve Biosystems Inc, Davis, CA) suspended in 5 mL of their mother’s breastmilk. Mothers collected their own fecal and breastmilk samples as well as fecal samples from their infants, and filled out daily questionnaires about infant feeding and gastrointestinal tolerability. Safety and tolerability was determined from daily logs that prompted parents to assess their infants’ feeding, stooling frequency, consistency, gut symptoms and health outcomes. Infants were also weighed by study personnel 1 and 2 months postnatal. Compliance was determined by counting *B. infantis* sachets used biweekly and checking for questionnaire completion. Based on preliminary data (n = 24 in the Bi+LS, and n = 18 in the LS group), *B. infantis* was determined to be well-tolerated and did not result in any adverse events in the supplemented infants.

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Investigations on intelectin-1 (lactoferrin receptor) during chemically induced colitis using a knockout mouse model

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Intelectins (ITLNs) are carbohydrate-binding proteins implicated in innate immunity and highly conserved across chordate evolution, including both ascidians and humans. Genome-wide association studies identified human ITLN1 as a susceptibility locus for Crohn’s disease. ITLN1, which binds microbial but not host glycans, is highly abundant in the intestinal mucosa of humans. Since ITLN1 also acts as a receptor for lactoferrin, a major protein in human milk, our research group has developed a knockout mouse model to study the biological activities of Itln1 in vivo. Both mRNA and protein analysis of mouse tissues found that Itln1 is most highly expressed in the small intestine, particularly by Paneth cells, and expression is abolished in the Itln1−/− mice. We then investigated the effect of Itln1 deletion on disease activity during experimentally induced colitis. Itln1−/− and Itln1+/+ littermate mice were treated with 2% w/v dextran sulfate sodium (DSS) for 7 d. Itln1−/− mice exhibited increased sensitivity to DSS and impaired recovery upon DSS removal, including significantly enhanced fecal occult blood, weight loss, and colonic length shortening, indicating that Itln1 protects from chemically induced colitis. On-going studies aim to determine the underlying mechanism of Itln1 protection, including its potential role in mediating host-microbe interactions and/or immune-signaling pathways. These investigations may provide further insight into the role of ITLNs in innate immunity and in human inflammatory bowel disease.
The plausibility of maternal toxicant exposure and nutritional status as contributing factors to the risk of autism spectrum disorders

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Recent research suggests the maternal environment may be especially important for the risk of developing autism spectrum disorders (ASD). In particular maternal infections, micronutrient deficiencies, obesity, and toxicant exposures are likely to interact with genetic risk factors to disrupt fetal brain development. Objectives: The goal of this paper is to investigate the plausibility of maternal toxicant exposure and nutritional status as causal factors in the development of ASD. Methods: This paper reviews current research investigating the hypothesis that maternal toxicant exposure and prenatal micronutrient intake are important modifiable risk factors for ASD. Results: Zinc, copper, iron, and vitamin B9 are identified as specific micronutrients with relevance to the etiology of ASD. Specific toxicants induce a maternal inflammatory response leading to fetal micronutrient deficiencies that disrupt early brain development. Importantly, maternal micronutrient supplementation is associated with reduced risk of ASD. Furthermore, animal studies show that micronutrient supplementation can prevent the teratogenicity and developmental neurotoxicity of specific toxicants. Discussion: These findings lead to the hypothesis that maternal infection, obesity, and toxicant exposures (e.g. valproic acid, endocrine disrupting plasticizers, ethanol, and heavy metals) are all environmental risk factors for ASD that lead to fetal micronutrient deficiencies resulting from a maternal inflammatory response. It could be possible to use markers of inflammation and micronutrient status to identify women that would benefit from micronutrient supplementation or dietary interventions to reduce the risk of ASD. However, more research is needed to demonstrate a causal role of fetal micronutrient deficiencies and clarify the underlying mechanisms that contribute to ASD.
Decreased ERK1/2 phosphorylation is associated with disruption of fetal neurogenesis resulting from marginal zinc deficiency

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Prenatal zinc deficiency resulting from insufficient dietary intake, absorption, or transport can compromise development of the central nervous system leading to a spectrum of defects ranging from severe congenital malformations to subtle neurological and cognitive impairments. We previously found that marginal zinc deficiency disrupts the ERK1/2 signaling pathway leading to disruption of neural progenitor cell proliferation. This study investigated if marginal zinc deficiency during gestation in rats could affect fetal neurogenesis. Rats were fed a marginally zinc-deficient or adequate diet through gestation and offspring were analyzed at embryonic day (E)14, E19, postnatal day (P)2, and P56. ERK1/2 phosphorylation measured by Western blot in the cerebral cortex was decreased in the marginal zinc fetuses at E19. Immunofluorescence staining showed the pattern of ERK1/2 phosphorylation was specifically decreased in the ventricular zone of the frontal cortex at E19 and this was associated with decreased labile zinc levels measured by zinquin staining. Marginal zinc deficiency decreased neural progenitor cell proliferation measured by Western blot and immunofluorescence for the mitotic marker phospho-vimentin. Decreased proliferation led to decreased expression of the neural progenitor cell marker Sox2 and the neuronal marker (NeuN) at E19. However, the density of Tbr1 expressing glutamatergic neurons was increased in the superficial layers of the frontal cortex at E19. At P56 the density of cells expressing NeuN was decreased, demonstrating that disruption of fetal neurogenesis had lasting effects on the density of neurons in the frontal cortex following dietary repletion with adequate zinc levels. Disruption of neurogenesis could underlie irreversible neurobehavioral impairments observed after marginal zinc deficiency during fetal development.
Transcriptomic profiling of postprandial lipid-responsive gene in ROS signaling from RNA-seq data in brain microvascular endothelial cells

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Vascular dementia (VaD) is the second-most-common form of dementia after Alzheimer's disease and cause by problems in the supply of blood to the brain. Previous studies suggest a relationship of blood lipids and/or lipoproteins to vascular inflammation leading to dementia. However, the role of postprandial triglyceride-rich lipoproteins (TGRL) in the regulation of gene expression during abiotic stress situations mediated by cellular redox changes and/or by oxidative stress processes remains poorly understood. RNA sequencing (RNA-Seq) is a novel method to analyze the full transcriptional activity of a cell or tissue, thus allowing new insight into the impact of lipid-induced vascular inflammation. We conducted the first full-genome RNA-Seq analysis in human brain microvascular endothelial cells (HBMVEC) to analyze media control and TGRL. Transcriptome analysis with the aid of RNA-seq data revealed that the incubation of HBMVEC with TGRL lipolysis resulted in the modulation of 619 genes significant (p value=0.0005), of which 491 genes had a 0.5-log-fold-change, being 217 up- and 274 downregulated genes, respectively. Thus, RNA-seq data analysis showed that an important set of these genes were associated with the stress mediated signaling include NRF2-mediated oxidative stress response and hypoxia signaling. This study shows that lipolysis has the capacity to modulate the expression of genes involved in the response to abiotic stress conditions. We were able to identify new targets genes by RNA-Seq technology. Postprandial TGRL is capable of modulating new sets of genes involved in the signaling mechanism mediated by additional abiotic stresses and especially in stresses mediated by ROS.
The phytochemical study of *Tridax procumbens* for healing diabetic wound

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Diabetes is a chronic condition that can cause a number of serious complications. Problems with the feet are one of the most common. The purpose of this work is to provide information on what you need to know about feet and diabetes and what sort of help you should seek. The medicinal plant Tridax procumbens (L), commonly known as Tridax daisy, is a species of flowering plant in the daisy family. It is best known as a widespread weed. Tridax procumbens (L.) is a spreading annual herb found throughout India but unfortunately it is one of the neglected plants. Hence, the present work aims to open new avenues for the improvement of medicinal use of Tridax procumbens for various ailments and to bring the anti-diabetic medicinal plant to the scientists' notice, and raise awareness and add value to the resource. Traditionally, Tridax procumbens has been in use in India for wound healing. The essential values and uses of some medicinal plants have been worked out and published, but many of them remain unexplored to date. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to discover their medicinal properties. Hence, Present work deals with phytochemical analysis of Tridax procumbens for the improvement of medicinal use of Tridax procumbens for various ailments and raise awareness and add value to the resource.
Mitochondria-generated acetyl-CoA regulates gene expression in hepatic lipid synthesis

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During the absorptive state, the liver stores excess glucose as glycogen and synthesizes fatty acids for triglyceride synthesis. For de novo synthesis of fatty acids from glucose, the mitochondrial pyruvate dehydrogenase complex (PDC) plays a gatekeeper’s role for generation of acetyl-CoA. We previously showed that there was no incorporation of radioactive glucose-carbon into newly synthesized fatty acids in livers from liver-specific PDC-deficient male mice (L-PDCKO) due to the null mutation of the Pdha1 gene. In the present study, we show that L-PDCKO mice developed hyper-insulinemia with lower blood glucose levels in the fed state but their levels of triglyceride and cholesterol in both serum and liver remained unaltered. We further investigated expression of several key genes as well as their upstream regulators in lipid and glucose metabolism. Interestingly, there was significant downregulation of expression of several key genes in the de novo fatty acid synthesis pathway and their upstream regulators. Downregulation of several genes in the metabolism of glucose and fatty acid oxidation were also observed in livers of L-PDCKO mice. Although, the total hepatic acetyl-CoA content remained unaltered in these mice, modified acetylation profiles of several proteins in the nuclear compartment including the level of acetylated histone3-lysine9 in livers of L-PDCKO mice suggest an important role of PDC-generated acetyl-CoA from glucose-derived pyruvate in gene expression in hepatic lipid metabolism. This finding has important implications for regulation of hepatic lipid metabolism in obesity, type 2 diabetes and non-alcoholic fatty liver disease. (Supported in part by NIH Grant DK20478).
Anti-inflammatory and antioxidant activities, and total phenolic contents of flower extracts from *Azadirachta indica*

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*Azadirachta indica* or Neem is a tropical tree growing throughout Thailand and classified in the Meliaceae family. In Thai traditional medicine, many parts of neem tree are used for treatment of diseases. Neem flowers were used as bitter tonic, febrifuge, and nasal polyposis treatment. This research aimed to investigate anti-inflammatory and antioxidant activities of the ethanolic and water flower extracts using nitric oxide scavenging assay, and DPPH radical scavenging assay, respectively. Total phenolic content of the extracts was determined using Folin–Ciocalteu’s method. The results showed that the water and ethanolic extracts exhibited the potent NO-inhibitory activity with IC$_{50}$ values of 4.80±1.15 and 39.86±2.14 µg/ml, respectively. However, both showed lower anti-inflammatory activity than prednisolone as a positive control. The water extract displayed higher antioxidant activity (IC$_{50}$ = 9.43±0.26 µg/ml) than BHT as a positive control. Moreover, it showed a high content of total phenolic compounds. In conclusion, the water extract of *A.indica* flower displayed anti-inflammatory and antioxidant activities. Thus, it should be recommended for a good source of natural antioxidants for commercial uses and food applications.
**N-Acetylcysteine inhibits triglyceride accumulation in mouse embrionary fibroblasts during adipogenic differentiation**

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Assays were conducted using mouse embrionary fibroblasts (undifferentiated cells: CC), which are capable of differentiating into mature adipocytes (differentiated cells: DC). A significant increase in Triglyceride (Tg) content was observed in DC comparing to CC, which is set to 100: 100 ± 23 [CC] vs 169 ± 15 [DC], p< 0.01). We also determined Oil-Red-O stained neutral lipids; an increase was observed in DC (100 ± 6 [CC] vs 173 ± 18 [DC], p< 0.01) and, a decrease in DC-5 (129 ± 3 [DC-5] vs 173 ± 18 [DC], p< 0.01).

We evaluated mitotic cellular expansion (MCE) and observed an increase in DC of 1.5 cell number compared to CC at day 2. The highest doses of NAC inhibited MCE (Day 2: 7.87 x 10⁵ + 0.08 x 10⁵ cells/mL [DC-5] vs 11.77 x 10⁵ + 0.16 x 10⁵ cells/mL [DC], p< 0.01) and, an increase in expression levels of phosphoERK½ and phosphoJNK was observed during adipogenic differentiation. NAC did not affect pERK½, but 5mM NAC treatment decrease pJNK in almost 50% (pJNK/JNK: 0.91 ± 0.05 AU [DC-5] vs 1.76 ± 0.05 AU [DC], p< 0.01).

NAC could inhibit lipids accumulation and JNK phosphorylation in mouse embrionary fibroblasts during adipogenic differentiation.
Nitric oxide bioavailability in the heart of L-NAME treated rats: Effect of quercetin supplementation

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This study evaluates the effects of dietary quercetin (QC) supplementation on nitric oxide (NO) bioavailability in cardiac tissue of rats deficient in NO by treatment with L-NAME, an inhibitor of NO synthase (NOS).

QC treatment prevented the increases in systolic blood pressure (SBP), superoxide anion production, expression of p47phox NADPH oxidase (NOX) subunit, and SOD and GPx activities (p<0.05), observed in L-NAME treated rats. Regarding NOS activity, it was significantly lower in L-NAME treated rats (p<0.001) independently of the presence or absence of QC in the diet. The inducible isoform of NOS (iNOS) showed a significant increase in L-NAME treated rats (p<0.001) that was prevented by QC supplementation, while endothelial (eNOS) and neuronal (nNOS) isoforms had no change. GSSG/GSH² ratio was lower in rats receiving QC compared to L-NAME treated rats.

In conclusion, QC supplementation prevented the elevation of SBP and the oxidative alterations observed in cardiac tissue of L-NAME treated rats, suggesting that QC increases NO bioavailability through the modulation of the activity and/or the expression of NOS and NOX enzymes, being greater the effect on the modulation of superoxide anion metabolism.

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Fructose overload modifications in oxidative metabolism and corticosterone production in rat epididymal adipose tissue

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Fructose overload has shown to produce relevant metabolic effects in mammals. This study was focused in the modifications induced on rat epididymal white adipose tissue (EWAT) machinery involved in oxidative metabolism and glucocorticoid synthesis. Male Sprague-Dawley rats received fructose 10% p/v in the tap water (fructose overload group = F) or tap water (control group = C) for 7 weeks. At the end of the treatment animals from F showed significant increases in plasma triglycerides and insulin levels, as well as in blood pressure, respect to animals from C. Corticosterone plasma levels resulted 1.1 -fold increase in F respect to C. Ex vivo EWAT superoxide anion production showed an increment of 3.9-fold in F respect to C, in association with significant increases in NOX2 subunits expression (246%, 33% and 53% for p22phox, p47phox and gp91phox, respectively). Among the oxidative stress markers determined, only EWAT GSH levels were affected, showing a decrease of 55% in F respect to C. Microsomal fraction of EWAT was obtained to determine aspects related with corticosterone synthesis. 11-beta hydroxysteroid dehydrogenase 1 (11-βHSD1) and hexose-6-phosphate dehydrogenase (H6PD) activities were higher in F respect to C, in parallel with increased levels of their protein expressions (1.0 and 2.5-fold increase for 11-βHSD1 and H6PD respectively). Additionally, a higher microsomal NADPH content was found in F respect to C (62%). These results support that fructose overload modifies EWAT becoming it a potential source of superoxide anion and/or corticosterone.

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**Pinus species and parts: Health protection**

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Pinus Brutia, Pinus Halepensis, and Pinus Nigra are the most common pines of Greece. Fresh and dry needles, bark, wood and cones were studied in different cases. The needles and the bark extracts of Pinus Brutia and Pinus Halepensis are rich in polyphenols, proanthocyanidines, flavonoids, and flavonols, showing important reducing properties, while Pinus Nigra gave poor results. In fact both needles, dry and fresh, and bark extracts of Pinus Halepensis and Pinus Brutia could play a beneficial role in many aspects of health. Pinus Halepensis, the most studied type of pine in our laboratory, showed:

On skin, important protection from inflammatory phenomena, cancer, pain and oxidative stress induced by different factors such as cigarette smoke, UV light, X-rays, and organophosphate insecticide. It is to underline that the extracts favors normal skin cell proliferation and showed better results than antioxidants like ascorbic acid and rutin. On diabetes showed significant decrease of glucose levels and protection of diabetic skin. In ageing, provided as food supplement, contributed on significant life extension, and better health status by preventing wound creation, blindness, lowering of cholesterol and triglycerides levels. The whole water extract presented better action than each extract fractions.
Grape pomace extract induced mitochondrial biogenesis in visceral adipose tissue from spontaneously hypertensive rats receiving a high-fat diet and in primary rat adipocytes

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We determined the capacity of malbec grape pomace extract (GPE), containing high amount of polyphenols, to modulate inflammation and proteins related to mitochondrial biogenesis in epididymal visceral adipose tissue (eVAT) from spontaneously hypertensive rats (SHR) receiving a high-fat diet (HFD) and in primary cultures of rat adipocytes. Ten weeks old SHR (n=16) and its control WKY (WistarKyoto) rats (n=16) were divided into three groups: Control group (WKY/SHR; n=4 each); WKY/ SHR-HFD group receiving fat (40% (w/w), n=6 each); and WKY/SHR HFD supplemented with GPE (300 mg/Kg/d, n=6 each) for 10 weeks. HFD leads to increased eVAT in WKY but not in SHR, while the adipocyte diameter was higher in both groups. GPE supplementation decreased the adipocytes size, NOX4 and IL-6 and enhances UCP-1 and PPARγ expression in SHR-HFD. To evaluate the involvement of p38 and ERK MAPKs on UCP-1 activation, the stromal vascular fraction from eVAT were isolated from WKY and SHR and differentiated to adipocytes in presence or absence of SB203580 (p38 inhibitor) or U0126 (ERK inhibitor) (10 mmol/L) and GPE (30 µM). We first confirm that GPE enhance UCP-1 and PPARγ expression in adipocytes from SHR but not from WKY and that p38 and ERK were involved in GPE-induced UCP-1 expression in SHR adipocytes. GPE can stimulates the expression of protein involved in mitochondrial biogenesis and may protects against diet-induced adipose dysfunction supporting the utilization of winemaking residues in human health due to their high amount of bioactive compounds.
In vitro antioxidant and anti-inflammatory activities of Samanchan recipe and its plant ingredients used by Khampramong temple for cancer treatment

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Samanchan recipe consists of ten medicinal plants; Betula alnoides Buch. Ham., Ficus fovealata Wall., Ochna integerrima (Lour.) Merr., Coptosapelta flavescens Korth., Lissea cubeba Pers., Suregada multiflorum (A.Juss) Baill., Bauhinia strychnifolia Craib., Caesalpinia sappan Linn., Andrographis paniculata (Burm.f.) Wall. Ex Nees, Coscinium fenestratum (Gaertn.) Colebr., which has been used to maintain the cancer patients. This study was aimed to evaluate the antioxidant and anti-inflammatory activities of Samanchan recipe and its plant ingredients. The extraction method were maceration by 95% ethanol and decoction with water. All extracts were evaluated their antioxidant activity using DPPH radical scavenging assay and anti-inflammatory activity by determination of inhibitory activity on lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines using Griess reagent. The results found that the aqueous and ethanolic extract of C. sappan and B. strychnifolia and Samanchan recipe showed high antioxidant activity (EC$_{50}$ less than 10 µg/ml). Moreover, the ethanolic extract of C. sappan exhibited the highest nitric oxide inhibition with IC$_{50}$ was 4.26±1.39 µg/ml. And the ethanolic extract of A. paniculata and the aqueous extract of B. strychnifolia exhibited high nitric oxide inhibition (IC$_{50}$ were 19.25±4.77 and 21.93±0.76 µg/ml, respectively). The ethanolic extract of Samanchan recipe exhibited nitric oxide inhibition with IC$_{50}$ was 44.75±4.01 µg/ml but the aqueous extract of Samanchan recipe had no nitric oxide inhibition. This study has confirmed the biological activity that related to cancer of Samanchan recipe.
Investigating the mechanisms of trigeminal nerve pain:
How pH-based interventions affect nerve sensitivity in horses

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Trigeminal nerve pain can cause debilitating pain in horses and is known as headshaking. This is characterized by sudden onset of itching, tingling or electric-like sensations due to sensitivity in the trigeminal nerve and many horses are euthanized due to the pain. Changes in pH related to strong ion content of the diet has been documented in horses and cattle. A randomized controlled trial of intravenous pH manipulation was performed in affected horses to evaluate the influence of pH on trigeminal nerve pain. Affected horses were exposed to treatments of becoming metabolically acidic, basic or given control fluids and headshaking behavior and blood pH values were evaluated. Horses receiving the acidic treatment had no significant effects on headshaking behavior. Horses receiving the basic treatment had a significant reduction in headshaking behavior ($p < 0.01$). This proof of concept allows for further research on dietary manipulations in horses and trigeminal nerve pain, which would improve the lives of many horses.
Consumption of either high-fructose corn syrup or sucrose increases uric acid, postprandial triglycerides, LDL-cholesterol and ApoB, in men and women

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Previous studies from our laboratory have demonstrated that the consumption of fructose-sweetened beverages at 25\% of daily energy requirements (Ereq) reduces insulin sensitivity, increases hepatic de novo lipogenesis (DNL) and elevates LDL-cholesterol and lipoproteins. The two most common fructose-containing sweeteners include: 1) high fructose corn syrup (HFCS), composed of monosaccharides fructose (55\%) and glucose (45\%) and 2) sucrose, composed of equal proportions of fructose and glucose as a disaccharide. Controversy remains whether there are differential effects of these two sources of added sugars in our diet. We studied 75 participants matched for age and BMI in 3 sweetened beverage groups: 1) aspartame, 2) sucrose or 3) HFCS at 25\% of Ereq. After 2 weeks consuming sugar-sweetened beverages, only subjects consuming HFCS-sweetened beverages exhibited significant weight gain compared with aspartame (+0.8 kg, p=0.03). Compared with aspartame, both sucrose and HFCS increased plasma concentrations of uric acid (+0.31 \pm 0.09 mg/dL, p=0.02 and +0.46 \pm 0.09 mg/dL, p=0.0005), postprandial TG (54 \pm 8 mg/dL, p<0.0001 and 37 \pm 7 mg/dL, p=0.003), apolipoprotein B (+6.2 \pm 1.8 mg/dL, 0.04 and +10.1 \pm 1.7 mg/dL, p=0.0003), and plasma LDL (+12.2 \pm 2.6 mg/dL, p=0.002 and +13.4 \pm2.4 mg/dL, p=0.0004), respectively. These results demonstrate that HFCS- or sucrose-sweetened beverages consumed at 25\% of Ereq are equally capable of increasing uric acid and established lipid/lipoprotein risk factors risk for cardiovascular disease in only 2 weeks in young men and women.
Effects of grape consumption on gene expression of inflammatory mediators in peripheral blood mononuclear cells of obese individuals

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Obesity is a pro-inflammatory state involving activation of inflammatory mediators and increased oxidative stress. Polyphenols have been associated with protection against obesity-associated chronic diseases. We investigated whether consumption of polyphenol-rich whole grapes alters expression of genes related to inflammation and oxidative stress in peripheral blood mononuclear cells (PBMC) in obese individuals following 4 weeks of grape consumption and acutely following a high fat, high carbohydrate (HFHC) meal challenge. Using a crossover design, 20 obese subjects (BMI 37.0 ± 9.9) were randomized to consume either 60 g of freeze-dried whole grape powder (Grape; 297 mg polyphenols) or matched placebo powder (Control; 0 mg polyphenols) daily in 500 ml of water for 4 weeks separated by a 2-week washout. PBMC were isolated from fasted blood before and after each intervention. Meal challenge days consisted of a HFHC meal accompanied by Grape or Control consumption with PBMC isolated at time points 0, 1, 3, and 5 hr. Results show no changes in fasting expression of TNFα, NF-κB, IκBα, CREB, IL6 or Nrf2 following 4 weeks of either treatment. Following the HFHC meal, there were no significant differences between treatments, however effects of time were observed. Postprandial NF-κB expression differed at each time point relative to baseline within Grape and Control treatments (p<0.0001). CREB and Nrf2 expression differed at 1 hr and 3 hr relative to baseline following Grape treatment (p<0.05). Results indicate activation of inflammatory and oxidative stress response following HFHC meal, however no differences between Grape and Control treatments were observed.

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Developmental zinc deficiency impairs cortical astrogliogenesis by disrupting the STAT3 signaling pathway

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We previously observed that gestational marginal zinc deficiency (MZD) disrupts the STAT3 signaling pathway in the developing rat brain and in IMR-32 cells. We observed altered STAT3 phosphorylation patterns, low STAT3 nuclear translocation, DNA binding, and transactivating activity. Given that STAT3 is critical for astrogliogenesis, this work investigated the effects of MZD on STAT3 signaling at different stages in brain development and its potential impact on astrogliogenesis. Pregnant rats were fed diets containing 10 (MZD) or 25 mg Zn/g (control) from embryonic day (E) 0 through postnatal day (P) 2, after which all animals were fed the control diet until P56. Gestational MZD impaired cortical STAT3 activation, which coincided with the timing of astrogliogenesis. Low STAT3 phosphorylation in MZD brain was associated with: i) low JAK2 activation; ii) high PTP1B expression and activity; iii) low PTP1B ubiquitination/degradation. MZD also affected STAT3/cytoskeleton interactions and STAT3 acetylation. Consistent with an impaired STAT3 signaling, gestational MZD caused a deregulation of astrogliogenesis. This was evidenced by altered brain cortex patterns of expression of the astrogliogial markers GFAP and S100β. At P56, we observed, by immunohistochemistry, both a lower number of astrocytes in MZD cortex, and a disruption in the migration of astrocyte precursors. In summary, a low Zn availability during early brain development affects astrogliogenesis as a consequence of a disruption in STAT3 signaling. Thus, developmental MZD could alter brain cell number, type (astrocytes) and distribution, potentially causing long lasting effects on brain homeostasis and function. Supported by NIFA-USDA (CA-D*-XXX-7244-H) and PackerWentz Endowment.
Mechanisms underlying gliadin peptides-induced Caco-2 monolayer permeabilization

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Celiac disease (CD) is an autoimmune enteropathy triggered by dietary gluten. Gluten is an alcohol-soluble protein fraction present in cereals such as wheat, rye and barley. A main protein in wheat gluten is gliadin, and the peptides derived from the gastro-intestinal digestion of gliadin are proposed to underlie CD pathophysiology. Gliadin peptides can affect the tight junctions leading to an increased intestinal permeability which triggers local inflammation. The objective of this study was to characterize the signaling pathways involved in gliadin peptides-mediated intestinal permeabilization. Caco-2 cells were differentiated for 12 d into an epithelial cell monolayer. Cells were incubated in the presence of the peptides derived from gliadin trypsin digestion (PTG). Treatment with 1 mg/ml PTG for 6 h caused Caco-2 cell monolayer permeabilization as evidenced by a decrease in transepithelial electrical resistance and increased FITC-dextran transport. The activation of the NF-κB pathway, measured as p65 phosphorylation, was not affected by PTG. On the other hand, PTG treatment caused the activation (phosphorylation) of the mitogen activated kinase ERK1/2 and of the upstream kinase MEK within 1-6 h incubation. PTG did not affect the expression of claudin 2 and myosin light chain kinase (MLCK), two proteins that promote intestinal permeabilization and that are regulated by ERK1/2 and NF-κB. In summary, gliadin peptides could promote intestinal permeability via the activation of the MEK/ERK signaling pathway. Future studies will investigate the possibility that PTG-mediated ERK1/2 activation could lead to MLCK phosphorylation and consequent activation.
(-)-Epicatechin prevents high-fat diet-induced intestinal permeabilization and endotoxemia in mice: a link to insulin sensitivity

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We previously observed that dietary supplementation with the flavan-3-ol (-)-epicatechin (EC) improves insulin sensitivity in rodent models of overnutrition-induced type 2 diabetes. Intestinal permeabilization and the associated endotoxemia are proposed as major contributors to obesity-induced insulin resistance. We currently investigated if the prevention of barrier permeabilization could underlie the capacity of EC to mitigate high fat diet (HF)-induced insulin resistance. Male C57BL/6J mice were fed for 15 w control, or HF diets without or with 2, 10 or 20 mg EC/kg body weight. The consumption of a high fat diet caused obesity, insulin resistance and intestinal permeabilization. This was evidenced by findings of high fasted and fed plasma glucose and insulin levels, impaired responses to ITT and GTT tests, increased permeability to FITC-dextran and endotoxemia. Although EC only partially reduced body weight gain and adiposity, it inhibited HF diet-induced increase in intestinal permeability, and improved insulin sensitivity. Among the groups, fasted glucose levels were positively correlated with intestinal permeability to FITC-dextran (r: 0.89) and endotoxemia (r: 0.86), and as expected, endotoxemia correlated to FITC-dextran permeability (r: 0.80). EC in part acted preventing HF-induced changes in select tight junction proteins. In summary, dietary EC supplementation improved insulin sensitivity in high fat-fed mice. This could in part be due to the prevention of intestinal permeabilization and mitigation of endotoxin-induced fatty liver development. Thus, the above results support the concept that consumption of EC-rich foods can constitute a dietary strategy to mitigate obesity-associated insulin resistance. Supported by NIFA-USDA (CA-D*-xxx-7244-H)
Acute changes in NADPH Oxidase 4 in post-traumatic arthritis

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Knee injuries can initiate changes that lead to post-traumatic osteoarthritis (PTOA). Aside from structural changes, a knee injury initiates acute inflammation and generation of reactive oxygen species (ROS) that potentially cause further harm to the joint. The role of ROS in both normal joint homeostasis and joint injury is poorly understood. NADPH oxidase 4 (Nox4) is a member of a family of proteins whose sole purpose is the generation of ROS, and it plays a pivotal role in normal development and signaling of many cell types in the joint, including osteoclasts, osteoblasts, and chondrocytes. Inhibition of excessive ROS production by Nox4 after joint injury could be protective to the joint, decreasing oxidative stress and the initiation of PTOA. This study used cultured primary human chondrocytes and a non-invasive mouse model to elucidate the role of ROS in PTOA pathogenesis in the acute phase after joint injury. Our results show that there is an acute decrease in Nox4 mRNA, protein, and enzymatic activity within 24 hours after injury followed by a subsequent sustained low level increase for several days, a novel finding not seen in any other system. Inhibition of Nox4 activity by GKT137831 was protective against early structural changes after non-invasive knee injury in a mouse model. We conclude that Nox4 plays a significant role in the acute phase after joint injury, and that targeted inhibition of inflammation caused by Nox4 may be protective against early joint changes in the pathogenesis of PTOA.
Bioavailability and kinetic profile of anthocyanin and chlorogenic acids from wild blueberry in humans

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Diets abundant in phenolic compounds have been linked to decreased risk of cardiovascular disease, obesity and diabetes. Wild blueberries (WBB) are one of the richest sources of dietary phenolic compounds, particularly anthocyanin compounds. They also contain hydroxycinnamic acids, namely chlorogenic acids (CGA). Understanding their metabolic fate may provide insight to their health benefits and protection against chronic disease development. The purpose of this study was to evaluate the bioavailability and biotransformation of WBB anthocyanin and CGA in humans. This is a pilot study with healthy male and female volunteers (n=12). Plasma samples were collected at baseline (t=0h), 0.25h, 0.5h, 1h, 2h, 4h, 6h, 8h, 10h and 24h after consuming a WBB drink (25 g freeze dried WBB powder) with a standard polyphenol free breakfast. The anthocyanins in the WBB drink and their metabolites in human plasma were identified using HPLC-ESI-Q-TOF and quantified by dynamic MRM transitions on HPLC-ESI-QQQ. A total of 30 anthocyanins were identified in the WBB drink. These included glycosides of cyanidin, delphinidin, malvidin, petunidin and peonidin, which were detected in positive mode. Glucose was the most abundant glycoside attachment followed by galactose, arabinose, acetylated glucose, acetylated galactose and xylose. CGAs were also found in the WBB drink in negative mode and confirmed with standards. All the anthocyanins in the WBB drink were found in plasma as well as 3-CGA. The bioavailability for delphinidin, cyanidin, malvidin, peonidin, and petunidin were each less than 1%. The bioavailability for 3-CGA was 0.29% after consuming 25g wild blueberry powder. The Cmax (maximum concentration) of anthocyanins and 3-CGA was ~2h post ingestion. Cmax of phase II metabolites, including peonidin-glucuronide, delphinidin-glucuronide, cyanidin-glucuronide was ~ 8h. These results suggest that anthocyanins and CGAs from a WBB drink can be partly absorbed in their intact form in humans. The relationships between absorption kinetics of WBB anthocyanins and CGAs with indicators of metabolic health are under further investigation.
Comparison of ultracentrifugation and chemical precipitation methods for preparative high density lipoprotein separation

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Multiple methods are used to separate high density lipoprotein (HDL) from plasma, including an array of density-based ultracentrifugation methods, as well as dextran sulfate separation. Ultracentrifugation is the gold standard for lipoprotein isolation and has been used to assess HDL for decades. However, it is thought that the high shear stress and high salt concentrations in density ultracentrifugation based methods may lead to the loss of specific molecular species in HDL. Moreover, none of the current methods have been optimized and validated for subsequent omic analysis. In this study, several HDL separation methods were optimized and validated, including two sequential flotation ultracentrifugation methods, dextran sulfate and polyethylene glycol precipitation, and fast protein liquid chromatography. These methods were compared using a standard pooled HDL sample and analyses were completed in triplicate. ApoA-I, albumin, and cholesterol were measured in the separated HDL. Our results show that there were differences between methods in the content of these HDL constituents, indicating that the specific HDL isolation protocol influences the compositional analysis of HDL. Proteomic, lipidomic, and glycomic analyses are under way.
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