

H&N 1: Omega-3 Fatty Acids: Brain Health and Function

Chairs: R. Sharpee, NOW Foods, USA; and R. Watson, University of Arizona, USA

Overview of Omega-3 Fatty Acids and Health. F. De Meester, DMF Ltd. Co., Marche, Belgium.

Essential fatty acids fit the modern paradigm of being dangerous in the wrong amounts and types, while being health promoting otherwise. The involvement of EPA and DHA in inflammation and cognition is firmly established. Attempts at defining their appropriate daily intakes are being made for the current standards appear all but in-appropriate for maintaining brain health. Much of aging humanity with limited fish or supplemental omega-3 fatty acids are at greater risk. The book reviews functional and structural changes that are concomitant with the apparent deprivation – either through low intake of omega-3 or through high intake of omega-6 – of EPA and DHA in modern human diets. The neurological consequences as described at psychological and psychiatric levels are, for some cognitive dysfunction conditions impressive. For others they are less conclusive but current research and understanding is provided to define apparent or potential effects. Clearly with a worldwide aging population understanding of a dietary supplement, omega-3 fatty acids, that can affect or ameliorate some of the mental dysfunctions is critical. It is good to see the industry involved in the reasoning because no long term solution to the ramping – not to say acute – threat can reasonably be envisaged and cast into stone without the economic engine to provide omega-3 fatty acids for those that do not eat much fish.

n-3 Fatty Acid-derived Lipid Mediators Against Neurological Oxidative Stress. A.A. Farooqui, Ohio State University, Columbus, OH, USA.

In brain DHA is mainly esterified with ethanolamine plasmalogen and phosphatidylserine. From PlsEtn, it is released by Ca²⁺-independent PlsEtn-selective PLA₂. The breakdown of DHA by 15-LOX-like enzyme results in the synthesis of D-series resolvins. 15-LOX also oxidizes DHA into protectin D₁ through the formation of epoxide intermediate at the 16(17) position. The occurrence of PD₁ has also been reported in brain, where it is called as neuroprotectin D₁. These lipid mediators not only antagonize the effects of PGs, LTs, and TXs, but also modulate leukocyte trafficking and down-regulate the expression of cytokines in glial cells. Resolvins, neuroprotectins, and maresins regulate immune

systems by modulating signal transduction processes associated with neuroinflammation and neurodegeneration. Like DHA, oxidation of EPA either by acetylated COX-2 or via a cytochrome P450 pathway results in the generation of E-series resolvins. DHA and EPA-derived metabolites have potent anti-inflammatory and proresolution properties. They retard excessive inflammatory responses and promote resolution by enhancing clearance of apoptotic cells and debris from inflamed brain tissue. These actions may underlie the beneficial effects of EPA and DHA in human health and neurotraumatic and neurodegenerative diseases.

The Effects of Omega-3 Polyunsaturated Fatty Acids on Maternal and Child Mental Health. M.P. Judge, A.F. Diallo, and C.T. Beck, University of Connecticut School of Nursing, Storrs, CT, USA.

Mental health disorders pose a national health issue for women, children and adolescents resulting adverse financial, developmental and family health outcomes. Essential omega 3 fatty acids, particularly docosahexaenoic acid (DHA, 22:6, n-3), play critical roles in building and maintaining a healthy central nervous system affecting cognition and mood regulation. Given these roles, omega-3 fatty acids are being investigated as a complementary or alternative approach in the treatment of mental health disorders in maternal and child health. Of particular interest is the state of the literature as it relates to the role of omega-3 fatty acids in alleviating symptoms of postpartum depression, attention-deficit hyperactivity disorder, childhood depression, and autistic spectrum disorder. Links between omega-3 fatty acid and related psychopathologies affecting maternal and child health were reviewed. The literature points to a need for an expansion of our current knowledge of the role of omega-3 as an alternative or adjunctive treatment in this population for these disorders. Further, given poor general intake, increased efforts are necessary in maternal and child health to ensure adequate intake of omega-3 in this population.

Fatty Acid Levels and the Aging Brain. Z. Tan, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA.

The human brain undergoes structural, chemical

and cognitive changes during the aging process. Fatty acids are essential for maintaining the brain's structural integrity and physiologic functions. Several studies have suggested that the omega-3 polyunsaturated fatty acids (PUFAs) docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) have protective effects against age-related neurological pathology. Dementia is a highly prevalent age-related brain disease and recent research supports the role of omega-3 PUFAs in preventing or slowing the pathological cascade of this disease. Regular consumption of omega-3 fatty acids through its main dietary source, fatty fish, shows promise as an intervention to help optimize brain aging, though further research will be necessary to confirm its effectiveness as well as clarify recommend forms, amounts and duration of intake.

Imaging Brain DHA Metabolism *in vivo*, in Animals and Humans. S.I. Rapoport, National Institute on Aging, Bethesda, MD, USA.

Docosahexaenoic acid (DHA, 22:6n-3) is a precursor of antiinflammatory resolvins and neuroprotectins and a second messenger in neurotransmission and other brain processes. The rate of incorporation of unesterified circulating DHA into brain phospholipids from plasma, J_{in} , equals the rate of metabolic DHA loss within brain, and is unchanged by changes in cerebral blood flow (CBF). It can be imaged with quantitative autoradiography in unanesthetized rodents or positron emission tomography (PET) in humans, using intravenous [1- 14 C]DHA or [1- 11 C]DHA, respectively. Rodent drug and knockout studies indicate that resting state and drug activated DHA incorporation do not depend on extracellular Ca^{2+} but are mediated by Ca^{2+} -independent phospholipase A_2 (iPLA $_2$); lacking this enzyme (type IVA) leads to synaptic loss and motor disability in mice. PET imaging in humans shows higher DHA consumption in gray than white matter, and a normal brain DHA consumption rate of 3.8

mg/day, but elevated DHA incorporation and CBF following alcohol withdrawal in chronic alcoholics. In summary, quantitative regional imaging of brain DHA metabolism at rest or during functional or pharmacological activation, can "biomark" specific roles for DHA in brain structure and function in health and disease.

Omega-3 Fatty Acids and Cognitive Behavior. G.E. Giles and R.B. Kanarek, Tufts University, Medford, MA, USA.

The majority of research into cognitive effects of *n*-3 PUFA supplementation has focused at either end of the lifespan: in infants and older adults. Multiple studies have found positive effects of maternal and formula supplementation on infant cognitive development, particularly in problem solving, memory and language development, generally suggesting that although *n*-3 PUFA supplementation may not influence global cognitive development among infants, it may aid particular cognitive functions. The opposite may be true in older adulthood, as higher *n*-3 PUFA intake and plasma levels are associated with reduced overall cognitive decline, but less so for specific cognitive domains. Epidemiological results are supported by some RCTs showing that *n*-3 PUFA supplementation reverses age-related cognitive decline, more so in otherwise healthy individuals than in those with mild cognitive impairment and Alzheimer's disease. Research in young adults remains fairly limited, and although some data suggest positive effects of *n*-3 PUFA supplementation on mood and executive function, other studies have failed to replicate these effects. We will discuss extant evidence assessing *n*-3 PUFA and cognition throughout the lifespan, with a particular focus on young adults, including recent results from our lab looking at how *n*-3 PUFA and stress interact in their cognitive and emotional outcomes.

H&N 2/ EAT 2: Digestive Processing: Lipid Structure and Metabolism

Chairs: A. Wright, University of Guelph, Canada; and M.C. Michalski, INSA Lyon, France

Impact of the Food Matrix on Dietary Fat Digestion and Absorption. M.C. Michalski, CarMeN laboratory, INRA USC1362, INSERM U1060, Villeurbanne, France.

Dietary fats and oils present various fatty acid compositions that are widely studied regarding their nutritional impact. However, these fatty acids are organized into various lipid molecules, which can exist in different food products under several types of supramolecular structures such as emulsion droplets. This presentation will review current knowledge on the impact of lipid structures and the food matrix on lipid hydrolysis in the digestive tract and subsequent intestinal absorption and postprandial metabolism. A specific focus will concern emulsified structures and dairy products, which have been the subjects of great advances in the last years.

Membrane CD36 and Fatty Acid Signaling Coordinate Fat Absorption and Utilization. N.A. Abumrad, Washington University School of Medicine, St. Louis, MO, USA.

The role of CD36 in cellular fatty acid (FA) uptake, which was identified in 1993, is now supported by strong evidence generated in CD36 deficient rodents and humans. Common polymorphisms in the CD36 gene have been linked to alterations in plasma lipids (fatty acids, triglycerides, cholesterol), to risk of metabolic syndrome and stroke. CD36 functions in high affinity cellular uptake of long chain FA and under excess fat supply CD36 can contribute to lipid accumulation and metabolic pathology. Recent evidence supports the view that CD36 FA uptake and signaling coordinate cellular fat utilization. This view is based on newly identified CD36 actions that involve gustatory fat perception, intestinal fat absorption, secretion of the peptides cholecystokinin and secretin, hepatic lipoprotein output and the production of the FA derived bioactive eicosanoids. Thus abnormalities of fat metabolism and the associated pathology might involve dysfunction of CD36-mediated signal transduction in addition to changes of FA uptake.

In-mouth Mechanism Leading to the Perception of Fat and Its Consequence on Post-prandial Lipid Metabolism in Humans: The Particular Role of Saliva. G. Feron⁵, C. Vors^{1,4}, E. Guichard⁵, and M.C. Michalski^{2,3}, ¹Université de Lyon 1, Villeurbanne, France, ²INRA, U1362 Laboratoire CarMeN, Villeurbanne, France, ³INSA-Lyon, IMBL, Villeurbanne, France, ⁴CRNH-RA and CENS, Pierre-Bénite, France, ⁵INRA, UMR1324 Centre des Sciences du Goût et de l'Alimentation, Dijon, France.

In humans, the in-mouth perception of dietary fat in food is a complex process involving many sensory modalities (texture, aroma, taste and trigeminal). During food oral processing, a bolus is formed in which saliva is significantly incorporated thus contributing significantly to the perception of fat. In particular, it has been shown that the levels of some salivary characteristics (lipolysis, lysozyme, flux, antioxidant status, level of carbonic anhydrase and proteins) are related to the sensitivity of the individual to triolein and oleic acid. Interestingly, the involvement of some of these salivary variables in the perceived intensity and preference towards model oil emulsions was also shown. In addition to fat perception and preference, recent results show an indirect relation between these salivary components, BMI and post-prandial lipid metabolism in human subjects. It is hypothesized that this relationship should be linked to a difference in fat sensitivity and liking according to the BMI. The role of saliva as a key factor in fat perception in one side and post-prandial lipid metabolism in another side is discussed.

Enhancement of Palm-oil Derived γ -Tocotrienol Intestinal Uptake and Oral Bioavailability. S. Alqahtani¹, B. Abuasal², A. Alayoubi¹, S. Nazzal¹, P.W. Sylvester¹, and A. Kaddoumi¹, ¹University of Louisiana at Monroe, Monroe, LA, USA, ²US Food and Drug Administration, Silver Spring, MD, USA.

γ -Tocotrienol (γ -T3) is one form of naturally occurring vitamin E present in palm oil that provide significant health benefits, including anticancer and anticholesterolemic activity, besides acting as a potent antioxidant. γ -T3 is a lipophilic compound with low oral bioavailability. The objectives of this work were to evaluate γ -T3 intestinal uptake mechanism and to improve its bioavailability. *In situ* rat intestinal perfusion studies showed that γ -T3

intestinal uptake is a saturable carrier-mediated process and its intestinal uptake is mediated by Niemann-Pick C1-like 1 (NPC1L1) transporter. Thus, to overcome the effect of this saturable process we have hypothesized that enhancing γ -T3 passive permeability would increase its oral bioavailability. Solid lipid nanoparticles and self-emulsifying drug delivery systems were tested as the delivery systems of choice to enhance γ -T3 permeability and bioavailability. *In vitro*, *in situ* and *in vivo* studies demonstrated both delivery systems to significantly enhance the permeability and relative oral bioavailability of γ -T3 when compared to γ -T3 prepared as mixed micelles as control. In conclusion, our results showed for the first time that γ -T3 intestinal absorption is partly mediated by NPC1L1 and we successfully were able to improve its bioavailability using two different delivery systems.

e-Polylysine Decreases Micellar Lipids Solubility and Enhances the Fecal Lipids Excretion in Rats. R.

Hosomi¹, D. Yamamoto¹, T. Nishiyama², M. Yoshida¹, and K. Fukunaga¹, ¹Dept. of Life Science and Biotechnology, Faculty of Chemistry, Materials and Bioengineering, Kansai University, Suita, Osaka, Japan, ²Dept. of Public Health, Kansai Medical University, Hirakata, Osaka, Japan.

e-Polylysine (EPL) has been used as food preservation substrate and has anti-microbial and anti-viral activities. In addition, EPL has also the hypotriglyceridemic effect through the inhibition of pancreatic lipase activity. In present study, we demonstrated that the effect of EPL on lipid absorption *in vivo* and micellar lipids solubility *in vitro*. Groups of male Wistar rats were fed AIN93G diet containing 1% EPL or 1% L-lysine. After 4 weeks of feeding EPL diet, markedly decreased serum cholesterol (CHOL) and triacylglycerol levels in partly due to enhanced fecal excretions of fatty acids (FAs), CHOL and bile acids (BAs) compared with AIN93G and L-lysine diets. To elucidate the mechanism of enhancing fecal lipid excretions by fed EPL, effect of lipid absorption was evaluated using *in vitro* gastrointestinal digestion model. Lipase activity, micellar solubility of lipids were significantly lower and BAs binding activity was higher in the presence of EPL digests compared with casein digests and L-lysine. These results suggest that the hypolipidemic effect of EPL is mediated by increased fecal FAs, CHOL, BAs excretions, which is due to the digestion products of EPL having reduced lipase activity and micellar solubility of lipids, and increased BAs

binding capacity.

Digestion, Absorption, and Potential Toxicity of Edible Nanoemulsions. D.J. McClements, Dept. of

Food Science, University of Massachusetts, Amherst, MA, USA.

Edible nanoemulsions are increasingly being used within the food industry to encapsulate and deliver lipophilic functional agents, such as oil-soluble colors, flavors, preservatives, vitamins, and nutraceuticals. Nanoemulsions are thermodynamically unstable systems that contain oil droplets with radius < 100 nm. Nanoemulsions have some advantages over conventional emulsions for certain food and beverage applications: higher optical transparency; greater physical stability; and, higher oral bioavailability of encapsulated lipophilic agents. However, there are also some potential risks associated with reducing the size of the lipid droplets in nanoemulsions that should be considered before they are widely utilized: alterations in the fate of bioactive agents within the gastrointestinal tract; potential toxicity of some of the ingredients used in their fabrication. This presentation provides an overview of the current status of our understanding of the biological fate and potential toxicity of edible nanoemulsions suitable for used in the food and beverage industry.

Influence of Emulsifier Structure on Lipid

Bioaccessibility in Oil-water Nanoemulsions. M.

Rogers, Y. Lan, and A. Speranza, Rutgers University, New Brunswick, NJ, USA.

The influence of several nonionic surfactants (Tween-20, Tween-40, Tween-60, Span-20, Span-60, or Span-80) and anionic surfactants (sodium lauryl sulfate, sodium stearyl lactylate, and sodium stearyl fumarate) showed drastic differences in the rank order of lipase activity/lipid bioaccessibility. The biophysical composition of the oil and water interface has a clear impact on the bioaccessibility of fatty acids (FA) by altering the interactions of lipase at the oil-water interface. It was found that the bioaccessibility was positively correlated with the hydrophilic/lipophilic balance (HLB) of the surfactant and inversely correlated to the surfactant aliphatic chain length. Furthermore, the induction time in the jejunum increased as the HLB value increased and decreased with increasing aliphatic chain length. The rate of lipolysis slowed in the jejunum with increasing HLB and with increasing aliphatic chain length.

H&N 2.1: Sterols

Chairs: R.A. Moreau, USDA, ARS, ERRC, USA; W.D. Nes, Texas Tech University, USA; and E.J. Parish, Auburn University, USA

Using Sterols to Probe the Structure and Function of Membrane Organization. E. London (*Schroepfer Medal Award Winner*), Stony Brook University, Stony Brook, NY, USA.

Eukaryotic plasma membranes are believed to contain sphingolipid and sterol rich ordered domains ("lipid rafts") which co-exist with disordered domains rich in unsaturated phospholipids. Using lipid vesicles (liposomes) the role of sterols in organizing membrane domains was investigated. Sterol structures were found to vary between strongly promoting and strongly inhibiting ordered domain formation. Variation in sterol domain forming ability along the cholesterol biosynthetic pathway was also found, and may have implications for cholesterol biosynthesis diseases. To refine studies of membrane domains we developed methods to prepare sterol-containing liposomes which, like natural membranes, have lipid asymmetry (a difference in inner and outer leaflet lipid composition). They are being used to study membrane domain formation in vesicles closely imitating natural membranes. Membrane domains in cells have also been studied. To study membrane domains in the sterol-containing bacterium *Borrelia burgdorferi*, which causes Lyme disease, we used a sterol substitution approach. It was found that a sterol having the ability to support ordered domain formation in liposomes was necessary and sufficient for it to support ordered domain formation and maintain membrane integrity in *B. burgdorferi*. Extending this approach may help define the formation and function of lipid domains in eukaryotes.

Steryl Glycosides and UGT80 Enzymes in the Seed Coat Mucilage. K. Schrick, Kansas State University, Manhattan, KS, USA.

Sterol compounds and their derivatives are ubiquitous to animals, plants and fungi. Sugar-conjugated sterols called steryl glycosides are especially widespread among plants, yet little is known about their specific biological functions. Steryl glycosides are synthesized by membrane-bound UDP-glucose:sterol glucosyltransferase 80 (UGT80) enzymes encoded by the *UGT80A2* and *UGT80B1* genes in *Arabidopsis*. Based on *in vitro* enzyme activity assays and steryl glycoside

compositional analysis of mutants, these enzymes appear to have overlapping but also distinct roles in steryl glycoside production. Intriguingly, only *ugt80B1* and not *ugt80A2* mutants display striking defects in the seed, including abnormal morphology of cellulosic rays within the pectin-rich seed coat mucilage, a specialized cell wall. Mucilage produced by various plant tissues is edible and harbors medicinal properties as a demulcent, in addition to serving as an ingredient of glue when mixed with water. Several genes that are required for mucilage production in seeds have been identified from *Arabidopsis* including the *CESA5* cellulose synthase gene and the *SOS5* gene that codes for an arabinogalactan protein implicated in cell adhesion. Using a combination of biochemical and molecular genetic approaches, the mechanistic relationship between steryl glycosides and these other mucilage components is being further investigated.

The Major Cycloartenols of *Sutherlandia frutescens* (L) R.Br.: Possible Roles *in planta* and in Human Health. W. Folk¹, C. Thomas², and W.D. Nes², ¹University of Missouri, Columbia, MO, USA, ²Texas Tech University, Lubbock, TX, USA.

Sutherlandia frutescens is widely used in traditional and contemporary medicines in subSaharan Africa for treatment of stress, fatigue, cancer, diabetes, stomach ailments and a variety of infections, including HIV. Vegetative parts obtained from wildcrafted and commercially cultivated plants are consumed in teas and capsules, and contain significant levels of cycloartane glycosides which may be responsible for the claimed health benefits. Clinical trials of the consumption of *S. frutescens* by healthy and HIV+ adults at early stage disease, as well as ongoing studies in cell culture and animal models are providing insights into biological targets for the secondary metabolites. These studies are informed by understanding of the roles, biosynthesis and deposition of the cycloartenols *in planta*, about which new and novel insights will be provided.

On the Regulation of Isoprenoid Biosynthesis in Plant Systems. T.J. Bach, IBMP, CNRS UPR 2357, Université de Strasbourg, Strasbourg, France.

We elucidate regulatory interactions of compartmentalized pathways that lead to the

production of universal isoprenoid precursors IPP and its isomer DMAPP, with one focus being on phytosterol biosynthesis, another one on protein isoprenylation, for which special test systems need to be established. These are based on transformed tobacco BY-2 cells, but also on the usage of Arabidopsis mutants. In this way we have checked the regulation of the rate-limiting enzyme in the cytoplasmic mevalonic acid (MVA) pathway, HMG-CoA reductase. It is apparently feedback-regulated by an intermediate in the phytosterol pathway as revealed by inhibitor studies and amiRNA approaches. New observations argue also for some implication of a geranylgeranylated protein, for instance in the elicitation of MVA-dependent sesquiterpenoid biosynthesis. The geranylgeranyl residue stems from the plastidial methylerythritol phosphate (MEP) pathway and might constitute a link through which the two pathways conspire, besides the transport of building blocks. The problems of silencing genes implied in phytosterol biosynthesis by amiRNA can now be explained by the dependence of the machinery for RNAi formation on the MVA/sterol pathway. Thus available inhibitors like mevinolin and others still yield better results.

Plant Sterol Biology, Specificities, and Functions. C. Villette¹, V. Compagnon¹, B. Grausem¹, E. Forestier¹, T.J. Bach¹, E. Gas-Pascual^{1,2}, A. Berna¹, and H. Schaller*¹, ¹Plant Metabolic Networks, Institut de Biologie Moléculaire des Plantes du CNRS and Université de Strasbourg, Institut de Botanique, Strasbourg, France, ²Dept. of Horticulture and Crop Science, Ohio State University, Wooster, OH, USA.

Plant cells use a sterol pathway that is peculiar compared with that of other eukaryotes. They transform 2,3-oxidosqualene into 9b,19-cyclopropylsterols which are then further converted into sterols and brassinosteroids, and possibly other steroid derivatives according to family, genus, or species specificities. In addition, the capability of tracheophytes to produce 24-alkyl sterols has been assigned to unique botanical evolutionary features. The physiological significance of lanosterol synthase in plants remains however an open question. Contrasting with some of the biosynthetic features mentioned above, sterol catabolism in plant recruits components that are highly similar to those at play in animal systems. The large array of biological functions that maybe fulfilled by particular sterols (and described at many places), ranging from innate immunity and biotic interactions to development, requires a thorough examination of chemical

phenotypes down to the organ level. Indeed, analysis of biosynthetic mutants in a restricted number of organs or cells may shed new light on functional aspects. Here we present data to understand the biological functions of cycloartenol derivatives, which have only been considered until recently as biosynthetic intermediates.

Novel Sterol Biosynthesis Inhibitors as Anti-trypanosomal Agents. D.J. Leaver and W.D. Nes, Texas Tech University, Lubbock, TX, USA.

Trypanosomes are the causative agents of several wide-spread neglected diseases in Africa and Central and South America, with Chagas disease creeping into the southern United States as the latest parasite threat. The current chemotherapy of the human trypanosomiasis relies on only six drugs, five of which were developed > 30 years ago. In addition, these drugs display undesirable toxic side effects and the emergence of drug-resistant trypanosomes has been reported. Recently, inhibitors of ergosterol biosynthesis in *Trypanosoma brucei* and *Trypanosoma cruzi* have been tested against these organisms. Both substrate-based and non-substrate based inhibitors show high specific activity and in some cases curative results. In this talk, we present an overview of recent progress in identifying novel lead compounds targeted to inhibit essential enzymes of the ergosterol biosynthesis pathway of trypanosomes. Particular emphasis is placed on mechanism-based inactivators showing promising and selective anti-trypanosomal activity.

Interesting Effect of Phytosterol Structure on Antioxidant Activities of Phytosteryl Ferulates. J. Moser, H.S. Hwang, and E. Bakota, USDA, ARS, NCAUR, Peoria, IL, USA.

In nature, phytosteryl ferulates usually occur as mixtures where the profiles of the phytosterols are influenced by the cereal source. When evaluating phytosteryl ferulates from corn and rice as potential antioxidants for frying, we found that phytosteryl ferulates from corn were superior to those from rice. To understand why, steryl ferulates were synthesized from pure commercial sterols with different structural features, including saturated sterols, double bond at the 5C-position, and the 4,4'-dimethyl group common to rice phytosteryl ferulates. The antioxidant activities of these synthetic steryl ferulates were compared to rice phytosteryl ferulates by several methodologies, including by *in vitro* antioxidant assay, by oxidative stability index (OSI) of soybean oil, and by analysis of

antioxidant activity during frying. Synthetic steryl ferulates and oryzanol did not differ much in antioxidant activity *in vitro* or by OSI. However, in their evaluation as frying oil antioxidants, steryl ferulates with a saturated sterol group had the best antioxidant activity, followed by sterols with one double bond in the C5 position. The results indicate that a dimethyl group at C4 as well as a C9, C19-cyclopropane group, as found in rice steryl ferulates, negatively affects antioxidant activity in frying oils.

Sterol Profiles in Plant Foods Revisited. L. Nyström, ETH Zurich, Zurich, Switzerland.

Sterol contents and profiles in foods are commonly analyzed with methods that include alkaline hydrolysis (saponification) and possibly acid hydrolysis. This procedure liberates sterols from their conjugates and allows their easy separation from saponifiable lipids that could otherwise interfere with the analysis. This analytical scheme is

applied in the official methods for sterol analysis, and most of the literature available on sterol contents and profiles in plants are obtained with such procedures. However, this approach often leads to either underestimation of sterols, if acid hydrolysis is not included, or on the other hand, to a falsified sterol profile with several artifacts, as the acid treatment leads to an isomerization or degradation of labile sterols. In extreme cases the underestimation of sterol content may be more than 50% (when acid hydrolysis is not done for samples rich in glycosylated sterols), or over 50% of the sterols may be misidentified (if acid hydrolysis is done on a sample rich in labile sterols). To enable accurate estimations of dietary intakes of sterols, renewed analysis of sterol contents and profiles in selected plant foods with other techniques is of vital importance, either via softer (enzymatic) sample pretreatment or direct analysis of the intact conjugates.

H&N 3: Evidence-Based Claims for Food vs. Drugs

Chairs: R. Ward, Utah State University, USA; and K.J. Hintze, Utah State University, USA

Research Designs for Understanding Unique Health Benefits of Whole Foods and Food Bioactives. P.M. Kris-Etherton (*Ralph Holman Lifetime Achievement Award Winner*), Dept. of Nutritional Sciences, Penn State University, University Park, PA, USA.

Dietary guidance for health has transitioned from nutrient-targeted to food-based recommendations. The 2013 AHA/ACC Guideline on Lifestyle Management to Reduce CVD Risk recommends a “dietary pattern that emphasizes intake of vegetables, fruits, and whole grains; includes low-fat dairy products, poultry, fish, legumes, non-tropical vegetable oils, and nuts; and limits intake of sweets, sugar-sweetened beverages and red meats”. Research is needed to increase our understanding of the unique health benefits of individual foods within food groups to further evolve dietary recommendations. This presentation will briefly describe clinical nutrition studies we have conducted that evaluated unique effects of specific foods. In one study we evaluated the effects of a whole food by comparing the isocaloric substitution of 1.5 oz of almonds per day with a banana muffin on CVD risk factors. We also have evaluated the effects of bioactives on CVD risk markers by comparing an avocado diet (one per day) with an isocaloric macronutrient controlled diet. This study demonstrated benefits of avocado bioactives beyond the favorable fatty acid profile of the diet. We also studied the components of a whole food by evaluating the postprandial effects of walnut components (the skin, nut meat, oil and whole walnut) on various CVD risk markers to identify the component(s) that may explain the beneficial effects. Lastly, we evaluated the role of a specific bioactive in a weight loss study that compared whole grains versus refined grains. In this study, we observed an inverse relationship with plasma alkylresorcinols (a measure of whole grain intake) and a loss in visceral adipose tissue. The use of a variety of research designs in clinical nutrition research provides a powerful approach to better understand the role of whole foods and their bioactives on health. This information is important for evolving future dietary recommendations.

Optimizing Rodent Models for Dietary Bioactives and Chronic Disease. K.J. Hintze, R.E. Ward, A.D. Benninghoff, and M. Lefevre, Utah State University, Logan, UT, USA.

Rodent studies that test the effects of bioactive dietary components on chronic disease generally use purified basal diets that have nutrient profiles optimized for growth and fertility such as the AIN93-G diet. However, these diets are not reflective of typical American macro and micronutrient intake. Previously, we used NHANES data to formulate the Total Western Diet (TWD), a rodent diet that emulates average American intake levels for macro and micronutrients using nutrient density that increases basal colorectal cancer by ~2-fold compared to the AIN93-G diet. However, the TWD is comprised of purified ingredients and does not recapitulate the complex food matrix consumed by Americans. Using the latest version of the USDA Food Intakes Converted to Retail Commodities Database, we modified the TWD by using the most commonly consumed whole foods as ingredients. The TWD2 has the same micro and macronutrient content as the previous TWD but contains 25 ingredients. Moreover, we have developed a generalizable method to humanize non germ-free mice with human microbiota using antibiotic treatment and human fecal transfer. The TWD2 coupled with humanized mouse models will help narrow the gap between human nutrition and rodent models as the food matrix, secondary compounds, cooking byproducts and microbiota have all been shown to influence chronic disease risk.

Physiological Functions of Coffee Polyphenols. A. Shimotoyodome, Kao Corporation, Haga-gun, Tochigi, Japan.

Coffee is one of the most popular beverages throughout the world that have been consumed for thousands of years for their attractive flavors and physiological effects. Many studies have demonstrated a relationship between the consumption of coffee and their potential health benefits which might be linked to their polyphenol content. Chlorogenic acid (a caffeic acid ester of quinic acid) is the most abundant polyphenol in coffee. A single cup of coffee contains 70–350 mg of chlorogenic acids. A series of our studies in animals

and humans demonstrated that dietary coffee polyphenols exhibit the beneficial effects in regulating vascular endothelial function and fat metabolism. This presentation will summarize the evidences which show the inhibitory effects of dietary coffee polyphenols on 1) hypertension and 2) obesity, and the molecular mechanisms. Our human studies showed that daily intake of approximately 300 mg coffee polyphenols either improved blood pressure or reduced body fat and weight in Japanese healthy subjects. These studies suggest that daily consumption of coffee polyphenols might prevent the development of metabolic syndrome.

Metabolic Adjustments of Normal and Overweight Subjects During Overfeeding Revealed by Metabolomics.

J.L. Sebedio^{1,2}, B. Morio¹, J.F. Martin^{1,2}, E. Chanseume¹, M. Alligier^{4,5}, C. Junot³, B. Lyan^{1,2}, Y. Boirie¹, H. Vidal^{4,5}, M. Laville^{4,5}, B. Comte¹, and E. Pujos-Guillot^{1,2}, ¹INRA, UMR 1019, UNH, CRNH Auvergne, Clermont-Ferrand, France, ²INRA, UMR 1019, Plateforme d'Exploration du Métabolisme, UNH, Clermont-Ferrand, France, ³CEA-LEMM, Gif-sur-Yvette cedex, France, ⁴Institut National de la Santé et de la Recherche Médicale Unit 1060, CarMeN Laboratory and Centre Européen Nutrition Santé, Lyon 1 University, Oullins, France, ⁵Centre de Recherche en Nutrition Humaine (CRNH) Rhône-Alpes, Centre Hospitalier Lyon-Sud, Pierre Bénite, France.

The purpose of this work was to compare early changes in metabolic status of male overweight (OW) and lean subjects (NW) during a moderate weight gain.

19 NW and 19 OW subjects were submitted to a lipid-enriched overfeeding protocol. Metabolic explorations, as well as plasma and urine metabolomic profiles acquired using UPLC-MS, were determined along 8 weeks to compare metabolic trajectories and identify early changes in metabolic processes.

Urinary metabolomic profiles evidenced differences in metabolic trajectories between groups, characterized by an increase over time of short-, medium-chain acylcarnitines, and bile acids in OW. For most of the anthropometric, metabolic parameters and plasma metabolomics data, the two phenotypes were discriminated but the time-course evolution of all subjects was similar. Plasma abundances of unsaturated lysophosphosphatidylcholines decreased over time more importantly in NW while those of the saturated isomers increased in both groups.

These findings not evidenced with classical parameters, indicate a differential response to overfeeding in urine metabolomes, suggesting different nutrient metabolic fate with weight status. Subtle metabolic changes, mostly related to inflammation and difference in β -oxidation indicate a lower metabolic flexibility of OW subjects facing weight gain.

Flaxseed: Health Benefits and a New Health Claim in Canada. K.C. Fitzpatrick, Flax Council of Canada, Winnipeg, MB, Canada.

Flaxseed contains high levels of the omega 3 fatty acid, linolenic acid (ALA) which may be converted to longer-chain (n-3) PUFA, such as eicosapentaenoic acid (EPA) and possibly docosahexaenoic acid (DHA). The efficiency with which this conversion occurs and the factors that may modify it could have important public health implications. ALA is the most commonly consumed n-3 fatty acid in the typical Western diet. Flaxseed is unique among oilseeds because it contains an exceptionally high concentration of phytoestrogenic compounds known as lignans, compounds which possess antioxidant activity. Flaxseed contains other antioxidants including phenolic acids, anthocyanin pigments, several flavonols and flavones, and phytic acid. Flaxseed and other oilseeds contain various antioxidants including phenolic acids, anthocyanin pigments, several flavonols and flavones, and phytic acid which protect the oil in the germinating seed. Proper processing is required to ensure that antioxidants remain viable. The seed is also high in dietary fibre (approximately 28% of its composition,) the principle of which soluble fibre found primarily in the form of mucilage. In October 2013, Health Canada's Food Directorate approved a health claim for flaxseed and serum cholesterol lowering – the only tenth allowed. Such a claim will open opportunities for food manufacturers.

Regulatory Roadblocks to Probiotic Functional Foods and Supplements. M.E. Sanders, Dairy & Food Culture Technologies, Centennial, CO, USA.

The successful development and marketing of probiotic supplements, conventional foods or medical foods depends on: (1) providing consumers safe and efficacious products, (2) conducting well-controlled research documenting efficacy, and (3) having a clear means to communicate product benefits to consumers and healthcare providers. Significant regulatory roadblocks exist to these pursuits, especially for conducting research and

communicating benefits. The roadblocks are different in the United States and the Europe Union. At a time when evidence of probiotic efficacy is revealed in numerous systematic reviews and exciting new relationships between probiotics and health endpoints and emerging, regulatory constraints seem to be the biggest hindrance. Waving the flag of consumer safety and protection, and constrained by inadequate legislation, regulators prevent consumers from getting useful information on probiotics and obstruct research needed to advance the field.

Challenges of Evidence-based Food Guidance. J. Slavin, University of Minnesota, St. Paul, MN, USA.

The search for the holy grail of diets, what we should and should not eat and in what quantities, continues to be debated. Diet wars are waged by the low-carbohydrate and low-fat camps. Yet, one must remember that human are omnivores with gastrointestinal tracts most similar to pigs. Thus, humans can adapt to a wide range of diets and food intakes. Nutritional needs vary greatly through the life cycle so a diet that promotes healthy growth and development for a child may not be optimal for the elderly. This talk will describe the evidence-based procedures used in the Dietary Guidelines for American, including the procedure to evaluate the strength of the relationship between food intake and disease outcomes. Unfortunately, most food and disease relationship are not particularly strong, leading to disappointed consumers who adopt diets with the hope of prevention of disease. Generally, a balanced diet contains adequate protein (both in terms of quantity and quality) alongside sufficient amounts of essential vitamins and minerals. Such diets can be either high or low in carbohydrates and fats, and the choice will reflect the cultural norms and traditions of consumers.

How the US Food and Drug Administration Evaluates the Scientific Evidence for Health Claims.

K. Ellwood, College of Southern Maryland, La Plata, MD, USA.

Health claims were first authorized through the Nutrition Labeling and Education Act of 1990. Health claims describe the relationship between a substance (food or component of food) and a disease or health-related condition. There are two types of health claims, authorized health claims and qualified health claims. In January 2009, the Food and Drug Administration (FDA) issued a guidance document entitled "Evidence-Based Review System for the Scientific Evaluation of Health Claims." The process used by the FDA to review the scientific evidence for health claims and qualified health claims will be described.

Research Evidence to Support Claims of Efficacy for a Food Product. J.W. Finley, USDA, ARS, Beltsville, MD, USA.

The US FDA is the primary agency that regulates food products in the US, and the FDA makes a clear distinction between food and drugs. Drugs must follow a carefully controlled process proving safety and efficacy before that go on the market, whereas food must be safe, not adulterated and labeled correctly. There are myriad means of meeting these requirements; launching a new product, and the amount of research needed is dictated by how the manufacturer wishes to position the product. Meeting regulations for various label claims as well as meeting the guidelines of the FTC regarding advertising may require human studies. Human studies are quite expensive, different types of studies provide varying levels of quality and in the end may not provide the level of evidence needed. Animal/in vitro studies are useful for generating hypotheses, and if the product does not require label claims, they may be sufficient. The research needed to support label claims is illustrated by the process used to develop label claims for barley.

H&N 4: Healthy Aging: From the Inside Out

Chairs: E. Bailey-Hall, DSM Nutritional Lipids, USA; and T. Yanagita, Nishikyushu University, Japan

Fatty Acids at the *sn*-1, 3 Positions of Triacylglycerols are Crucial in Alleviating Fat Accretion Illustrated in C57BL/6 Mice. S.W. Gouk (Honored Student Award Winner and Health and Nutrition Division Student Excellence Award Winner), S.F. Cheng, A.S.H. Ong, and C.H. Chuah, University of Malaya, Kuala Lumpur, Malaysia.

Most reports on adipogenic edible oils were merely based on the total saturated, monounsaturated, *n*-6 and *n*-3 polyunsaturated fatty acid contents in oils. Nevertheless, it is well established that the fatty acids at different *sn*-position are not subjected to the similar rate of intestinal absorption. Long chain saturated fatty acids (SFA) which originated from *sn*-1,3 positions of triacylglycerols, will suffer delayed absorption by virtue of formation of insoluble calcium soaps. Henceforth, this prompts us to investigate the effect of positional distribution of long chain SFA in triacylglycerols, on fat deposition using C57BL/6 mice model. In this paper, the results of 15 weeks *in vivo* study will be presented in terms of body mass gain, subcutaneous, visceral fat deposition and fat excretion in feces. A negative correlation was observed between fat/feed and the total SFA content at *sn*-1,3 positions. Moreover, stearic acids at the *sn*-1,3 positions were more efficient than palmitic acids in reducing fat deposition. As a result, we confirmed our hypothesis that if long chain SFA resides at the *sn*-1,3 positions of triacylglycerols, they tend to alleviate the fat deposition, while higher obesity risk will be expected if fatty acids at the *sn*-1,3 positions are predominantly unsaturated.

Omega3-PUFA Containing Lipids in Metabolic Syndrome. K. Nagao¹ and T. Yanagita², ¹Saga University, Saga, Japan, ²Nishikyushu University, Kanzaki, Japan.

The metabolic syndrome is a cluster of metabolic disorders that contribute to increased cardiovascular morbidity and mortality. Although the pathogenesis of metabolic syndrome is complicated, dietary lipids have been recognized as contributory factors in the development and the prevention of cardiovascular risk clustering.

We investigated the physiological functions and molecular actions of omega3-PUFA containing lipids, such as EPA-TG, DPA-TG, DHA-TG, omega3-PC and EPA-polar lipids, in the development of metabolic

syndrome.

Results indicate that dietary omega3-containing lipids would be useful to prevent or alleviate metabolic syndrome in obese animals. In particular, the function of omega3-containing lipids as dietary adiponectin inducers deserves attention with respect to alleviation of metabolic syndrome by dietary manipulation.

Smart Prevention—Health Care Cost Savings Resulting from the Targeted Use of Dietary Supplements. S. Mister¹ and C. Shanahan², ¹Council for Responsible Nutrition, Washington, DC, USA, ²Frost & Sullivan, Alexandria, VA, USA.

Policymakers, public health experts, and consumers question whether health care costs can be avoided if more preventive measures are adopted. Approximately three quarters of total U.S. health care expenditures are spent on preventable diseases, but only 3% of health care expenditures are invested in disease prevention programs. This talk will present an economic analysis which confirms that notable cost savings can be realized through the use of selected dietary supplements. For this presentation, omega-3 fatty acids and vitamin D/calcium will be examined. A state-of-the evidence review was utilized to determine an overall reduction in the risk of disease-related events attributable to the use of the supplements. Then, these impact variables are used in a cost-benefit analysis to determine the potential economic benefits – in terms of avoided hospital utilization costs – that could be realized if a specified high-risk population was to use these dietary supplements at specified intake levels. These monetary benefits could be an element in reducing health care costs of vulnerable, high-risk populations. Understanding this link will help key stakeholders – including patients, physicians, governments, and private insurance companies and employers – make recommendations on the best course of action to help minimize costs and maximize benefits.

Lipids, Genes, and Age-related Macular Degeneration. J.P. SanGiovanni, National Eye Institute, National Institutes of Health, Bethesda, MD, USA.

Age-related macular degeneration (AMD) is a common, complex, and chronic progressive sight-

threatening disease of the neural and vascular retina. In this presentation I will discuss research on the origin, fate, and actions retina-resident lipids and their derivatives in the context of a unified theory of AMD pathogenesis. Inferences will be made on findings integrated from applied clinical studies, model systems, and reports on the molecular genetics of AMD-associated genes that encode constituents (cleavage and biosynthetic enzymes, transmembrane and nuclear receptors, transporters, ligands, and hormones) of processes involved in lipid biosynthesis, uptake, transport, concentration, cleavage, oxidative modification, and metabolism. The cellular and molecular organization and biophysical-biochemical function of these processes (n.b. our focus is on *organization* of processes, and not solely factors) supports the core structural, bioenergetics, and signaling roles of lipids. I will finish by describing my approach to identify drugs and small molecules with promise for modulating signaling systems acting on lipid-based molecular probes or targets.

DO-HEALTH: A Clinical Study to Support Healthy Aging. E. Stoecklin¹, K. Yurko-Mauro¹, and H.A. Bischoff-Ferrari², ¹DSM Nutritional Products, Columbia, MD, USA, ²University of Zurich & University Hospital Zurich, Zürich, Switzerland.

Background: The global aging population is expected to triple from 524 million in 2010 to 1.5 billion in 2050 (WHO, 2011). Although this represents one of society's greatest achievements, this poses challenges for social and health care costs. Nutrition and physical exercise represents a promising public health intervention to extend healthy life expectancy and reduce healthcare costs. Therefore, the specific aims of the DO-HEALTH study are to establish whether vitamin D, omega-3 fatty acids, and a simple home exercise program will prevent disease at older age. The study will also evaluate the cost-benefit of these interventions.

Methods: This randomized, double-blind, placebo-controlled, 2X2X2 factorial design study will enroll 2152 community-dwelling European men and women aged 70 years and older. The multi-center study is recruiting from 8 centers in 5 EU countries. The nutritional interventions include vitamin D3 (2000 IU/d) and an algal source of omega-3 fatty acids, DHA+EPA (1 g/d) for 3 years' supplementation period.

Outcomes: Bone fracture incidence, muscle strength, blood pressure, cognitive decline and rate of infection will be monitored throughout the study.

These outcomes will be supplemented with relevant biomarker data.

Study status: The study is currently in recruitment phase with more than half of the subjects enrolled.

Plasma Levels of Long Chain Omega-3 Fatty Acids are Related to Markers of Decreasing Oxidative Stress and Aging: Results from a Randomized Controlled Trial. M.A. Belury, Ohio State University, Columbus, OH, USA.

Inflammation and oxidative stress each exacerbate conditions associated with aging including the development of heart disease and cancer. Pro-inflammatory cytokines and oxidative stress regulate the enzyme, telomerase and are associated with shortened telomere lengths in people. In order to determine whether dietary fat could alter this pattern, we conducted a randomized clinical trial in older adults (N=106 healthy, overweight men and women) with either a comparative control supplemental oil or "low-" or "high-" dose fish oil supplement containing eicosapentaenoate (EPA; 20:5n3) and docosahexaenoate (DHA; 22:6n3) at doses of 1.25 or 2.50 g / day fish oil. Supplementation with fish oil decreased plasma ratios of n6:n3 fatty acids. The decreased ratio of plasma n-6: n-3 fatty acids was significantly related to longer telomere length. In addition, F2-isoprostane, a key marker of oxidative stress, was significantly decreased in people consuming LC n-3 supplements for 4 months. These data suggest long chain n3 fatty acids may be protective against telomere shortening that is characteristic of aging and that may lead to degenerative diseases.

Docosahexaenoic Acid and Lutein: Bioactive Lipids of Interest in Cognitive Health. E.J. Johnson, Carotenoids & Health Laboratory, Tufts University, Medford, MA, USA.

The central nervous system selectively accumulates the bioactives docosahexaenoic acid (DHA) and lutein. There is strong scientific support that these bioactive lipids function in ocular health. Scientific evidence is accumulating for their role in cognitive health. In vitro research suggests that DHA and lutein tend to serve complementary roles in neural cell membranes. DHA increases membrane fluidity, while lutein provides structural support. DHA tends to oxidize quickly under conditions of oxidative stress, while lutein is a potent antioxidant. Our research indicates that serum and brain

concentrations of lutein, alone and in combination with DHA is related to cognitive function in a healthy cohort of older adults and that their relationships to cognitive function are dependent on each other. Further, we have shown that supplementation with a combination of DHA and lutein in healthy older adults improves cognitive function, more so than either alone. Given that intakes of each of these dietary lipid components may be suboptimal in the U.S. population, efforts aimed at increasing intakes of these lipids is warranted.

The Potential Role of n-3 Fatty Acids on Skeletal Health. R. Jackson, Ohio State University, Columbus, OH, USA.

Osteoporosis has become a pervasive public health problem, especially in women. It accounts for more than 1.5 million fractures annually, including 329,000 hip fractures. Determining the relation of nutritional components to fracture outcomes is an important first step in developing dietary recommendations to decrease the burden of this disease. In the last decade, interest has arisen in the differential roles of the omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFAs) on skeletal

health. Data support a beneficial role of n-3 PUFAs on osteoporosis through (1) decreased pro-inflammatory cytokines; (2) positive effects on calcium balance; and (3) modulating transcription factors involved in regulation of bone turnover. Although animal studies support favorable effects of n3 FA on bone mass and turnover, the results from human studies remain inconclusive. Observational studies have reported inconsistent findings; for bone mineral density, a proxy for bone strength, some studies show positive associations with total PUFA, n-3, n-6 and linolenic FAs while others show no association. There is some suggestion that these modest associations may translate to reduced fracture risk. Several cohort studies have demonstrated a protective association of AA, total PUFAs or ALA with fragility fractures. A systematic review also suggests evidence of a favorable effect of n-3 FA on BMD and bone turnover which appears to be enhanced by concurrent administration of calcium. Additional insights from prospective studies and clinical trials will be critical to ultimately determine the benefits of PUFA supplementation for prevention of osteoporosis.

H&N 5: General Health and Nutrition

Chairs: H. Durham, Pennington Biomedical Research Center, USA; and M. Drewery, Louisiana State University, USA

Vitamin E and Vitamin E Acetate Solubilization in Mixed Micelles: Physicochemical Basis of Bioaccessibility. Y. Yang (*Honored Student Award Winner*), and D.J. McClements, Dept. of Food Science, University of Massachusetts Amherst, Amherst, MA, USA.

Vitamin E is an essential micronutrient for humans due to its antioxidant and non-antioxidant activities. In this study, an emulsion titration assay was used to quantify the kinetics and extent of VE and VE acetate solubilization in model mixed micelles. The composition of the mixed micelles was designed to mimic those produced during digestion of lipids in human bodies: bile salts, phospholipids, and free fatty acids. Initially, the optimum conditions required to form model mixed micelles were established. The solubilization capacities of VE and VE acetate in the mixed micelles were then compared. The solubilization capacity of the mixed micelles for VE was higher than that for VE acetate, which was attributed to differences in the ability of the vitamin molecules to be incorporated into the micelle structure. The solubilization capacities also depended on the composition of the mixed micelles: micelle solubilization of VE was increased by the presence of phospholipid, but did not depend strongly on the presence of free fatty acid (octanoic acid or linoleic acid). Overall, this research has important implications for understanding the digestion, absorption, and transportation of VE in the human gastrointestinal tract and for designing delivery systems to increase its bioaccessibility.

The Effects of Prenatal and Early Postnatal Tocotrienol-Rich Fraction Supplementation on Spatial Memory in Male Offspring Rats. G.

Nagapan¹, G.Y.Meng², I.S. Addul Razak², K. Nesaretnam¹, M. Ebrahimi², ¹Malaysiam Palm Oil Board (MPOB), Kajang, Selangor, Malaysia, ²Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, University Putra Malaysia, Serdang, Selangor, Malaysia.

Recent animal studies have demonstrated that tocotrienols have potential memory enhancing effects but there is limited evidence on prenatal and postnatal influences of tocotrienols. Therefore, this study was aimed to investigate potential prenatal and early postnatal influence of Tocotrienol-Rich Fraction (TRF) supplementation on spatial memory

development in male offspring rats. Adult female Sprague Dawley (SD) rats were randomly assigned into five groups of two animals each. The animals were fed control (CTRL), vehicle (VHCL), docosahexanoic acid (DHA), Tocotrienol-Rich fraction (TRF), and docosahexanoic acid and tocotrienol rich fraction (DTRF) diets for 2 weeks prior to mating. The females were maintained on their respective treatment diets throughout the gestation and lactation periods. The male pups were weaned at 8 weeks postnatal, after which they were grouped into five groups of ten animal each and fed with the same diets as their dams for another eight weeks. Learning and behavioural experiments were conducted in these male off-spring rats using the Morris water maze. TRF supplementation elevated brain and plasma alpha-tocotrienol levels and improved spatial memory in male progeny rats.

Comparative Flow Cytometric Analysis of Flavonoid Rich *Oxalis corniculata* Extract in Synergy Versus Its Individual Pure Principal Fractions (Rutin and Ferulic Acid) in Ameliorating Mitogen Induced Oxidative Stress in Human PBMCs. S. Mukherjee^{1,2}, D. Paul^{1,2}, M. Ghosh^{3,2}, and P. Dhar^{1,2}, ¹Laboratory of Food Science and Technology, Food and Nutrition Division, University of Calcutta, Kolkata, West Bengal, India, ²Center for Research in Nanoscience and Nanotechnology, University of Calcutta, Kolkata, West Bengal, India, ³Laboratory of Chemical Technology, University of Calcutta, Kolkata, West Bengal, India.

Oxalis corniculata, an herbaceous plant of the *Oxalidaceae* family is being used as an ethnic medicine by the native people of South Eastern Asia. Present study deals with the biochemical evaluations and analysis of major flavonoids present in this leaf. The chromatogram showed the predominance of the active principal components, i.e. flavonoids in the following order: Rutin>p-hydroxybenzoic acid>Ferulic acid. The uptake of flavonoid rich extract by human PBMC and cell viability assay were performed by FACS. Flow-cytometric analysis were also done to investigate the protective effect of extract(500ng/mL) and two major flavonoids (Rutin and Ferulic acid) from *Oxalis corniculata* against PHA and LPS induced ROS in human PBMC. Extract significantly reduced the intracellular ROS generation. The nitrite production in culture

supernatants was assessed by the Griess reaction. A significant decrease in NO production was observed on treatment with extract. It can therefore be concluded that the synergistic effect of different flavonoids present in the extract has better efficiency than individual flavanoids in circumventing *in vitro* mitogen induced generated ROS.

Lipid Peroxidation Biomarker HODE and Its Application to Diagnosis. Y. Yoshida and A. Umeno, National Institute of Advanced Industrial Science and Technology, Takamatsu, Kagawa, Japan.

Recently, the biological roles of molecules induced by oxidative stress have received a great deal of attention not only for elucidating pathological mechanisms but also for practical clinical applications as biomarkers. For example, lipid peroxidation has been the subject of extensive studies from the viewpoints of mechanisms, dynamics, product analysis, involvement in diseases, inhibition, and biological signaling in the last 50 years.

We have developed a novel method to measure hydroxyoctadecadienoic acid (HODE) from biological fluids and tissues; using this method, a considerable amount of the oxidation products of linoleic acid can be measured. Reduction and saponification enabled us to measure hydroperoxides and hydroxides of both free and esterified forms of linoleic acid as totally assessed HODE, which includes enzymatic and non-enzymatic products; 9- and 13-(Z, E)-HODE, non-enzymatic free radical-mediated products; 9- and 13-(E, E)-HODE, and specific non-enzymatic singlet oxygen-mediated products; 10- and 12-(Z, E)-HODE. In this presentation, the usefulness of HODE will be introduced showing animal and human experiments and its application to early diagnosis for several diseases will be discussed.

Lymphatic Lipid Transport Regulated by Dietary Components and Drug. B. Shirouchi and M. Sato, Kyushu University, Fukuoka, Japan.

Researchers have often measured lymphatic transport of dietary lipids in rats by infusing a lipid emulsion into the gut. This method has several advantages in which they evaluate quantitative lipid absorption measurement without contamination and/or using a small amount of a radioisotope-labeled substance. In fact, foods are complex mixtures of various components. From this aspect, this method fails to take into account the interaction of the lipid emulsion with other dietary components, such as proteins and carbohydrates. Additionally, the

method is difficult to assess physiological lymph flow due to continuous infusion of saline and restraint stress.

Based on the above understanding, we developed a permanent thoracic lymph duct cannulation method to evaluate actual dietary lipid absorption from a normal diet without restraint stress and then investigated the effects of dietary components and drug on the lymphatic lipid transport in rats under near-physiological conditions. Our researches provide the first evidence that guar gum, a water-soluble dietary fiber, reduces lymphatic lipid transport *via* the reduction of lymph flow and that ezetimibe, a cholesterol absorption inhibitor, inhibits lymphatic transport of esterified cholesterol without affecting lymph flow and lymphatic transport of free cholesterol, triacylglycerol, and α -tocopherol.

Comparative Study of Gastrointestinal Absorption of Rice Bran Oil and Medium Chain Fatty Acid Rich Rice Bran Oil in Rat Model. M. Ghosh and A. Sengupta, University of Calcutta, Kolkata, West Bengal, India.

The present study dealt with the preparation of different medium chain fatty acid rich rice bran oils and investigation of their *in vivo* intestinal absorption in the single-pass perfusion rat model. Medium chain fatty acid (MCFA) rich rice bran oil (RBO) was prepared by enzyme catalysis technique which resulted in substitution of long chain fatty acids (LCFA) present in rice bran oil with MCFA. *In situ* absorption efficiency of the oils was measured in laboratory acclimatized adult Sprague–Dawley rats. A comparative study of lipid absorption between MCFA rich rice bran oil and native rice bran oil of similar dilution level, in cannulated small intestine of rats with time gradient, had been done. Subsequent analysis (e.g. percent volume absorption, percent lipid absorption) had shown that the medium chain fatty acid rich rice bran oil significantly enhanced absorption of lipids from the emulsion system in the small intestine of the rats. This better absorption capacity of MCFA comparison to LCFA can be utilized in treating the infants, patients with low absorption or reduced absorption capacity.

Inhibitory Effect of α -tocopherol and the Analogues on Release of Chemical Mediators from Mast Cells. M. Takasugi¹, J. Kirikoshi², S. Sasayama², M. Hirata², A. Utsunomiya¹, R. Hosomi³, K. Fukunaga³, H. Arai², ¹Kyushu Sangyo University, Fukuoka, Japan, ²Kitami Institute of Technology, Kitami, Hokkaido, Japan,

³Kansai University, Suita, Osaka, Japan.

It has been suggested that reactive oxygen species may play an important role in inflammation including allergic diseases. In type I allergy, chemical mediators (CM) like histamine and leukotrienes (LT) are released from mast cells. α -Tocopherol (Toc), a strong lipophilic antioxidant, has been implicated as an allergy inhibitor, although the mechanism is still unclear. In this study, we investigated effects of Toc and the analogues on the release of CM from mast cells *in vitro*.

Rat basophilic leukemia cell line (RBL-2H3) and mouse mast cell line (PB-3c) were used for histamine and LTB₄ releasing assay, respectively. RBL-2H3 and

PB-3c treated with Toc or Toc analogues were stimulated with calcium ionophore. The secreted histamine and LTB₄ were determined by HPLC.

The releases of histamine and LTB₄ from mast cells were dose-dependently suppressed by Toc. On the other hand, Toc quinone, an oxidized form of Toc, showed no inhibitory effect on CM releasing. 2,2,5,7-Pentamethyl-6-chromanol (PMC) suppressed LTB₄ releasing more strongly than Toc, while Trolox, a hydrophilic analog of Toc, did not decrease LTB₄ releasing. These data suggest that Toc may have the anti-allergic function through suppression of CM releasing, to which the antioxidant activity and the localization of Toc may contribute.

H&N-P: Health and Nutrition Posters

1. Sugar Alcohol Esters, Mutation Inhibitor Containing the Esters or *Hericium erinaceum* Extract, and Food and Cosmetic Containing the Mutation Inhibitor. M. Miyazawa¹, T. Takahashi¹, and R. Ishikawa², ¹Kinki University, Higashiosaka, Osaka, Japan, ²Taiyo Food Co., Daito, Osaka, Japan.

An mutation inhibitor, useful for inhibiting mutagenic substances inducing cancer, contains a sugar alc. ester I (n = 0, 1; R = alkanoyl, alkenyl) or an ext. of *H. erinaceum*. Also claimed are a food additive, food, a cosmetic additive, and a cosmetic contg. the mutation inhibitor. Thus, erythritol 1-acetate, D-arabitol 1-acetate, and D-arabitol 1-linoleate, extd. from *H. erinaceum* or chem. synthesized, showed antimutagenic activity against Trp-p-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indol).

2. Characteristic Odors and Food Security of Japanese Food, Agitake. A. Usami and M. Miyazawa, Kinki University, Higashiosaka, Osaka, Japan.

The chemical composition of volatile oil from agitake (*Pleurotus eryngii* var. *ferulae*) was established for the first time using gas chromatography (GC) and GC-mass spectrometry. Sixty-seven and 24 components were extracted by hydrodistillation (HD) using diethyl ether (DE) and dichloromethane (DM), respectively. Thirteen and 48 components of were extracted by the solvent-assisted flavor evaporation (SAFE), using DE and DM, respectively.

Odor evaluation of the volatile oil from agitake was also carried out using GC-olfactometry, aroma extraction dilution analysis (AEDA), and the odor activity value (OAV). This study proved that HD and SAFE can be used as complementary extraction techniques for the complete characterization of volatile oil from agitake. These oils were assayed to determine their antioxidant activity by the oxygen radical absorbance capacity (ORAC) assay using fluorescein as the fluorescent probe. The ORAC values varied from 1413 ± 102 trolox equivalents (µmol TE/g) for HD using DE, from 1134 ± 15 µmol TE/g for HD using DM, from 405 ± 147 µmol TE/g for SAFE using DE, and from 358 ± 161 µmol TE/g for SAFE using DM. The difference in the antioxidant activities among method and solvent were attributed to their different composition. These data provided evidence that the volatile oil from agitake is a good dietary source of antioxidant.

3. Placental Fatty Acid Trafficking in Women with and without Gestational Diabetes. M.L. Drewery¹, C.L. Clouatre^{1,2}, M.L. Thibodaux², W.T. Cefalu², C.J. Lammi-Keefe^{1,3}, and H.A. Durham², ¹Louisiana State University, Baton Rouge, LA, USA, ²Pennington Biomedical Research Center, Baton Rouge, LA, USA, ³Louisiana State University AgCenter, Baton Rouge, LA, USA.

Newborns of women with gestational diabetes mellitus (GDM) have compromised docosahexaenoic acid (DHA) and arachidonic acid (AA) status at birth, which is unrelated to maternal fatty acid status. Normally, DHA and AA are preferentially transferred from maternal to fetal circulation during gestation. We hypothesize placental transfer and retention of DHA and AA is altered in GDM and aim to investigate the effect of GDM on gene expression in placental fatty acid transport. Specifically, this research will focus on optimization of conditions and techniques by which placental mRNA is extracted and cDNA is synthesized to ultimately measure gene regulation in human placental tissue. We will collect full-term placentas by caesarean section and vaginal deliveries from women with 1) normal glucose tolerance and 2) GDM. Total RNA will be purified from human placental tissue using a commercially available kit and reverse transcribed. Quantification of mRNA expression levels will be performed with real-time PCR by monitoring product formation with the fluorescent dye SYBR Green I. Target genes will include fatty acid transport protein 4, fatty acid binding protein (FABP) 4, plasma membrane FABP, PPAR γ , and adipophilin with GAPDH as a reference gene. Optimization of these procedures will allow us to further investigate the mechanism by which placental fatty acid trafficking is altered in GDM.

4. Effects of Dietary Egg White Hydrolysate on Muscular Fat Metabolism in Insulin Resistance Model Rats. M. Ochiai and T. Matsuo, Kagawa University, Kita, Kagawa, Japan.

Some dietary proteins are reported to prevent from obesity and insulin resistance (IR), but the detailed mechanisms in skeletal muscles and the anti-IR effects of egg white (EW) ingredients are not clear. We investigated the effects of dietary EW or EW hydrolysate (EWH) on skeletal muscular fat metabolism in non-obese diabetic Goto-Kakizaki (GK) rat (Exp A) and Wistar rat fed a high-fat and high-sucrose diet (HFSD) (Exp B).

In Exp A, Wistar and GK rats were respectively fed 20% (w/w) casein or EHW contained normal diet

for 6 weeks. In Exp B, Wistar rats were fed a HFSD containing 20% (w/w) casein, EW or EWH for 8 weeks. Triglyceride (TG) accumulation and stearoyl-CoA desaturation (SCD) indices (C16:1/C16:0 and C18:1/C18:0), a kind of lipogenic markers, in skeletal muscles were mainly investigated.

In Exp A, TG contents and SCD indices of the soleus muscle were decreased by dietary EWH in paralleled with the glucose metabolism. In Exp B, these biochemical parameters of the soleus and gastrocnemius muscles were also decreased by dietary EW and EWH.

These data conclude that EW and/or EWH are effective dietary protein ingredients to improve muscular fat metabolism in various different kinds of IR model rats.

5. Lipidomic Analysis of Whole Blood and Dried Blood Spots. J.J.A. Henao¹, A.H. Metherel¹, R.W. Smith², and K.D. Stark*¹, ¹Dept. of Kinesiology, University of Waterloo, Waterloo, ON, Canada, ²University of Waterloo Mass Spectrometry Facility, Dept. of Chemistry, University of Waterloo, Waterloo, ON, Canada.

Lipidomics is an expanding field but the use of lipidomics with rapid blood sampling techniques is relatively limited. We examined the lipidomic profiles (n=5) of lipid extracts from venous whole blood (50uL) after storage in cryovials (WB), or after applying to chromatography paper, drying and storing (DBS) using both an Orbitrap MS/MS (Thermo Scientific Q-Exactive with Dionex UPLC/HPLC) and single-quadrupole (Waters ZQ with Waters HPLC) instruments. Both were set to positive electrospray mode, and used identical solvent systems and chromatography programs. The Waters ZQ required more concentrated lipid extracts (10-20-fold) for spectral analysis, and using the LipidBlast library with the Orbitrap MS/MS allowed for discrimination of isobaric species (ex. 16:0/20:4 vs. 18:2/18:2 glycerophosphocholine). In DBS samples, ion intensities tended to be less than WB samples. 16:0/18:2 glycerophosphocholine [M+H]⁺ ion was the most abundant ion in all samples with 16:0/18:1/18:2 [M+NH₄]⁺ being the most abundant triacylglycerol ion. Cholesteryl ester ions (18:2 and 20:4 [M+NH₄]⁺) and a cholesteryl ester fragment ([M-OH]⁺, m/z 369.35170) were proportionally higher in the DBS samples. In conclusion, lipidomic analysis of DBS samples is possible. An examination of extraction efficiency of glycerolipids from chromatography paper is necessary before using DBS for quantitative assessments.

6. A Phospholipid-Peptid Complex from Krill Reduces Plasma Lipid Levels in Low-fat Fed Rats.

M.S. Ramsvik^{1,2}, B. Bjørndal¹, I. Bruheim², J. Skorve¹, and R.K. Berge^{1,3}, ¹Dept. of Clinical Science, University of Bergen, Bergen, Norway, ²Olympic Seafood, Ålesund, Norway, ³Dept. of Heart Disease, Haukeland University Hospital, Bergen, Norway.

Health benefits from eating seafood rich in n-3 polyunsaturated fatty acids have been documented in several studies. There is growing evidence that proteins from marine origin affects mitochondrial fatty acid metabolism in animals. The objective of this study was to investigate the effect of a phospholipid-peptid complex (PPC) from krill on plasma levels of lipids and hepatic fatty acid oxidation, in a dose-dependent manner.

Male Wistar rats were fed low-fat diets (10% fat, 20% protein, wt/wt) for 4 weeks, given either a control diet (2% soy oil, 8% lard, 20% casein), or an experimental diet where casein and lard were replaced with PPC at 3, 6, or 10% (wt/wt). Plasma lipids and fatty acid composition were determined, as well as hepatic enzyme activities and gene expression.

The effect of dietary treatment with PPC resulted in significantly reduced levels of plasma triacylglycerols (TAG), phospholipids and cholesterol in the 10% group. There were no differences in hepatic fatty acid oxidation between the groups, whereas fatty acid synthase (FAS) activity was reduced by PPC treatment. Results of enzyme activity and gene expression will be presented. The present data suggest a triacylglycerid- and cholesterol-lowering property of PPC from krill. Results indicate this property explained by reduced lipogenesis.

7. Biomarkers for Early Detection of Type 2 Diabetes, Including 10- and 12-(Z,E)-hydroxyoctadecadienoic Acids (HODE).

A. Umeno¹, K. Yoshino², Y. Hashimoto¹, M. Shichiri², and Y. Yoshida¹, ¹National Institute of Advanced Industrial Science and Technology, Takamatsu, Kagawa, Japan, ²National Institute of Advanced Industrial Science and Technology, Ikeda, Osaka, Japan.

Current diagnostic tests such as glycemic indicators have limitations for early detection of impaired glucose tolerance (IGT), which leads to diabetes. We have found that fasting plasma levels of totally assessed 10- and 12-(Z,E)-hydroxyoctadecadienoic acid (HODE) revealed the fairly well correlations with those of HbA1c and glucose during oral glucose tolerance tests (OGTT),

of which levels were determined by performing LC-MS/MS after reduction with triphenyl phosphine and saponification by potassium hydroxide (A Umeno et al, PLOS ONE, e63542, 2013). We then applied this marker with the combination of other biomarkers including adiponectin and high sensitivity-C-reactive protein (hs-CRP) to OGTT (n = 57), including normal type group (n = 44), "high-normal" (fasting plasma glucose, 100–109 mg/dL) and borderline type (IGT) group (n = 11), and diabetic type group (n = 2) in order to tighten these markers for the early detection of type 2 diabetes. It was found that an algorithm led by these markers in fasting plasma was able to prospect the glucose levels and insulin resistance index during OGTT. In conclusion, multi-markers especially including 10- and 12-(Z,E)-HODE may be prominent biomarkers for the early detection of IGT and "high-normal" type without OGTT.

8. A Novel Method for Selective Recovery of Tocotrienol from Rice Bran Deodorizer Distillate Using Ion-exchange Resin without Molecular Distillation.

K. Hiromori, N. Shibasaki-Kitakawa, K. Nakashima, and T. Yonemoto, Dept. of Chemical Engineering, Tohoku University, Aoba-ku, Sendai, Japan.

We proposed a novel method to selectively recover tocotrienol, received much attention as bioactive compound, from deodorizer distillate obtained during refining edible oil. Tocotrienol was retained on the anion-exchange resin by ion-exchange reaction of hydroxyl group, and then released and recovered from the resin by ion-exchange reaction of acetic acid group. Prior to the ion-exchange reactions of tocotrienol, free fatty acid (FFA), a major component of deodorizer distillate, was converted to fatty acid ester, not participating in the reactions. This method was applied to the mixture of rice bran deodorizer distillate (1.5wt% of tocotrienol content) with stoichiometric molar ratio of ethanol. Sixty-eight percent of tocotrienol was recovered and the solutes in the product were 24wt% of tocotrienol, 21wt% of tocopherol with similar structure and 55wt% of FFA from triglyceride. In the conventional method with molecular distillation, the tocotrienol yield was very low, about 25% due to loss by thermal decomposition. The contents of tocotrienol, tocopherols and FFA were 10wt%, 10wt% and 9wt%, respectively and the other solutes were glycerides, sterols and hydrocarbons. Therefore, this method using ion-exchange reactions was very effective for selective recovery of

tocotrienol.

9. Easy Short Time Cooking, No Waxy and More Digestible Brown Rice Using Supercritical Carbon Dioxide.

M. Matsubara¹, Y. Nakatou², and E. Kondoh¹, ¹University of Yamanashi, Kofu, Yamanashi, Japan, ²Koa Electronics Co. Ltd., Shimoina-Gun, Nagano, Japan.

Brown rice is 100% whole grain, and drastically rich in 7 vitamins and 8 minerals mainly including Vitamin E, B6, niacin, and potassium, magnesium, phosphorus, with antioxidant, phytonutrients and dietary fiber compare to white rice. Brown rice has been founded to help reduce the risk cardiovascular disease, type 2 diabetes and metabolic syndrome.

Eating more brown rice is great, but not popular more than white rice due to not easy cooking and waxy bad smell.

We studied and developed new brown rice required no further rinsing and soaking, short time cooking and no waxy bad smell more than regular brown rice using supercritical carbon dioxide (scCO₂). These new brown rice extracted with scCO₂ was resulted in no waxy smell and the formation of micro-fracture in the bran and inside of rice which is possible to absorb water efficiently. These results enabled us to be short time cooking, more aromatic, chewy and digestible, and longer shelf life.

10. The Antioxidant Potential Against Reactive Oxygen and Nitrogen Species of an Extract from *Byrsonima crassifolia* Fruit.

L.R.B. Mariutti¹, R.C. Chisté², E. Rodrigues¹, E. Fernandes², and A.Z. Mercadante¹, ¹Dept. of Food Science, Faculty of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil, ²REQUIMTE, Dept. of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.

Murici (*Byrsonima crassifolia*) is an unexploited fruit native to Brazil. In our study, a methanolic extract of murici was prepared and evaluated in relation to its antioxidant potential against reactive oxygen and nitrogen species (ROS and RNS), as determined by both *in vitro* chemical and cellular assays. The major phenolic compound in the extract, determined by HPLC-DAD-MSⁿ, was quercetin (2.72 mg/mL). The extract was able to scavenge ROO[•] (0.3 mmol of trolox equivalent/mg of extract), HOCl (IC₅₀=10 µg/mL), HO[•] (IC₅₀=7 µg/mL) and ONOO⁻ (IC₅₀=21 µg/mL in the absence of NaHCO₃ and 17 µg/mL in the presence of NaHCO₃). It also showed a low capacity (46%) to scavenge H₂O₂ at 734 mg/mL (maximum tested). Human erythrocytes were

subjected to oxidative damage caused by ROO^{*} and murici extract was not able to inhibit hemolysis, even at the highest tested concentration (1000 mg/mL). However, the extract showed capacity to inhibit both hemoglobin oxidation (IC₅₀=354 µg/mL) and lipid peroxidation by 48%. Murici extract was also able to inhibit the depletion of glutathione (GSH) by 44% and the formation of its oxidized form (GSSG) by 87%, both at 100 mg/mL). Our results demonstrate that murici, containing mainly phenolic compounds, may impart health benefits against oxidative stress.

11. Synthesis and Biological Evaluation of Unsaturated Fatty Acid-based β-hydroxy 1,2,3-triazoles. M. Vijay, B.L.A. Prabhavathi Devi, R.B.N. Prasad, U. Ramesh, and A. Singh, CSIR-Indian Institute of Chemical Technology, Hyderabad, Andhra Pradesh, India.

N-Heterocyclic compounds such as 1,2,3-triazole structure moiety does not occur in nature, may display biological activities. There are numerous examples in the literature stating that, compounds with triazole functionality having anti-microbial activity against Gram positive bacteria, anti-HIV, antifungal, anti bacterial, anti-allergic, anti-convulsant, β-lactamase inhibitory activities, and so on. A series of b-hydroxy 1,2,3-triazole derivatives of unsaturated fatty acids namely, oleic, ricinoleic and 10-undecenoic acids were synthesised in excellent yields. All these derivatives were characterised by ESI-MS, IR and NMR spectra as well as HRMS. The b-hydroxy 1,2,3-triazoles were screened in vitro for their anticancer activity against four human cancer cell lines namely, DU-145, HeLa, MCF-7 and A549 in comparison with doxorubicin, a standard anticancer drug. Among all the triazoles, oleic acid-based b-hydroxy 1,2,3-triazole ester with -CH₂OH side chain (5a_{ii}) exhibited good anti cancer activity with IC₅₀ values 10.73, 13.61, 11.93, and 16.54 µM against DU-145, HeLa, MCF-7 and A549 cancer cell lines respectively.

12. Short-term Soybean β-conglycinin Feeding Improves Lipid and Carbohydrate Metabolism in GK Rats. N. Inoue¹, A. Funayama¹, N. Tachibana², M. Kohno², T. Tsuduki¹, and I. Ikeda¹, ¹Tohoku University, Sendai, Miyagi, Japan, ²Fuji Oil Co. Ltd., Izumisano, Osaka, Japan.

Previously, we suggested that soybean β-conglycinin (β-CG) exerted hypolipidemic effects and prevented the development of diabetes in rats. However, we do not know whether β-CG affects

carbohydrate metabolism directly or improves carbohydrate metabolism as a result of the hypolipidemic effect. Therefore, for analyzing the parameters in the early stage of feeding β-CG, we investigated the effect of short-term soybean β-CG feeding in GK rats. Male GK rats were fed an AIN-93G diet containing casein or β-CG for 1wk. As the result of energy metabolism measurement, carbohydrate consumption was higher in the β-CG-fed rats. Serum and hepatic adiponectin levels were significantly higher in the β-CG-fed rats. In the protein expression of insulin signaling pathway, the phosphorylation of IRS-1 and Akt was increased in the β-CG-fed rats. Serum and hepatic TAG levels in the β-CG-fed rats were significantly lower. The feeding of β-CG suppressed the activity of FAS and enhanced the activity of CPT in the liver. In summary, we show that short-term soybean β-CG feeding significantly enhances carbohydrate consumption and adiponectin secretion. These effects were partly attributable to the enhancement of adiponectin incorporation into liver, and the improvement of insulin signaling.

13. Supplementation of Laying-hen Feed with Annatto Tocotrienols for Egg Nutrient and Cholesterol Reduction. H. Hansen¹, T. Wang¹, H. Xin¹, and D. Dolde², ¹Iowa State University, Ames, IA, USA, ²DuPont Pioneer, Johnston, IA, USA.

Changing what is in the feed for laying hens can vastly change the composition of the egg produced. Annatto is the only known source that is rich in tocotrienols (delta and gamma forms) that does not contain alpha-tocopherol. Tocotrienols have been shown to have many health benefits, including the ability to reduce cholesterol, neuro-protection, and suppression of some type of cancer cells. The impact of higher consumption of tocotrienols by the hens on the cholesterol content in resulting egg is also being evaluated. In this study various amounts of Annatto, both with and without added alpha-tocopherol, were supplemented to hen feed to determine the transfer efficiency to the yolk. Literature has shown that transfer proteins in the absorption process have the highest affinity for alpha-tocopherol, causing other forms of vitamin E to be to have poor absorption. By adding Annatto tocotrienols to the feed, it may be possible to increase consumption and bioavailability of these giving the various benefits they can provide to the consumer.

14. The Linoleic Acid to α -Linolenic Acid Ratio of High Oleic Edible Oils. K.V.S. Sweetman and C. Tyburczy Srigley*, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, Food and Drug Administration, College Park, MD, USA.

The linoleic acid to α -linolenic acid ratio (LA/ALA) has been reported to be an important consideration when evaluating the healthfulness of edible oils. Oils with high LA/ALA ratios are reported to be associated with adverse effects on biomarkers of chronic disease, whereas those with low LA/ALA ratios reportedly support beneficial health outcomes and long chain omega-3 polyunsaturated fatty acid (PUFA) biosynthesis. Specialty-bred oils containing high concentrations of oleic acid and low concentrations of LA are now available. This study compared the LA/ALA ratios of these high oleic (HO) oils with those of the corresponding commodity oils. Fatty acid methyl esters from 13 HO oils (sunflower, safflower, peanut, soy, canola, n = 4, 4, 2, 2, 1, respectively) and 11 commodity oils (sunflower, safflower, peanut, soy, canola, n = 1, 1, 2, 3, 4, respectively) were analyzed by gas chromatography according to AOCS Official Methods. LA/ALA ratios varied from 4:1 to 262:1 for HO oils and from 2:1 to 113:1 for commodity oils. Of the HO varieties, LA/ALA ratios were lowest for soy (4:1) and canola (8:1) oils and highest for safflower oils. Commodity soy and canola oils had low LA/ALA ratios (2:1 - 7:1) but high total PUFA contents. Overall, HO varieties of soy and canola oil offer low LA/ALA ratios and their lower total PUFA contents support more shelf-stable products.

15. An Acute Toxicity Evaluation of a New Algal Oil that Contains Eicosapentaenoic Acid (EPA, 20:5n-3) and Palmitoleic Acid (16:1n-7). B. Connolly¹, M.L. Collins¹, A. Bull², A.S. Ryan¹, and J.D. Astwood¹, ¹Aurora Algae, Hayward, CA, USA, ²Huntingdon Life Sciences, Suffolk, UK.

Aurora Algae, Inc. has developed a new composition of algal oil (Algal-EE) that is naturally high in ethyl esters of EPA (25-30%), palmitoleic acid (20-25%), with no docosahexaenoic acid (DHA). This study evaluated the acute toxicity of Algal-EE as a food ingredient administered in the diet of Sprague-Dawley rats. Six female rats received a single oral gavage dose of Algal-EE, formulated in corn oil, at a dose level of 2,000 mg/kg body weight. Clinical observations were made in the morning and evening of each day for 14 days. All animals were sacrificed on Day 15. Macroscopic evaluation involved the examination of all organs in the cranial, thoracic, and

abdominal cavities. There were no deaths or clinical signs of treated-related adverse events. All animals achieved satisfactory body weight gains over the 14 day period. There were no abnormalities in any animal at the macroscopic level. The acute median lethal oral dose (LD50) of Algal-EE was greater than 2,000 mg/kg body weight. The administration of Algal-EE up to 2,000 mg/kg body weight did not produce any toxic-related signs of morbidity or affect body weight or organ appearance.

16. Genetic Toxicity Assessment of a Unique Algal Oil that Contains Eicosapentaenoic Acid (EPA) and Palmitoleic Acid. B. Connolly¹, M.L. Collins¹, W. Barfield², L. Pritchard², K. May², A.S. Ryan¹, and J.D. Astwood¹, ¹Aurora Algae, Hayward, CA, USA, ²Huntingdon Life Sciences, Suffolk, UK.

Aurora Algae, Inc. has developed and optimized algal strains of *Nannochloropsis sp.* to produce algal oil ethyl esters (Algal-EE) that are naturally high in EPA (25-30%), palmitoleic acid (20-25%), with no docosahexaenoic acid (DHA). This study evaluated the safety of Algal-EE based on three genetic toxicity tests. In the Ames test, Algal-EE was investigated for its potential to induce gene mutations using *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and the tester strain *Escherichia coli* WP2 uvrA. Concentrations of Algal-EE up to 5,000 μ g/plate were used. The tests were conducted with and without metabolic activation. The chromosome aberration test evaluated the ability of Algal-EE to induce structural chromosome aberrations using cultured human lymphocytes with and without metabolic activation. The micronucleus test investigated the potential of Algal-EE to induce micronucleated polychromatic erythrocytes in murine blood. A dose of 2,000 mg/kg of Algal-EE was selected as the maximum tolerated dose. Under the conditions of the three studies, Algal-EE showed no evidence of having any genetic toxicity potential.

17. Essential Oil Components in Macroalgae for Human Health. D. Özçimen, B. Inan, and B.Ö. Demirer, Yildiz Technical University, Bioengineering, Istanbul, Turkey.

In recent years, algae are generally used as a raw material for biofuels, but especially macroalgal lipids are used for health science. This type of algae which is known as seaweed have been used in food, pharmacy, cosmetic and biotechnology industries. The lipid content of algae changes in accordance with algae type, ambient temperature, environment conditions and seasons. The most common lipid type

in macroalgae is glycolipid, and macroalgae contain phospholipids, sterols and triacylglycerols structures besides glycolipid. In general fatty acids in macroalgae are omega-3 which are eicosapentanoic acid, α -linolenic acid, stearidonic acid and omega-6 which are arachidonic acid. Eicosapentanoic acid is used for treatment of cardiovascular disease, asthma and migraine. In addition to this, arachidonic acid has an important role in immune response systems and neuron structures. Both omega-3 and omega-6 are effective on rejuvenescence and dermal health protection. While macroalgae has a huge potential for fatty acid source, fatty acids in macroalgae can be easily oxidized and cause some problems with its use in diet, since these fatty acids contain high amount of double bond of lipid structure. Nowadays, many researches are worked especially for stabilization of algal fatty acids and macroalgal applications keep their importance today and also in future.

18. Development of Measuring System of Fucoxanthinol in Human Serum Using LC-MS/MS. N. Mikami¹, M. Abe², M. Hosokawa², K. Miyashita², and Y. Kokai¹, ¹Dept. of Biomedical Engineering, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan, ²Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan.

Fucoxanthin (Fx) is a marine carotenoid derived from brown algae and has been reported for carrying various health promoting effects those including anti-obesity, anti-oxidant, anti-cancer, anti-diabetic and anti-photoaging properties. Fx is metabolized to fucoxanthinol (FxOH) and amarouciaxanthin A in mice, though the presence has been known little in human. We here report the establishment of measurement of human sera FxOH by LC-MS/MS.

We first tuned detection of standard FxOH on a triple quadrupole tandem mass spectrometer using APCI and in multiple reaction monitoring (MRM) mode equipped with LC. Ion transition monitored for quantitation was m/z 617.5 \rightarrow 109.0 for FxOH. Calibration curve of FxOH was linear ($r^2 = 0.99$) and CV values were $< 5.0\%$ for all concentrations in the range of 10-10000 ng/mL (50-50000 pg on column). We then analyzed FxOH in serum by using the MRM channel above. Two difficulties have been raised in preparation for MS analysis, such as, (1) increase of noise peak of chromatogram by unknown substances in serum and (2) condensation of samples by volatilization in according to Asai's

method. Using ultrafiltration instead of filtration and spiking of Fx standard before extraction, we have overcome these obstacles. Finally, we have developed a measurement system of FxOH in human serum by LC-MS/MS.

19. The Influence of Dietary Oil Sources on Hatchability of Alligator Eggs from a Captive Population. A. Mikolajczyk¹, W. Holmes^{1,5}, M. Zappi^{1,5}, R. Hernandez^{1,5}, T. Joanen², M. Staton³, and E. Falgout⁴, University of Louisiana, Lafayette, LA, USA, ²Louisiana Department of Wildlife and Fisheries, Rockefeller, LA, USA, ³Mark Staton Co., Lafayette, LA, USA, ⁴T-Bois Alligator Farms, LaRose, LA, USA, ⁵UL Energy Institute, Lafayette, LA, USA.

Breeding of alligators in captivity is essential for conservation and commercial purposes, but a major problem of embryonic deaths exists. Eggs from captive alligators commonly display a hatchability of 50 percent in comparison with a hatchability of wild type having 95 percent. In 1993, Noble et. al indicated differences in fatty acid composition of egg yolks from captive and wild alligators which advanced the supplementation of fatty acids into the diet to improve hatchability of eggs from captive breeders.

A study was done to analyze and compare the fatty acid content and hatchability of captive alligator eggs with two different diets. These farmed eggs were also compared with that of wild populations.

20. Palm Oil in Trans-free Formulations - The Nutritional Benefits. G. Nagapan, Malaysian Palm Oil Board, Kajang, Selangor, Malaysia.

Palm oil is the major produced and exported edible oil in the world. The majority of palm oil produced is used in food applications. As palm oil has an equal content of saturated and unsaturated fatty acids, it offers a viable alternative to Trans Fatty Acids (TFAs) in food formulations. The benefits of palm oil as a substitute for TFAs are supported by scientific observations from a number of human intervention trials. Studies specifically investigating the effects of palm oil in a diet in substitution with monounsaturated (MUFA) and polyunsaturated (PUFA) rich vegetables oils have found similar lipid profile in healthy subjects. When palm oil was consumed as a major dietary fat, it did not elevate Total and Low Density Lipoprotein (LDL) cholesterol as predicted by Keys and Hegsted for palmitic acid (C16:0). There is possible evidence to suggest that the Total Cholesterol (TC) and LDL Cholesterol raising

effects of palmitic acid are lower for vegetable oils than animal fats, because it is present predominantly in the *sn*-1 and *sn*-3 positions. On the other hand, the greatest dietary CVD risk culprit is TFAs, which is found in hydrogenated fats. Even low levels of TFAs (1 or 2% en) in the diet were associated with increase in the incidence of coronary heart disease.

21. Use of Hydrolysable Tannins from Sweet Chestnut (*Castanea sativa* Mill.) in Food and Feed Ingredients and Products. E. Bargiacchi¹, A. Romani², P. Pinelli², A. Scardigli², and S. Miele¹, ¹Consortium INSTM, Firenze, Italy, ²Phytolab, University of Firenze, Polo Scientifico e Tecnologico, Sesto Fiorentino, Italy.

Hydrolysable tannins, water-extracted from

sweet chestnut biomass (CHT) and membrane concentrated, have several remarkable effects as antioxidant, antimicrobial, and metal complexing agents. To formulate more use-oriented products, several whole water extracts and process streams, obtained by membrane separation technology, were analyzed and characterized. Each one of the analyzed fractions was tested for its antioxidant and antiradical activity. Selected extracts and fractions of chestnut tannins, and their mixtures with other polyphenols, were evaluated as antioxidant, antiradical, and antimicrobial agents for food and feed ingredients and products. A method to reduce mycotoxin in wheat grain was also developed. Some examples of applications are presented. All the applications are patent pending.

H&N-P: Sterols Posters

Chair: E.J. Parish Auburn University, USA

1. Oxidation of Steroidal Olefins at Room Temperature Using Vanadyl Acetylacetonate and TBHP. E.J. Parish and W. Grainger, Dept. of Chemistry and Biochemistry, Auburn University, Auburn, AL, USA.

Traditionally, oxidation of steroidal olefins has required esterification of hydroxyl groups to prevent formation of overoxidized side products. However, through the addition and removal of the protecting group, overall yield is decreased. Several catalyst used to oxidize steroidal olefins are also toxic and, or, degrade quickly. Vanadyl acetylacetonate, which is safe and readily available, was found to oxidize the allylic sites of Δ^5 steroidal olefins without protecting the hydroxyl group. Cholesterol, dehydroepandrosterone, pregnenolone, and related steroids were oxidized to 7-keto, products at moderately high yields at room temperature in a one pot reaction.

2. A Comparison of the Phytosterol Classes in Corn Oils and Sorghum Oils. R.A. Moreau¹ and V. Singh², ¹Eastern Regional Research Center, USDA, ARS, Wyndmoor, PA, USA, ²University of Illinois, Urbana, IL, USA.

Our laboratories have conducted extensive research on the composition of corn oil extracted using various organic solvents and using several aqueous processes from ground corn and from corn milling fractions. We also published a previous report (Singh et al, Cereal Chem 80:126-129, 2003) on the phytosterols and other lipids in wet milled fractions of grain sorghum (milo). In recent experiments we extracted sorghum oil from a variety of sorghum milling fractions using various solvents. The levels of phytosterols and other lipid classes in the various sorghum oils was impacted by both milling method and type of solvent.