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## Health and Nutrition Interest Area Technical Program Abstracts

### Table of Contents

H&N 1: Vitamin D and Human Health .....	2
H&N 2: The Role of Endocannabinoids and Fatty Acids in Shaping Human Health.....	3
H&N 3: The Role of Lipid Mediators in Essential Pro- and Anti-inflammatory Responses .....	5
H&N 3.1/PHO 3: Delivery Systems .....	7
H&N 3.2: Sterols .....	9
H&N 4: Health and Nutrition Needs of Children and Young Adults.....	11
H&N 5: General Health and Nutrition.....	14
H&N 5.1/EAT 5: Satiety and Sensory .....	17
H&N-P: Health and Nutrition Poster Session .....	19

*The presenter is the first author or otherwise indicated with an asterisk (\*).  
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## H&N 1: Vitamin D and Human Health

This session sponsored in part by Nu Skin and Nutrilite.

Chairs: H.A. Durham Zanetti, Nutrilite, Amway, USA; and R.E. Ward, Nutrition, Dietetics, & Food Sciences, Utah State University, USA

**The D-lightful Vitamin D for Good Health.** M.F. Holick<sup>1,2</sup>,  
<sup>1</sup>Boston Medical Center, USA, <sup>2</sup>Boston University School of  
Medicine, USA.

Most humans obtaining their vitamin D from sun exposure. There are a variety of factors that influence the cutaneous production of vitamin D including skin pigmentation, aging, time of day, season, altitude and latitude. To overcome the vagaries of the various factors influencing the cutaneous production of vitamin D an app dminder.info has been developed which provides information about how much vitamin D a person can make and provides the user when enough sun exposure has occurred to prevent a sunburn. Vitamin D deficiency is one of the most common medical conditions worldwide. It is not only has a negative consequence on the growing skeleton but also precipitates and exacerbates osteoporosis and the painful bone disease osteomalacia. Vitamin D deficiency has also been associated with a variety of acute and chronic illnesses including preeclampsia, asthma, infectious diseases, type 1 diabetes, multiple sclerosis, deadly cancers, type 2 diabetes, cardiovascular disease and neurocognitive dysfunction. The Endocrine Society recommends that all children over 1 year of age receive 600-1000 IUs vitamin D daily and adults 1500-2000 IUs daily.

### What Are Vitamin D Tests Actually Measuring?

J.A. Straseski<sup>1,2</sup>, <sup>1</sup>University of Utah, USA, <sup>2</sup>ARUP Lab., USA.

There is a great deal of interest in Vitamin D in both the popular and scientific literature. As testing volumes have increased, questions regarding available methods, what they

test, and overall test accuracy have been raised. The biases that have been reported for certain Vitamin D assays may confound monitoring of therapy, therefore knowledge of assay limitations is useful. We will review the current state of 25-hydroxyvitamin D testing including what forms should be measured to assess adequate Vitamin D storage, optimal decision points for defining deficiency, currently available methods and how they compare, measurement issues specific to Vitamin D testing, and current and future standardization efforts.

**Vitamin D: How to Define Deficiency.** R.I. Thadhani and C.E. Powe\*, Div. of Nephrology, Massachusetts General Hospital, USA.

Based on the IOM report that defined vitamin D deficiency as less than 20ng/ml, or the Endocrine Society that defined it as less than 30ng/ml, the prevalence of vitamin D deficiency is 20-40% worldwide. Vitamin D circulates bound to a binding protein, and based on the free hormone hypothesis, the fraction that is biologically active is that which circulates free and that which circulates bound to albumin. This latter fraction (combination of the two) is defined as bioavailable D. In this talk I will discuss the data to support the use of bioavailable D to define deficiency. This is especially important among Blacks, who have low total levels but have excellent bone health and low risk for fractures. I will also discuss the upcoming VITAL study, and various ancillary studies we are involved in. These include progression of diabetic nephropathy and progression of left ventricular hypertrophy.

## H&N 2: The Role of Endocannabinoids and Fatty Acids in Shaping Human Health

This session sponsored in part by DSM and Nestlé.

Chairs: C.J. Lammi-Keefe, Louisiana State University, USA; and M.A. Belury, Ohio State University, USA

### Modulators of the Endocannabinoid System as Nutritional and Therapeutic Medications.

A. Makriyannis, Center for Drug Discovery, Northeastern University, USA.

Among the lipid modulators endocannabinoids play a key role. Our current knowledge of the endocannabinoid system includes CB1 and CB2, two G<sub>i/o</sub> GPCRs involved in a number of signaling mechanisms. The endogenous molecules that modulate this biochemical system, which include ethanolamides as well as 2-glycerol esters of long fatty acids are represented by arachidonylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG). The levels of these endocannabinoids are modulated by a number of membrane associated enzymes, including the amidase, fatty acid amide hydrolase (FAAH) and the esterase, monoacylglycerol lipase (MGL), as well as by a transporter system that remains to be fully characterized.

Regulation of the levels of endocannabinoids and related lipids in different organs can provide significant nutritional or therapeutic opportunities. Modulation of the endocannabinoid system either directly (through CB1/CB2) or indirectly (through enzymatic or transport inhibition) provides opportunities for the design and development of small ligands capable of effecting physiological changes and, thus, serve as potential drug candidates. This target-based drug design utilizes a combination of computational and biophysical methods.

The biochemistry of the endocannabinoid system and approaches involving its modulation for nutritional or therapeutic opportunities will be discussed.

Research Supported by grants from NIH: DA009158, DA003801, and DA023142

### Peripheral Endocannabinoid Signaling in Diet-induced Obesity.

N.V. DiPatrizio, Div. of Biomedical Sciences, School of Medicine, University of California, Riverside, USA.

Endocannabinoid (eCB) signaling pathways throughout the body participate in the control of feeding and energy balance. For example, we reported that both tasting dietary fats and fasting drives food intake by initiating biosynthesis of eCBs in the small intestine of rats. These studies suggest that eCB signaling in the gut provides positive feedback to the brain, which serves functionally as a general hunger signal. Furthermore, recent experiments from our laboratory reveal that eCB pathways in the gut becomes dysregulated in diet-induced obesity. For these studies, mice were fed a test diet that mimicked the "Western Diet" (i.e., high levels of fats and carbohydrates) for 60 days, and eCB levels in the gastrointestinal tract and blood were analyzed via LC/MS/MS. Western Diet dramatically elevated eCB levels in the small intestine and circulation when compared to controls maintained on a standard lab chow. Ongoing studies are

investigating specific roles for this aberrant signaling in obesity. Collectively, this work highlights exciting novel approaches for the treatment of obesity via therapeutic interventions that target the eCB system in the gut.

NIH R00 DA034009 to NVD

Food intake is regulated by a complex interplay of many signaling pathways, including those under the control of the endocannabinoids [i.e., the body's natural "cannabis-like" molecules (eCBs)]. This presentation will review recent studies from the DiPatrizio laboratory that suggest an important role for gut-brain eCB signaling in obesity.

### Lipidomics Unveils the Healthy Biosignature of "Omega-3" Transgenic Mice.

G. Astarita<sup>1</sup> and J. Kang<sup>2</sup>, <sup>1</sup>Georgetown University, USA, <sup>2</sup>Harvard Medical School, USA.

Omega-3 intake has been linked to health benefits and the prevention of many chronic diseases. Current dietary intervention studies using fish oils often lack of appropriate control diets, hindering a correct evaluation of the physiologic effects deriving from high omega-3 intake. Here we used the fat-1 transgenic mouse model to evaluate the molecular phenotype of long-term omega-3 supplementation in a controlled manner. Such transgenic mouse is able to convert omega-6 to omega-3 fatty acids protecting it against a wide variety of diseases including chronic inflammatory diseases and cancer.

Wild type and fat-1 mice were put under an identical 6-month diet containing 10% corn oil to mimic the modern Western diet, which is enriched in omega-6 and low in omega-3 PUFAs. We used high-throughput MS assays, including a prototype microfluidic MS platform, to conduct both untargeted and targeted lipidomic analyses of plasma samples from fat-1 and wild-type mice. Untargeted profiling showed a significant modulation of the cholesteryl esters composition. Targeted lipidomics profiling highlighted a marked increase in selected bioactive mediators, corresponding to the activation of CYP450 pathway. In conclusion, our study highlights a panel of lipid mediators as potential biomarkers of omega-3 PUFAs intake, suggesting their involvement in the health benefits associated with a balanced omega-3/omega-6 ratio.

### Changes in Tissue Omega-3 Content Modulate Endocannabinoid Biosynthesis.

G. Carta, E. Murru, C. Manca, S. Lisai, L. Muredda, A. Sirigu, D. Demurtas, and S. Banni\*, Dip. di Scienze Biomediche, Università di Cagliari, Italy.

The endocannabinoid system contributes to the regulation of lipid and energy metabolism influencing body composition. Overweight and obese subjects are often characterized by high levels of endocannabinoids in plasma associated to dyslipidemia and unfavorable body fat

deposition. Dietary n-3 HPUFAs by modulating arachidonic acid availability in phospholipids, may modify the levels of endocannabinoid biosynthetic precursors. Our studies in experimental models and humans indicate that dietary strategies aimed at increasing the incorporation of n-3 HPUFAs in tissue phospholipids influenced endocannabinoid levels and ameliorated dyslipidemia and body fat distribution. However, this was not merely the result of a decreased arachidonic availability, but rather to a modified n-6/n-3 HPUFA ratio irrespective of the absolute levels of arachidonic acid in phospholipids.

Therefore, the biosynthesis of the endocannabinoids is modified by dietary fatty acids and their tissue metabolism, influencing the onset of the metabolic syndrome and its cardiovascular consequences.

**The Putative Role of the Endocannabinoid System on Sleep Modulation.** E. Murillo-Rodríguez, Lab. of Molecular & Integrative Neuroscience, School of Medicine Health Sciences Div., Anahuac Mayab University, Mexico.

During the 1990s, transmembrane proteins in the central nervous system (CNS) that recognize the principal compound of marijuana, the delta-9-tetrahydrocannabinol ( $\Delta^9$ -THC) were discovered. These receptors were classified as CB<sub>1</sub> and CB<sub>2</sub>. At this date, it has been widely accepted that specific endogenous lipids bind to the CB<sub>1</sub>/CB<sub>2</sub> receptors. These lipids have been named endogenous cannabinoids or endocannabinoids and include oleamide, *N*-arachidonylethanolamine (ANA), 2-arachidonylglycerol, virodhamine, noladin ether and *N*-arachidonyldopamine. Endocannabinoids promote cannabinomimetic effects by modulating diverse neurobiological functions, including sleep. For instance, administrations of ANA promote sleep whereas the blockade of CB<sub>1</sub> receptor promotes wakefulness. From

the pharmacological and pharmaceutical perspective, the endocannabinoid system might be considered as an effective approach to the prevention and management of sleep disturbances such as insomnia or excessive somnolence.

Acknowledgements: This work was supported by funding (given to E.M.R.) from Escuela de Medicina, Universidad Anáhuac Mayab.

**Farmed Atlantic Salmon (*Salmo salar*) Influences Lipoprotein Concentration and Particle Size in Healthy Men and Women.**

S.K. Raatz<sup>1,2</sup>, L.K. Johnson<sup>1</sup>, and M.J. Picklo<sup>1</sup>, <sup>1</sup>USDA, ARS, Grand Forks Human Nutrition Research Center, USA, <sup>2</sup>University of Minnesota, USA.

Farmed Atlantic salmon is a good source of n-3 fatty acids which have known positive lipid modifying effects; however, little is known about the effect of salmon intake on lipoprotein particle size and concentration. Adult participants (n=19) were enrolled in a cross-over designed clinical trial evaluating lipoprotein responses to dose-dependent intake of farmed Atlantic salmon. In random order, participants were assigned to 90, 180, and 270g of salmon twice weekly for 4-week treatments. Twice weekly intake of farmed Atlantic salmon at all intake levels reduced triglyceride concentrations and increased HDL-cholesterol concentrations. Measures of serum lipoprotein particle (P) concentration and size were modified in response to salmon intake at all levels. The concentrations of large VLDL-P and CM-P were reduced and the large LDL-P concentrations were increased in a dose dependent manner. The mean size of VLDL-P was reduced and that of LDL-P was increased after consuming salmon. Twice weekly intake of farmed Atlantic salmon influences plasma lipids and serum lipoprotein particle size and concentration in a manner associated with cardiovascular disease risk reduction.

## H&N 3: The Role of Lipid Mediators in Essential Pro- and Anti-inflammatory Responses

This session sponsored in part by Johnson & Johnson Consumer Inc. and Waters Corp.

Chairs: A.P. Kitson, University of Toronto, Canada; and K.D. Stark, University of Waterloo, Canada

### **The Role of Bioactive Lipid Mediators in the Regulation of Cutaneous Inflammation.** A.C. Kendall and A. Nicolaou, Manchester Pharmacy School, University of Manchester, UK.

The skin is the body's barrier against the environment, and employs a unique profile of lipids to prevent water loss and protect against external insults such as infection and ultraviolet radiation. From structural ceramides, to smaller lipid mediators including eicosanoids and related docosanoids and octadecanoids, and the endocannabinoids, the skin produces a huge array of lipid species with biological activity. The polyunsaturated fatty acids from which these derive are provided to the skin systemically, and so the profile depends markedly on diet, as well as local cell populations and enzyme expression. Interactions between different lipid pathways add to the complexity of the cutaneous lipid network. Using targeted mass spectrometry-based lipidomics, we have explored the lipids involved in maintaining skin homeostasis under normal conditions, as well as changes in the lipid profile evident in a number of inflammatory skin conditions, including sunburn, wound healing, and irritant dermatitis. Additionally, we have studied the effects of nutritional intervention on the cutaneous environment, exploring dietary fatty acid supplementation as a potent tool with which to manipulate the bioactive lipid profile. Further elucidation of cutaneous lipid networks will provide additional mechanistic insight to skin health, and help identify biomarkers and therapeutic targets for skin disease.

### **Regulation of Brain Oxidized Linoleic Acid Metabolite Concentrations by Diet and Hypoxia.** A.Y. Taha, Dept. of Food Science & Technology, University of California, Davis, USA.

Omega-6 linoleic acid (LA) is a precursor to oxidized linoleic acid metabolites (OXLAMs) whose regulation and function in brain are not known. One way to test the involvement of OXLAMs in brain signaling is to determine whether mechanisms exist to regulate their formation in brain. The present experiments tested the hypothesis that dietary LA or hypoxic stress regulate brain OXLAM concentrations. Liquid chromatography coupled to tandem mass spectrometry was used to quantitate OXLAM concentrations in rat brain following graded increments in dietary LA or acute hypoxia. Increasing dietary LA from 0.4% to 10.5% energy increased unesterified OXLAM concentrations without altering concentrations of LA's elongation-desaturation product, arachidonic acid. Acute hypoxia induced regional increases in OXLAM concentrations. The response of brain LA metabolites to diet and hypoxic stress suggest regulatory pathways that modulate brain OXLAM metabolism and possible roles in brain signaling.

### **Effect of Dietary Fat Composition on the Development and Severity of Metabolic Inflammation.** T. Xu, K.H. Hintze, M. Lefevre, and R.E. Ward\*, Nutrition, Dietetics, & Food Sciences, Utah State University, USA.

Does the PUFA composition of the US diet affect acute and chronic inflammatory processes? Four diets were formulated to mimic high and low n-6 and n-3 intakes based on US intakes in a 2x2 design. In Experiment 1, mice were fed for 28d and inflammation was induced with i.p. injection with LPS. In Experiment 2, mice were fitted with osmotic pumps to deliver a chronic, low-grade LPS inflammatory stress and fed for two months. In Experiment 1, high n-6 diets promoted food intake, weight, and fat mass gain. However, in the acute inflammatory stress, n-3 fatty acids were associated with a more vigorous cytokine response. In Experiment 2, n-3 fatty acid intake was positively associated with fasting glucose, but higher glucose sensitivity. In adipose, on the other hand, high n-6 fatty acid intake promoted an inflammatory phenotype with upregulation of MCP-1, HMGB1, NFKB, and TNF $\alpha$ . A limitation of the LPS pump delivery method is the length of duration (8 weeks), and it will be interesting to see longer-term effects. Nonetheless, these data indicate that modification of the plant-derived PUFA composition of the diet, within levels consumed in the US, affects both metabolism and susceptibility to inflammatory stress.

### **Oxygenated Bioactive Lipids Derived from Fatty Acids: Composition Data Reveals Novel Effects of Dietary Fatty Acids.** H. Aukema, University of Manitoba, Canada.

Bioactive lipid mediators synthesized from fatty acids are major mediators of fatty acid effects on physiology. A major class of these bioactive lipids is referred to as oxylipins because they are formed by metabolism of fatty acids into oxygenated metabolites such as prostaglandins, thromboxanes, leukotrienes, resolvins, protectins, and many others. While early studies focused only on the few oxylipins formed from arachidonic acid, recent advances have revealed the presence of hundreds of these oxylipins derived from many polyunsaturated fatty acids, including those found in high amounts in edible oils. Our studies on the composition of tissue oxylipins have revealed that the oxylipin composition cannot reliably be predicted from fatty acid composition, possibly due to differences in the synthesizing enzyme levels and apparent fatty acid selectivity of these enzymes. Examples of both similar and diverging conclusions based on tissue oxylipin compared to fatty acid composition will be discussed. These examples will demonstrate how oxylipin data compared to fatty acid data can potentially lead to potential therapeutic interventions in inflammatory renal disease, differing conclusions on inflammatory effects of dietary fatty acids and differing conclusions on fatty acid metabolism.

**Specialized Pro-resolving Mediator Biosynthesis from Omega-3 Fatty Acids Brings Resolution of Inflammation.**

R.E. Abdulnour<sup>1,2</sup>, <sup>1</sup>Brigham & Women's Hospital, USA, <sup>2</sup>Harvard Medical School, USA.

Acute inflammation is the host response to tissue injury in an attempt to restore homeostasis. If left unchecked, acute inflammation can injure healthy host bystander tissues and cause organ damage. In health, specialized proresolving mediators (SPMs) are enzymatically derived from essential fatty acids to restrain the inflammatory response and signal for resolution. In murine models of acute lung inflammation, lipid mediator metabolomics uncovered selective temporal regulation of SPM generation. In a model of acute sterile lung inflammation, maresin 1 (MaR1; 7R,14S-dihydroxy-4Z,8E,10E,12Z,16Z,19Z- docosahexaenoic acid)

biosynthesis was increased early after lung injury, and dependent on a previously undescribed vascular biosynthetic route during human platelet–neutrophil interactions. Intravascular MaR1 was organ-protective, leading to decreased lung neutrophils, edema, and proinflammatory mediators. In a murine model of pneumonia, aspirin-triggered resolvin D1 (AT-RvD1; 7S,8R,17R-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid) production was temporally regulated. AT-RvD1 engaged host-responses for enhanced clearance of gram-negative bacteria, and resolution of lung inflammation. Together, these pro-resolving actions suggest potential new therapeutic approaches for inflammatory diseases that emphasize SPMs and their mechanisms.

## H&N 3.1/PHO 3: Delivery Systems

This session developed in conjunction with the Phospholipid Division.

This session sponsored in part by Johnson & Johnson Consumer Inc.

Chairs: M. Rebmann, Perimondo, USA; and K. Mahmood, Johnson & Johnson Consumer Inc., USA

### Topical Delivery Enhancement of Actives into Skin by Lipid Based Vesicular Systems. J. Paturi, Johnson & Johnson Consumer Inc., USA.

Delivering actives into skin is challenging due to resistance offered by the stratum corneum. A wide variety of enhancement techniques continue to evolve to address this challenge ranging from simple solvents acting as penetration enhancers to the more complicated nanoparticles. Developing a delivery system which can incorporate small as well as large molecules irrespective of their polarity is important to enhance penetration across biological membranes. Liposomes are vesicular systems with the ability to encapsulate both hydrophilic and lipophilic actives and enhance their topical penetration profiles. Ample studies have already established this with phospholipid based liposomes. This talk focusses on non-phospholipid vesicular systems and their characterization. It also provides insights into delivering actives *in vitro* through these vesicular systems for skin benefits.

### *In vitro* Digestibility and Bioaccessibility of Lipid-based Delivery Systems Obtained via Enzymatic Glycerolysis:

**A Case Study of Rosemary Extract Bioactivity.** M. Corzo-Martínez<sup>1</sup>, T. Vargas<sup>1</sup>, L. Vázquez<sup>2</sup>, G. Reglero<sup>1,2</sup>, A. Ramírez de Molina<sup>1</sup>, and C.F. Torres<sup>2</sup>, <sup>1</sup>Inst. of Food Science Research, CIAL (CSIC-UAM), Spain, <sup>2</sup>IMDEA-Food Inst. CEI (UAM-CSIC), Spain.

Association to lipid-based delivery systems (LDS) is a formulation strategy usually used for improving bioaccessibility and, hence, efficacy *in vivo* of bioactive compounds. In this sense and attending to the multiple biological activities of alkylglycerols (AKG), their utilization as LDS provides additional interest. However, AKG show poor digestibility which limits their applicability. This highlights the importance of the development of effective lipid formulation strategies.

The aim of this work was to study the effect of enzymatic glycerolysis on digestibility of ratfish liver oil rich in AKG, as well as the glycerolysis product capability as LDS. For that, it was combined with a rosemary extract and bioaccessibility and bioactivity of the formulations was assessed. An *in vitro* intestinal digestion model simulating *in vivo* conditions was used for digestibility and bioaccessibility studies and antiproliferative activity was determined using human pancreatic cancer cells.

The glycerolysis product showed higher digestibility than the original oil. The combination of rosemary extract with this product improved its bioaccessibility.

Enzymatic glycerolysis is, therefore, an efficient formulation strategy leading to more bioaccessible and

bioactive LDS which could be used to design effective functional foods.

### Increasing Bioavailability of Lipophilic Nutraceuticals:

**The Effects of Mixed Micelles.** J. Chen<sup>1,2</sup>, F. Li<sup>1</sup>, D.J. McClements<sup>1</sup>, and H. Xiao<sup>1</sup>, <sup>1</sup>University of Massachusetts Amherst, USA, <sup>2</sup>State Key Lab. of Food Science & Technology, Jiangnan University, China.

Poor bioavailability of lipophilic nutraceuticals negatively affects their health-promoting efficacy. Polymethoxyflavones (PMFs) is a group of lipophilic nutraceuticals with many beneficial activities. In this study, we investigated the effects of mixed micelles in the intestinal uptake of PMFs. Mixed micelles from canola oil and olive oil were obtained by *in vitro* digestion of canola oil and olive oil-based nanoemulsions, respectively. 5, 3', 4',-Tridemethylnobiletin (THN, a representative PMF) was then dispersed in the micelles. The micelles were then added to culture media and incubated with intestinal epithelial cell (Caco-2) growing on glass slides. At different incubation times, the cells were fixed and stained with 2-Aminoethyl diphenylborinate (DPBA). Our results showed when excited by laser with certain wavelength, the DPBA and THN conjugate were fluorescent. The cells were observed under a fluorescent microscope. In the mean time, micelles-treated cells were also harvested, processed, and analyzed with flow cytometer. Results from both fluorescent microscope and flow cytometer analysis showed that mixed micelles significantly increase cellular uptake of THN. This study is the first to use DPBA to monitor the intestinal cellular uptake of PMF, and to show the potential of mixed micelles in enhancing cellular uptake of PMF.

### Characterization of Intestinal Digestion of Ceramide 2-aminoethylphosphonate, a Marine Sphingolipid.

N. Tomonaga, D. Qi, Y. Manabe, and T. Sugawara, Div. of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Japan.

Ceramide 2-aminoethylphosphonate (CAEP) is one of the major sphingolipids of marine origin and generally contained in Mollusca, such as squid and shellfish consumed around the world. CAEP has unique structures such as a phosphorus-carbon bond in the polar head and triene type of sphingoid bases. Notwithstanding, it is known that some dietary sphingolipids are digested and absorbed in the intestinal tract, there is no information about CAEP. To investigate the efficacy of dietary CAEP, we examined its digestion in the intestine of mice.

CAEP was extracted and purified from the skin of jumbo flying squid *Dosidicus gigas*. CAEP was incubated with homogenate of small intestinal mucosa of mouse. Ceramides

and free sphingoid bases which are the degradation products from CAEP were determined by LC-MS.

We found that intestinal mucosa can remarkably hydrolyze CAEP to ceramides and sphingoid bases. Our results suggest that dietary CAEP is probably hydrolyzed in digestive tract and generated sphingoid bases may be absorbed from intestinal epithelial cells, similar to other dietary sphingolipids.

#### **Improved Stabilisation of Concentrated Oil-in-Water Emulsions by Complexing Soy Protein with $\kappa$ -carrageenan.**

I. Tavernier<sup>1</sup>, P. Van der Meeren<sup>2</sup>, K. Dewettinck<sup>1</sup>, and A.R. Patel<sup>1</sup>, <sup>1</sup>Lab. of Food Technology & Engineering, Ghent University, Belgium, <sup>2</sup>Particle & Interfacial Technology Group, Dept. of Applied Analytical & Physical Chemistry, Belgium.

Biopolymers such as hydrophilic proteins are commonly used stabilizers in O/W emulsions. However, for the development of highly concentrated emulsions ( $\phi_{oil} \geq 0.6$ ), the stabilization provided by only proteins is often insufficient. The emulsion stabilizing properties of proteins can markedly be enhanced by complexing them with suitable polysaccharides. In this work, we report for the first time the use of soy protein isolate –  $\kappa$ -carrageenan (SPI: $\kappa$ -CG) complexes as stabilizing agents for concentrated oil-in-water emulsions prepared at  $\phi_{oil} = 0.6$ .

The colloidal stability of concentrated emulsions stabilized with SPI was compared to emulsions stabilized by SPI: $\kappa$ -CG complexes using advanced microscopy and light scattering experiments. These measurements showed that a better long-term stability was achieved by using the SPI: $\kappa$ -CG complexes compared to using only SPI. Rheological studies revealed that the stability enhancement effect was due to interfacial accumulation of particles rather than the increase in the bulk viscosity. Cryo-SEM analysis and Confocal Scanning Laser Microscopy of the emulsions confirmed the formation of SPI: $\kappa$ -CG particles and visualized their interfacial accumulation.

The potential of using SPI: $\kappa$ -CG complexes as stabilizers for concentrated emulsions could open up new opportunities for the development of emulsifier-free food products.

#### **Interfacial Behavior of Milk Polar Lipids and Their Influence on Gastric Lipase Adsorption: A Natural Effective Delivery System.**

C. Bourlieu<sup>1,4</sup>, W. Mahdoueni<sup>1</sup>, G. Paboeuf<sup>2</sup>, S. De Oliveira<sup>1</sup>, S. Pezenec<sup>1</sup>, J.F. Cavalier<sup>3</sup>, S. Bouhallab<sup>1</sup>, D. Dupont<sup>1</sup>, P. Villeneuve<sup>4</sup>, F. Carrière<sup>3</sup>, and V. Vié\*<sup>2</sup>, <sup>1</sup>INRA-AGROCAMPUS, France, <sup>2</sup>IPR Inst. of Physics, Rennes University, France, <sup>3</sup>CNRS, Aix-Marseille Université, France, <sup>4</sup>CIRAD, UMR IATE, France.

The polar lipids in human milk fat globule membranes condition the accessibility and enzymatic digestibility of milk lipids. Their substitution by bovine polar lipids to produce infant formulas biomimetic of human milk has been suggested. However a comparison of the interfacial behavior of bovine and human polar lipids and of their interaction with gastric lipase is lacking.

Such comparison is here undertaken using complementary biophysical tools: tensiometry, ellipsometry, Brewster angle and atomic force microscopy, in the presence or in the absence of gastric lipase. Polar lipids extracts were obtained from a pool of human milk (n=5) or bovine butterserum and analyzed using GC and HPLC.

Human milk polar lipids (HMPL) had a higher compressibility than bovine milk polar lipids (BMPL). Despite the presence of liquid condensed domains in both extracts, their morphological aspect and growth differed in relation with the lipid composition. Lipid phase separation impacted on gastric lipase adsorption in both extracts with an exclusive adsorption onto the liquid expanded phase. Despite differences in their physico-chemical properties, both polar lipid extracts share close interfacial reactivity in gastric conditions. This biophysical characterization will be broadened to other milk polar lipids interesting for human milk substitution.



## H&N 3.2: Sterols

Chairs: L. Nyström, ETH Zurich, Switzerland; and J.K. Winkler-Moser, USDA, ARS, NCAUR, USA

**Sterols, an Ancestral Cytochrome P450 and Evolution to Resistance in Our Time.** S.L. Kelly (*George Schroepfer Medal Award Winner*), Centre for Cytochrome P450 Biodiversity, Inst. of Life Science, Medical School, Swansea University, UK.

Sterols can be used as biomarkers for present day life forms and in geological time to illustrate eukaryote emergence about two billion years ago as a possible route to detoxification of atmospheric oxygen. In fungi, the predominant sterol is ergosterol, the synthesis of which requires the oxygen requiring the enzyme cytochrome P450. As with so many areas of research, yeast provided fundamental insight in this biosynthetic pathway allowing the isolation of a cytochrome P450 family 51 (CYP51) gene for the first time. Since this discovery, the essential nature of CYP51 across species has been determined and consequently the interplay between it and a range of antifungals in healthcare and agriculture has been much studied. We now appreciate that the global burden on human health from fungi is similar to that of malaria and TB and there is an urgent need for new antifungals and to manage resistance to CYP51 inhibitors by understanding mechanisms and consequences. CYP51 evolution has been rapid giving rise to resistant forms both in the clinic and in treating plant pathogens and the relationship between both uses is the subject of debate.

**Translational Opportunities in Metabolic Engineering Plant Sterol Metabolomes.** W. Zhou<sup>1,2</sup>, J. Li<sup>2</sup>, D. Zhang<sup>2</sup>, J. Batley<sup>2</sup>, M. Anderson<sup>4</sup>, K. Wu<sup>5</sup>, S.M. Smith<sup>3,1</sup>, and W.D. Nes<sup>1</sup>, <sup>1</sup>Texas Tech University, USA, <sup>2</sup>University of Western Australia, Australia, <sup>3</sup>University of Tasmania, Australia, <sup>4</sup>Hexima, Australia, <sup>5</sup>Chinese Academy of Agricultural Sciences, China.

Phytosterols are considered one of the most important natural products; as universal membrane inserts and structure-specific signal molecules they control plant growth, resistant to abiotic and biotic stresses and produce desirable seed oils while as essential nutrients of insect pests they contribute to crop plant loss. Exciting recent new developments in the enzymology and molecular biology of phytosterols offer numerous opportunities to re-engineer the plant sterol metabolome to yield value-added traits. For example, successful targets of such modifications are soybean seeds genetically modified for increased sitosterol (24 $\alpha$ -ethyl sterol) production- relevant to human health and Arabidopsis thaliana plants genetically modified for increased clerosterol (24 $\beta$ -ethyl sterol) production, and stigmasterol – relevant in resistance to insect attack. Herein, we summarize recent progress in sterol biosynthesis as a principle model to fashion transgenic plants with novel sterol characteristics and discuss the factors that control productivity, focusing on the structural similarities and differences in phytosterols, rate-limiting enzyme steps in phytosterol biosynthesis (plants) and metabolism (insects) and biotechnological approaches

employing variant isoprenoid-sterol genes from green algae to animals.

**Susceptibility of Plant Sterols for Oxidation, Formation of Plant Sterol Oxidation Products in Foods, and Their Estimation of Daily Intake.** E.A. Trauwtein, Y. Lin, and D. Knol, Unilever Research & Development Vlaardingen, The Netherlands.

Plant sterols (PS) are compounds in plant-derived foods. At intakes between 1.5-3g/d, PS lower LDL-cholesterol by 7-12% (Ras et al., Br J Nutr 2014) by partially inhibiting intestinal cholesterol absorption. PS are subject to thermal oxidation forming oxidised PS (or PS oxidation products POP). We have systematically evaluated POP contents of foods with added PS and stanols (Lin et al., Eur J Lipid Sci Technol 2015). Heating temperature and time, the chemical form (i.e. free PS or esters), the amount of PS/stanols added, and the food matrix were important determinants for POP formation. Recently, we measured oxidation susceptibility of PS and POP contents in a variety of foods including meats, fish, vegetables, potato, and egg prepared with typical cooking and baking methods using margarines without and with added PS. In baked foods prepared with both margarines, median POP contents (0.12-0.24mg per portion) and PS oxidation rates were low. In cooked foods, median POP contents were 0.57 vs. 1.42mg per portion using margarines without and with added PS, respectively. POP contents did not exceed 21mg per portion. Daily upper POP intake considering a PS intake of 3g/d and a (90% quantile) PS oxidation rate of 1.6% was estimated to be 48mg/d. However, actual intakes from consuming foods prepared with PS-added margarine may be much lower.

**Distinct Functions of Cycloartenol-derived Sterols in Plants.**

H. Schaller, CNRS, IBMP, Université de Strasbourg, France.

Plants present distinct sterol biosynthetic features compared to metazoans as they produce multiple end-products. Their sterol profiles display several 24-alkyl- $\Delta^5$ -sterols: proportions of individual sterols vary quite a lot relatively to organs or even more to cell types. In addition, plants use a mandatory route to convert 2,3-oxidosqualene to cycloartenol then obtusifolliol via seven succeeding enzymatic steps, thereby defining a 9 $\beta$ ,19-cyclopropylsterol biosynthetic segment. This is shown to exert a specialized function in the gametophyte phase of the plant life cycle. It is proposed that the *raison d'être* of the chemical diversity of sterols exceeds the role of these lipids as reinforcers of higher plant cellular membranes. Indeed, a breakdown of sterol homeostasis led in all cases to severe and multiple damages throughout the life cycle. Plants harboring chemical or genetic inhibitions of the sterol pathway have distinct morphogenetic and developmental deficiencies according to

steroidogenic genes or enzymes that are affected. These biological effects are mostly a reduced polar auxin transport, an altered cytokinesis and cell elongation, a perturbed microRNA biogenesis or function, and at the organ level deficiencies in vascular development, for instance. Here, we will discuss physiological implications of 9 $\beta$ ,19-cyclopropylsterols and 24-alkyl- $\Delta^5$ -sterols towards a refined understanding of plant sterol biology.

**Radical Entrapping for Studying the Oxidation of Phytosteryl Phenolates.** M.J. Lehtonen, D. Zhu, S. Boulos, and L. Nyström, Inst. of Food, Nutrition, & Health, ETH Zürich, Switzerland.

Phytosterols and their conjugates are prone to oxidation during food processing and storage. In order to control their degradation, it is essential to study their oxidation mechanisms, especially the initial stages of the reaction. Our analytical approach utilizes spin trapping and mass spectrometry to detect the site of radical formation in phytosterols. The formed radicals are entrapped with DMPO (5,5-Dimethyl-1-pyrroline N-oxide) and their structures are further studied with +ESI-MS/MS and UHPLC-MS/MS. By this method, we have been able to detect carbon, peroxy and alkoxy radicals of sterol phenolates, and indicate their formation sites. For example, specific fragments of stigmasteryl ferulate ion  $[M+DMPO]^+$  with  $m/z$  701.5 and 703.5/705.5, and ion  $[M+2DMPO]^+$  with  $m/z$  798.5 indicated the location of carbon radical formation site in the ring structure of sterol, in the ferul moiety or in the side chain of sterol, respectively. In addition, the fragments of ion  $[M+O_2/O+DMPO+Na]^+$  with  $m/z$  756.5/740.5, and ion  $[M+O_2/O+2DMPO+Na]^+$  with  $m/z$  873.6/857.6 revealed the formation sites of peroxy and alkoxy radicals, respectively. This powerful combination of radical entrapping and mass spectrometry enables detailed future studies on the oxidation behavior of phytosteryl conjugates, and can be applied to study oxidation mechanism under varying conditions.

**Plant Sterols—Market Perspective and Emerging Research.**

J. Moritz, BASF Corp., USA.

Plant sterols are prominent examples of nutraceutical ingredients supported by science, with hundreds of clinical trials demonstrating their cholesterol-lowering efficacy. We will provide an industry perspective on the cholesterol-lowering market for plant sterols and present the public

health need motivating further sterols consumption. Open questions around safety and impact on cardiovascular endpoints will be discussed. An overview of emerging research in plant sterols will then be presented, with particular focus on characteristics of responders, blood triglyceride lowering, Omega-3-plant sterols combination products, and impact on liver health.

**Mechanism-based Inhibition of Ergosterol Biosynthesis in *Acanthamoeba castellanii* and Its Therapeutic Implications.**

C.D. Thomas<sup>1</sup>, S.L. Kelly<sup>2</sup>, M. Chaudhuri<sup>3</sup>, and W.D. Nes<sup>1</sup>,  
<sup>1</sup>Center for Chemical Biology & Dept. of Chemistry & Biochemistry, Texas Tech University, <sup>2</sup>Centre for Cytochrome P450 Biodiversity, Inst. of Life Science, College of Medicine, Swansea University, UK, <sup>3</sup>Dept. of Microbiology & Immunology, Meharry Medical College, USA.

In this study we investigate the amoebicidal activity of the steroidal inhibitor 25-azalanosterol which blocks sterol C24-methylation, and compare its efficacy to the medical azole itraconazole which blocks sterol C14-demethylation, against *Acanthamoeba castellanii* (Ac), the pathogenic protozoan responsible for corneal infections. Bioinformatic and chemical analyses reveal that AC operates a plant-based ergosterol biosynthesis pathway whereby trophozoites synthesize cycloartenol; the protosterol is converted to ergosterol (C<sub>28</sub>) and 28-ethyl ergosterol (C<sub>29</sub>), amphipathic compounds that act as membrane inserts. Alternatively, cysts contain predominantly the C<sub>29</sub>-poriferasterol and C<sub>28</sub>- and C<sub>29</sub>-ring B aromatic phenanthrenes derived from metabolism of the C<sub>28</sub>- and C<sub>29</sub>- $\Delta^{5,7}$ -sterols. Treatment of trophozoites with 25-azalanosterol and itraconazole resulted in a dose-dependent inhibition of trophozoite growth affording IC<sub>50</sub>/MIC (cell kill) values of 10 nM and 500 nM and 50 nM and 3.5  $\mu$ M, respectively. A small population of trophozoites treated with the test inhibitors transformed into nonviable cysts that possessed uncommon sterol profiles. Given that 25-azalanosterol supplied to cultured human cells (HEK) has no effect on growth or cholesterol biosynthesis to 50  $\mu$ M - that thereby, affords a selective index (SI) for the inhibitor of 100 - or effected mice health fed the drug (Haubrich et al. J. Lipid Res. 56, 331-3410), reveals 25-azalanosterol is a highly potent and safe anti-amoebic agent. We propose steroidal inhibitors of *Acanthamoeba* ergosterol biosynthesis could provide new leads and a new treatment strategy (e.g., in combination with medical azoles) for chronic keratitis.

## H&N 4: Health and Nutrition Needs of Children and Young Adults

This session is sponsored in part by Johnson & Johnson Consumer Inc.

Chairs: M.J. Picklo, USDA, ARS, Grand Forks Human Nutrition Research Center, USA; and M.L. Drewery, Louisiana State University, USA

### The Role of Omega-3 Fats in Neurodevelopment: Implications for ADHD and Comorbid Behavioral Disorders.

R.V. Gow, Section of Nutritional Neuroscience, National Inst. of Health, USA.

**Objective:** Neurodevelopment involves a number of sensitive processes occurring simultaneously including neuronal migration, neurogenesis, synaptogenesis and myelination, all of which implicate the role of omega-3 highly unsaturated fatty acids (HUFAs). Deficits in omega-3 are thought to increase risk for adverse neurodevelopmental trajectory outcomes including attention deficit hyperactivity disorder (ADHD), non-diagnostic attentional symptoms, aggressive and delinquent behaviors characteristic of conduct disorder, and mood symptoms including depressive disorders.

**Methods:** The evidence linking omega-3 HUFAs deficits in the pathogenesis of attentional and behavioral symptoms originates from: (1) animal and human studies, (2) studies of essential fatty acid blood levels in children and young adults with ADHD compared to controls, and (3) randomized, double-blind placebo-controlled, clinical trials of omega-3 HUFA supplementation.

**Results:** Several meta-analytic reviews have reported small-modest effect sizes for clinical efficacy of omega-3 in populations with ADHD, and associative behavioral and mood disorders. Compared to our paleolithic diets, modern diets are relatively depleted in brain critical nutrients which may adversely impact neurodevelopment and mental health.

**Conclusions:** Dietary adjustments to increase omega-3 and reduce omega-6 HUFA consumption are sensible recommendations for youth and adults based on general health considerations, while the evidence base for omega-3 HUFAs as potential psychiatric treatments is being developed.

**Maternal n-3 LCPUFA Status and Infant Heart Rate Variability.** M.L. Drewery<sup>1</sup>, A.V. Gaitán<sup>1</sup>, R.I. Pinkston<sup>1</sup>, S. Spedale<sup>2</sup>, and C.J. Lammi-Keefe<sup>3,4</sup>, <sup>1</sup>Louisiana State University, USA, <sup>2</sup>Infamedics, USA, <sup>3</sup>LSU AgCenter, USA, <sup>4</sup>Pennington Biomedical Research Center, USA.

Maternal intake of n-3 long chain polyunsaturated fatty acids (LCPUFA) during pregnancy is related to developmental advantages for the infant. Currently, there is a body of literature suggesting postnatal n-3 LCPUFA intake positively affects maturation of the autonomic nervous system, as reflected in measures of infant heart rate (HR) and heart rate variability (HRV). The last trimester of pregnancy is characterized by significant fetal brain growth and maturation, and is a critical period of vulnerability during which the underlying neuronal circuitry is sensitive to environmental insults, including nutritional deficits.

Accordingly, the aim of this study is to fill a gap in the literature and investigate whether maternal n-3 LCPUFA status, defined as erythrocyte eicosapentaenoic and docosahexaenoic acid concentrations, during the third trimester is associated with maturation of the autonomic nervous system, measured as HR and HRV, at 2 weeks, 4 months, and 6 months of age.

### Adequacy of n-3 and n-6 PUFA Intakes in European Children and Adolescents in Light of the Current Recommendations.

M. Fleith<sup>4</sup>, L. van Lieshout<sup>9</sup>, C. Campoy<sup>1</sup>, R. Mensink<sup>2</sup>, A. Eilander<sup>3</sup>, S. Eussen<sup>5</sup>, C. Petisca<sup>6</sup>, S. Forsyth<sup>7</sup>, G. Hornstra<sup>2</sup>, P.C. Calder<sup>8</sup>, I. Sioen<sup>10</sup>, and S. Lohner<sup>11</sup>, <sup>1</sup>University of Granada, Spain, <sup>2</sup>Maastricht University, The Netherlands, <sup>3</sup>Unilever Research & Development, The Netherlands, <sup>4</sup>Nestlé Research Center, Switzerland, <sup>5</sup>Danone Research Centre, The Netherlands, <sup>6</sup>Bunge Europe, Belgium, <sup>7</sup>DSM Nutritional Products Ltd., Switzerland, <sup>8</sup>University of Southampton, UK, <sup>9</sup>ILSI Europe, Belgium, <sup>10</sup>Ghent University, Belgium, <sup>11</sup>Pecs University, Hungary.

An overview on individual n-3 and n-6 PUFA intake in children is currently not available. The present study aims systematically reviewing dietary intakes of total and individual n-3 and n-6 PUFA in young European population, identifying the latest intake recommendations and highlighting gaps between intake and recommendations. The systematic review was performed according to PROSPERO guidelines. Twenty-eight studies conducted in 30 countries were recovered: three in infants 6-12 months, six in young children 1-3y, 11 in children 4-9y and eight in adolescents 10-18y. LA intake was below the EFSA recommendations of 4E% in 39% of the countries, with higher rates in adolescents, whereas ALA intake was below the recommendation of 0.5E% in 26% of the countries. In 88% of the countries, EPA and/or DHA intake was lower than the recommendations. The limited available data indicate that n-3 PUFA, especially EPA and DHA and to a lower extent n-6 PUFA intakes may be suboptimal in a significant part of the young population in Europe. Moreover, more nationally representative surveys are necessary to clarify the need for specific public health measures for these vulnerable groups in Europe.

### Docosahexaenoic Acid Status in Pregnancy is Lower in African-Americans Compared to Caucasians and Hispanics: Differences in Fatty Acid Metabolism.

A.V. Gaitán<sup>1</sup>, M.L. Drewery<sup>1</sup>, R.I. Pinkston<sup>1</sup>, C.A. Thaxton<sup>1</sup>, E. Seidemann<sup>2</sup>, K. Elkind-Hirsch<sup>2</sup>, and C.J. Lammi-Keefe<sup>1,3</sup>, <sup>1</sup>Louisiana State University, USA, <sup>2</sup>Woman's Hospital, USA, <sup>3</sup>LSU AgCenter, USA, <sup>4</sup>Pennington Biomedical Research Center, USA.

It is well established that docosahexaenoic acid (DHA,

22:6n3) benefits pregnancy and infant outcomes. We assessed DHA status in overweight (BMI=25.0-29.9kg/m<sup>2</sup>) pregnant women. Women's age, ethnicity, weeks of pregnancy, weight, and length were recorded. Dietary intakes were assessed by repeated 24h dietary recalls. Blood samples were collected (16.5-20 weeks of pregnancy) for red blood cells (RBC) fatty acid status, using gas chromatography. Women (n=21) were 19-34y of age; 62% were African-Americans (A-A), 29% Caucasians (C), and 9% Hispanics (H). On average, pregnant women consumed 72±63mg DHA/day. Age, ethnicity, and socioeconomic status did not affect the probability of achieving recommended DHA dietary intake (p>0.05). RBC DHA was 8.48±1.39wt%. By ethnic groups, A-A had lower RBC DHA (7.98±0.94wt%) versus C + H (9.29±1.68wt%) (p=0.05). RBC ALA (α-linolenic acid, 18:3n-3) did not differ between ethnic groups [0.11±0.04wt%, A-A and 0.11±0.03wt%, C+H (p>0.05)]. Only ethnicity affected the RBC DHA wt% (p=0.05). Our finding that ethnicity is a factor that influences n-3 DHA status in pregnant A-A women warrants further exploration.

**High Linoleic Acid Ready-to-use Therapeutic Foods (RUTF) Suppress Long Chain Omega-3 Status in Malnourished Toddler.** J.T. Brenna, Cornell University, USA.

RUTF are stable, low moisture, high fat (45-60%energy) peanut-based foods that are provided as the sole source of nutrition to children below age 5 years presenting at rural clinics with severe acute malnutrition. Physical recovery rates are greater than 90%, restoring normal weight and length growth. RUTF made by the usual recipe is high in omega-6 linoleic acid (LA) and low in omega-3 alpha-linolenic acid (ALA), suggesting that long chain omega-3 needed for brain development will be suppressed. Conventional RUTF has 21% LA and 0.4% ALA (w/w); using high oleic (HO) peanuts we reformulated RUTF to contain 13% LA and 13% ALA. In four weeks of a randomized controlled trial in Malawi, plasma phospholipid DHA and EPA dropped 25% in children (mean age=20 months) on conventional RUTF. Children on HO RUTF had stable DHA and EPA increased 60%, entirely from endogenous metabolism. Recovery from malnutrition was equivalent. Working independently and in parallel, another group reformulated RUTF to increase ALA with no change in LA; no improvement in plasma phospholipid was found. These human results match animal and in vitro studies on LA antagonism of omega-3 metabolism, and highlight the importance of dietary PUFA balance via modest LA levels.

**Cerebral and Hepatic Effects of Energy Restriction and Dietary n-3 Reduction in Growing Rats.** M.J. Picklo, USDA, ARS, Grand Forks Human Nutrition Research Center, USA.

Without dietary sources of long chain (LC) n-3 fatty acids, alpha-linolenate (ALA) is the precursor for docosahexaenoate (DHA). It is not known how energy restriction (ER) impacts ALA conversion to DHA. We tested the hypothesis that ER reduces LCn-3 content in growing rats. Male rats (23 days old)

were placed on AIN93G diets (4wks) made with soybean (ALA sufficient) or corn (ALA deficient) oils. For each diet, one group of rats (ER) was pair-fed at 75% of a control animal with ad libitum (AL) intake. AL rats on the corn oil diet had lower cerebral cortical linoleate (LA; 13%) and DHA (10%) compared to the soybean oil AL group. ER did not alter cortical LCn-3 status. Liver LCn-3 content was reduced in AL animals fed corn oil vs. soybean oil. ER reduced hepatic LA, ALA, and arachidonate regardless of oil. Corn oil had minor effects on cortical and hepatic expression of Fads1, Fads2, Elovl2, and Elovl5. ER reduced expression of uncoupling protein 3 (UCP3) by nearly 50% in cerebral cortex and liver. These data indicate that this level of ER does not exacerbate LCn-3 loss in brain and liver induced by ALA-deficient oils.

**Examining Changes in Fatty Acid Concentrations of Maternal Tissues Throughout Pregnancy and Postpartum in Rats Fed Diets with Different Levels of Fat and Docosahexaenoic Acid.**

D.M.E. Lamontagne-Kam, A. Chalil, J.J. Aristizabal Henao, and K.D. Stark\*, Dept. of Kinesiology, University of Waterloo, Canada.

Docosahexaenoic acid (DHA) is essential for proper brain development and fetal demand is high in the later stages of pregnancy. The effect of fetal demand on maternal tissue stores has not been examined comprehensively. Sprague-Dawley rats were fed either a standard rat chow diet or a Total Western Diet based diet with or without DHA. Rats were examined before pregnancy, at 15 and 20 days of pregnancy, and 7 days postpartum. Heart, liver, brain, adipose, placenta, and blood samples were collected and stored at -80°C until fatty acid analyses. Blood levels of DHA increased from baseline to day 28 of pregnancy and then decreased postpartum. This effect was higher when DHA was included in the diet. A similar pattern was observed in the liver, but the increase was limited in the chow and TWD without DHA. In the heart it appears that DHA decreased with pregnancy and then again with postpartum. In adipose, DHA rose during pregnancy and fell postpartum with chow and TWD with DHA, but there was a gradual decline in adipose DHA through pregnancy and postpartum. Interestingly, decreases in DHA appear to be the largest at postpartum. Further study on mechanisms of DHA tissue mobilization during pregnancy are needed, particularly the balance between mobilization and synthesis during postpartum.

**Maternal Docosahexaenoic Acid (DHA) Synthesis is Sufficient to Maintain Maternal Whole-body DHA Status During Pregnancy in Rats.** A.P. Kitson, A.H. Metherell\*, A.F. Domenichiello, and R.P. Bazinet, University of Toronto, Canada.

Transfer of docosahexaenoic acid (DHA) from the mother to the fetus is essential for fetal neurodevelopment. However, it is not known if DHA synthesis from ALA is sufficient to prevent maternal DHA depletion during pregnancy. Female rats were maintained on a DHA-free diet

providing 2% of fatty acids as ALA. At postnatal day 56 rats were randomized to three groups: sacrificed for baseline measures (n=7), mated and sacrificed at post-conception day 18 (n=8), and age-matched virgin controls (n=8). All rats were sacrificed by head-focused microwave fixation and DHA status of carcass, liver, cortex, and adipose depots was assessed. After 18 days of pregnancy, fetal+placental DHA represented 7.7±1.2% of maternal carcass DHA. There were no differences in DHA accretion rates in cortex, carcass or

inguinal and perirenal adipose pools, while periuterine adipose DHA was 28% lower in pregnant rats (p<0.05). Hepatic DHA accretion rate was higher in pregnant compared with non-pregnant rats (2153±305nmol/d and -502±184nmol/d, respectively, p<0.05). This indicates that DHA synthesis can be sufficient to prevent depletion of most maternal DHA stores in pregnancy despite the high demand of fetal development. Direct measures of DHA synthesis are currently underway to be presented at the meeting.

## H&N 5: General Health and Nutrition

Chairs: I. Vieitez, Universidad de la República (UDELAR), Uruguay; and A. Gugliucci, Touro University-California, USA

**Dietary Saturated Fat Promotes Omega-3 Polyunsaturated Fatty Acid Incorporation into Human Plasma and Erythrocytes.** C.B. Dias<sup>1,2</sup>, L.G. Wood<sup>1,2</sup>, and M.L. Garg<sup>1,2</sup>,  
<sup>1</sup>University of Newcastle, Australia, <sup>2</sup>Hunter Medical Research Inst., Australia.

Research in animal models suggests that dietary saturated fat (SFA) enhances omega-3 polyunsaturated fatty acids (n-3PUFA) incorporation in membranes and tissue cells, when compared to dietary omega-6 polyunsaturated fat (n-6PUFA). However, the effect of different dietary fat types on n-3PUFA incorporation has not been studied in humans. Therefore, we investigated blood lipid levels and n-3PUFA incorporation in plasma and erythrocytes following dietary supplementation with n-3PUFA [2.4g eicosapentaenoic acid (EPA): docosahexaenoic acid (DHA) daily], combined with either a high SFA diet or a high n-6PUFA diet. This was a randomized, dietary intervention trial, in parallel design involving 25 healthy adults aged 18 to 65 years. Blood samples were collected after an overnight fast at baseline and after 6 weeks of dietary intervention. A significantly higher increase in n-3PUFA into plasma and erythrocyte lipids was observed after the consumption of the SFA diet when compared with the n-6PUFA diet (P=0.017 and P=0.006, respectively). The SFA diet also caused an increase in total (P=0.021) and low density lipoprotein (LDL) cholesterol (P=0.011). Thus, n-3PUFA supplementation appears to be more effective in increasing tissue n-3PUFA levels when the background diet is rich in SFA rather than n-6PUFA, despite increase in total and LDL cholesterol levels.

**Effect of Dietary Carboxymethyllysine on Cecal Short Chain Fatty Acid Composition in Mice.** S. Xiao<sup>1</sup>, M.C. Michalski<sup>2</sup>, A. Geloën<sup>2</sup>, K.H. Hintze<sup>2</sup>, and R.E. Ward<sup>\*1</sup>, <sup>1</sup>Nutrition, Dietetics, & Food Sciences, Utah State University, USA, <sup>2</sup>Laboratoire CarMeN (CARDiology, Metabolism, & Nutrition), INSA-Lyon, France.

Carboxymethyllysine (CML) is an oxidized product of lysine and a reducing sugar that is formed in heated foods. Rodent studies indicate that dietary CML is proinflammatory via interaction with the advanced glycation endproduct receptor (RAGE). Oxidation of proteins reduces their digestibility and may influence the formation of short chain fatty acids (SCFA) in the hindgut by the microbiome. However, to date there have not been any reports on how this may affect the generation of specific SCFA. In this experiment we fed five groups of mice (n=10/gp) either a control diet (AIN93G), a high fat diet (DIO45% kcal from fat) or the Total Western Diet (TWD) with three different levels of CML. After 8 weeks mice were sacrificed and cecal contents collected. Seven SCFA (acetic, butyric, propionic, isovaleric, caproic, valeric, isobutyric) were measured in the cecal contents using GC-FID. The three TWD diets had the highest

levels of SCFA compared to the AIN and DIO diets, and had similar levels of acetate and butyrate. Interestingly, dietary CML (and associated heat-damaged protein) led to a dose-dependent increase in cecal and plasma propionic acid in mice fed the TWD diets. The long term effects of elevated plasma propionic acid are unclear.

**Tuscany Naturben: Quality and Wellness with Traced Tuscan Food for Patients Under Chemotherapy.** E. Bargiacchi<sup>1</sup>, M. Campo<sup>1</sup>, A. Romani<sup>2</sup>, P. Pinelli<sup>3,2</sup>, and S. Miele<sup>1</sup>,  
<sup>1</sup>Consorzio INSTM, Italy, <sup>2</sup>Phytolab-DiSIA, University of Firenze, Italy, <sup>3</sup>Lab QuMAP-PIN Prato, Italy.

Patients' stress after discovering to be affected by breast cancer, the related surgery treatment, and the effects of pharma and chemotherapy are conditions leading to increased oxidative stress. Previous research indicated that diets based on food and supplements high in natural antioxidants help to control the free radicals and oxidative stress-related blood parameters, with increased patients' wellness. Starting from the excellence of Tuscany traced foods, a new set of food, herbal teas, and supplements, sensorially appealing for taste, aroma, and "roundness", was prepared. These products, analytically characterized for their dietary, antioxidant and anti-radical constituents (analyses presented), were administered freely to a group of patients in the period between 2<sup>nd</sup>-5<sup>th</sup> chemotherapy cycles at the Senology and Oncology Dept. of the Hospital Agency of Pisa-Tuscany (Italy). Psycho-physical wellness, and oxidative stress-related blood parameters (tested by CR-4000 of Callegari-Italy) of the control and treated groups of patients were controlled through and after the test period. The treated group also expressed their evaluation on the administered products. Results indicated that patients response to the treatment was positive both for the blood parameters, and the psycho-physical wellness. This research was funded by Regione Toscana Food and Nutraceutical Projects for EXPO 2015.

**Antioxidants Clinical Trials Failed to Reduce Cardiovascular Outcomes: The End of the Oxidative Stress Hypothesis?**  
A. Gugliucci, Touro University-California, USA.

Oxidative stress has been proposed to be the dominant mechanism by which all clinical risk factors lead to vascular damage and, eventually, to atherosclerosis and its clinical outcomes. With this as the molecular linchpin of cardiovascular disease (CVD), the prevention of the oxidative stress caused by dyslipidemia, smoking, hypertension, and/or diabetes would avert the endothelial dysfunction and the ensuing process that leads to plaque rupture and thrombosis. Plausibly, therefore, the modulation of oxidative stress was projected to have a possibly great impact on CVD. However, eight randomized trials involving 138,113 patients comparing

the risk of cardiovascular death among those randomized to placebo or carotene (Breslow-Day); ATBC Alpha-Tocopherol, Beta-Carotene Cancer Prevention trial; CARET Beta-Carotene and Retinol Efficacy Trial; HPS Heart Protection Study; NSCP: Nambour Skin Cancer Prevention; PHS Physicians' Health Study; and WHS Women's Health Study failed to report benefits or actually produced increased deaths. The big question is: why? We will discuss the possibilities. The wrong drug or combination of drugs has been studied; Vit E may require a cooxidant to prevent LDL oxidation? Inappropriate doses or for inadequate durations? Wrong patients have been studied because intervention may be too late in the atherosclerotic process to make a difference?

**Mary Enig Memorial Lecture: Risk and Revelation of Trans Fat's Long-term Effects on Health.** J. Enig Stockhamer, Halpern Stockhamer, Barristers & Solicitor, Canada.

In 1978, Dr. Mary G. Enig began to look at the growing concern regarding the speculation between the relationship of dietary fat and cancer causation. She was one of the first scientists to conclude that correlations between increase in *per capita* dietary fat intake and total cancer mortality over a 60-year period showed significant positive correlations for total fat and vegetable fat, and negative correlation for animal fat. *But how could this be?* Moreover, her work showed that there was no discernible correlation between any fat or fat component and colon cancer mortality. Follow-up analysis by Dr. Enig showed significant negative correlation between animal fat and incidence of total cancer, breast cancer, and colon cancer. Partial correlations revealed that the positive correlation between total cancer mortality and vegetable fat intake over a 60-year period was explained by the *trans* fatty acid (TFA) component. And, so began a nearly four decade battle against established beliefs, her objective being the ultimate removal of TFAs from the food supply. Still, during her more recent retirement, the long-term consumption of TFAs was beginning to show negative consequences, in the population, not previously anticipated, and so began another fight.

**Awareness and Knowledge of Individual Omega-3 Fatty Acids in Young Adults.** K. Roke, J.I. Rattner, P. Brauer, and D.M. Mutch, University of Guelph, Canada.

**Objective:** The purpose was to determine the awareness and knowledge in young adults regarding individual omega-3 fats and the possible health effects. We also investigated the sources of information used to learn about omega-3 fats and health.

**Methods:** Focus groups and cognitive interviews were conducted to refine survey questions and response options. An online survey was created using Qualtrics software, where data from 834 well-educated young adults were analyzed.

**Results:** More respondents had heard of alpha-linolenic acid (~60%) compared to eicosapentaenoic and docosahexaenoic acid (~35%); however, fewer respondents

recognized the ALA abbreviation (~40%) compared to the EPA (~51%) and DHA (~66%) abbreviations. Field of study influenced response rates. Respondents who used Academic/Reputable sources, Health Care Professionals, and/or Social Media to acquire nutritional knowledge were most aware of the possible health effects related to increased EPA/DHA consumption. Finally, ~83% of respondents knew that EPA and DHA had possible effects on heart and brain health.

**Conclusions:** EPA and DHA abbreviations and the possible health effects with increased EPA/DHA consumption are well-known by young adults. Better understanding of the core messages that are taken up, and channels being used by young adults, are foundational to improved knowledge translation of the emerging evidence about omega-3 fats.

**Anti-aging Effect of Fish Oil and Polyunsaturated Fatty Acid Based on Redox State Regulation and Telomere Protection Mechanisms.** J.N. Chen, Y. Wei, J. Wang, J.H. Chen, and Y. Zhang, Zhejiang University, China.

To understand the anti-aging mechanism of polyunsaturated fatty acid (PUFA), we investigated the effect of fish oil (FO), docosahexenoic acid (DHA) and arachidonic acid (AA) on the redox state regulation and chromosome telomere protection in D-galactose-induced aging mice after 8-week oral administration. Results (i) Antioxidase activities (superoxide dismutase, catalase, glutathione peroxidase) in liver, heart, brain, and plasma were significantly improved by FO and PUFA interventions ( $P < 0.05$ ). And plasma  $F_2$ -isoprostane levels, malonaldehyde (MDA) and monoamine oxidase activities in brain were reduced by 20%-79%, 16%-54% and 50%-90% in all experimental groups, respectively; (ii) PUFA monomers in high and moderate doses dramatically increased liver MDA levels, while AA in all doses even enhanced heart MDA levels; (iii)  $\omega$ -3 rather than  $\omega$ -6 PUFA prevented liver against telomere shortening, while only FO protected chromosome telomere in testis; (iv) PUFA monomers exerted better inhibitory effect on c-myc expression if compared with FO ( $P < 0.05$ ). Both PUFA and FO could inactivate p53 response. Conclusions FO and PUFA monomers in low doses have positive redox regulation effect. Only  $\omega$ -3 PUFA is capable of rescuing age-related telomere shortening. The anti-aging effect of  $\omega$ -3 PUFA appears dose-dependent while the effect of FO is related to its PUFA composition.

**Rapeseed Oils Produced from Different Methods Had Significant Effect on High-fat-induced Hepatosteatosis in Sprague Dawley Rats.** P.R. Cao, L. Zhang, J. Jiang, and Y.F. Liu, School of Food Science & Technology, Jiangnan University, China.

Food oils produced from various oilseeds as major nutrient have some nuance effect on human health. Furthermore, oils from same oilseed with various technologies may also have different impact besides the

quality of oils including micronutrients and flavors. In this study, rapeseed oils extracted with methods of cold- and hot-pressing, aqueous enzymatic extraction (AEE), hexane extraction, and refined rapeseed oil were evaluated in a high-fat-diet feeding animal model. Fifty-six Sprague Dawley male rats were divided into seven groups feeding the high fat diets. The diets were different only in the fat contents of oils from various extraction method. After feeding ad libitum for 12 weeks, rat body weight from feeding with AEE method was about 4.6+/-0.5% less than other high fat diet groups. The

hepatosteatorosis induced by high fat was significantly improved in the AEE group and cold-pressing group in contrast to others. Consistently, the total blood cholesterol and LDL were also significantly lowered in AEE group. Since there are not significant different on the oils composition except for some micronutrients, it was concluded that oils produced by AEE is more benefit to health than by other methods which may relate to its high content of polyphenol and carotene.



## H&N 5.1/EAT 5: Satiety and Sensory

*This session developed in conjunction with the Edible Applications Technology Division.*

**This session sponsored in part by Nestlé and Young Living Essential Oils.**

*Chairs: S. Martini, Utah State University, USA; and F. Dionisi, Nestlé, Switzerland*

### **The Taste of Fat and Its Role in Dietary Fat Intake.**

T.A. Gilbertson, Utah State University, USA.

It has been well over a decade since our laboratory first identified the ability of free fatty acids to activate mammalian taste receptors cells, consistent with there being a “taste of fat”. Since that time, the ability of fatty acids to act as the proximate stimuli for fat taste has been validated in a number of species spanning the molecular, cellular and behavioral levels. We have recently identified several novel fatty acid-activated G protein-coupled receptors that, in conjunction with the fatty acid binding protein CD36, allow the recognition of chemically distinct classes of free fatty acids. Interestingly, this pathway also plays an important role in the control of dietary fat intake. Mice with genetic deletions in signaling proteins in this pathway show a reduced preference for dietary fat and concomitantly gain less weight and put on less body fat than wild type mice. Further, there are pronounced sex differences in this pathway and its role in fat intake suggesting that the ‘fat taste worlds’ of males and females are markedly different. This presentation will summarize what is known about the receptors and transduction pathway for free fatty acids and its contribution to dietary fat preference.

### **Gut-brain Endocannabinoid Signaling: Fatty Acid Sensing and Beyond.** N.V. DiPatrizio, Div. of Biomedical Sciences, School of Medicine, University of California, Riverside, USA.

The endocannabinoid (eCB) system is an important regulator of feeding, energy balance, and reward. We reported that tasting dietary fats – but not carbohydrate or protein – initiated production of the eCB, 2-AG, in the rat small intestine, and this signaling event at local CB<sub>1</sub>Rs drives the intake of fatty foods. Importantly, surgical disruption of the vagus nerve – which communicates neurotransmission between the brain and gut – blocked increases in intestinal eCB levels after fat exposure, suggesting that cholinergics are required for orexigenic eCB activity in the gut. We recently extended these findings and reported that similarly to tasting fats, fasting for 24 h drives production of intestinal 2-AG. This effect occurred in a time-dependent manner that paralleled increases in the 2-AG precursor, SAG. Importantly, vagotomy blocked fasting-induced rises in jejunal 2-AG, an effect that was mimicked by inhibiting muscarinic acetylcholine receptors (mAChRs) in the small intestine, and reduced refeeding after a fast. Similarly, administration of a peripherally-restricted CB<sub>1</sub>R antagonist inhibited refeeding

after a fast. This result suggests that vagal activity at intestinal mAChRs drives production of 2-AG, which in turn, functions as a general hunger signal. Thus, these investigations advance our understanding of gut-brain eCB signaling and suggest potential new treatment options for appetite control.

### **Small Intestinal Sensing of Lipid in Humans—Relationship with Appetite and Energy Intake.** C. Feinle-Bisse, Discipline of Medicine & NHMRC Centre of Clinical Research Excellence in Nutritional Physiology, Interventions & Outcomes, University of Adelaide, Australia.

Small intestinal receptors play a key role in sensing the arrival of nutrients in the intestinal lumen, initiating feedback loops that lead to adjustments in the rate of gastric emptying and the release of gut hormones, both of which are involved in the regulation of energy intake. Lipid has potent effects on these functions, requiring fat digestion and fatty acids with a chain length of =12 carbon atoms. There is evidence from studies in animals and humans that these GI functions can adapt to both dietary restriction and excess nutrient exposure, modifying the sensitivity to nutrients, particularly, with potential implications for the regulation of energy intake. For example, consumption of a high-fat, high-energy diet accelerates gastric emptying and small intestinal transit of a fat-containing meal. Since obese individuals have an increased energy/nutrient intake, it is conceivable that they may have a reduced ability to sense nutrients, both in the oral cavity and the lumen of the GI tract, associated with reduced modulation of gut functions, thus, compromising the capacity to limit their energy intake. In support, our recent studies demonstrate that habitual fat and energy intake and BMI are inversely related to the ability to taste fat in the oral cavity in healthy humans. Furthermore, obese individuals have reduced pyloric contractile and plasma CCK responses to intraduodenal oleic acid infusion, compared with lean individuals, and the oral and small intestinal responses to fat are correlated. Finally, recent evidence suggests that both oral and small intestinal fat sensitivity can be modulated by dietary interventions, ie enhanced in response to a low-energy diet and reduced in response to a high-fat diet. For example, both acute (for 4 days) and longer-term (for 12 weeks) dietary restriction markedly enhances the sensitivity to the gastrointestinal (GI) and appetite-suppressant effects of fat. Taken together, while fat has potent effects on those GI functions that contribute to energy intake regulation,

these effects are diminished by a high-fat, high-energy diet, but can be reinstated, at least in part, by dietary restriction. Much further research is required to investigate the mechanisms underlying the effects of fat on energy intake regulation to determine whether these findings can be translated into efficient, novel approaches to the prevention and management of obesity.

#### **The Taste of Non-esterified Fatty Acids in Humans.**

R. Mattes, Purdue University, USA.

Accumulating evidence indicates humans can detect non-esterified fatty acids (NEFA) through the sense of taste as well as other sensory systems. Support for a taste component stems from finding that: A) there is an adequate stimulus concentration in the oral cavity when fatty foods are masticated; B) fatty acid receptors have been identified on taste cells and taste thresholds reflect their functionality; C) oral signals of fat detection are conveyed centrally by gustatory nerves; D) central decoding of gustatory signals from oral fat exposure are not based on somatosensory cues; E) there is a unique quality percept for NEFA; and F) oral fat exposure alters lipid metabolism. The taste quality of NEFA is generally rated as unpleasant when sampled in simple systems, but the possibility that it enhances overall flavor acceptability at low concentrations remains to be examined. Improved understanding of the sensory detection of NEFA holds implications for the food industry, clinical practice, public health, and sensory science.

#### **Sensory Determinants of Fat (and Oil) Intake—The Consumer Perspective.** J.X. Guinard, University of California, Davis, USA.

Our consumer research model considers product, consumer, and context variables as determinants of consumer behavior. Humans may not be as sensitive to fats and oils as they are to most gustatory, olfactory or trigeminal stimuli, as measured by difference thresholds, and in simple emulsions and complex matrices. Sensory-specific satiety, the short term food intake regulation mechanism by which humans satiate to specific sensory attributes, is not as relevant to the regulation of fat intake as it is to that of nutrients with taste and smell properties. The main means of sensory perception of fats and oils are by touch and kinesthesia as well as olfaction of aromas associated with fats and oils more than direct chemoreception and transduction. Consistently, sensory preferences for fats and oils are primarily learned, based on exposure (as for olfactory stimuli) rather than innate (as for taste stimuli and chemical irritants).

It follows that a segment of the population actually prefers olive oils with some degrees of rancidity and fustiness, likely based on sustained exposure to oils with such qualities. Our Healthy Flavors Research Initiative shows how beef can be substituted with healthier and flavor-boosting (umami) mushrooms in taco blends without loss of consumer acceptance, and how olive oil can be used in place of butter in equally successful culinary strategies for healthier eating.

#### **Complexity of Structure-sensory Relations: Science and Application.** G.A. van Aken<sup>1,2</sup>, <sup>1</sup>NIZO Food Research, The Netherlands, <sup>2</sup>Insight FOOD Inside, The Netherlands.

Being the portal of the gastrointestinal tract, the mouth is functional for testing the safety and expected nutritional value, pleasure, and possible toxicity of the food, and to prepare the food to form a slippery, smooth bolus that can be swallowed safely. During this oral processing, the food is broken up into pieces and mixed with saliva, during which changes occur in food particle size, rheological properties, and adhesion to the oral surfaces, and tastants and volatiles are released. The perception of texture and flavor is intricately connected to the way the food behaves during oral processing and the way this elicits responses by tactile, taste, and aroma receptors. These responses function to adjust oral processing optimizing the release and composition of saliva and to adjust mastication time and intensity. The time of oral exposure to sensory stimuli and the masticational effort before the food can be swallowed has furthermore been shown to strongly affect the desire to eat more or to stop eating. Moreover, already starting during consumption, the food enters the gastrointestinal tract, where its volume, consistency, and restructuring in the stomach and the release of absorbable nutrients by digestion and absorption signals the brain about the nutritional properties of the food, reflected in sensations of fullness and hunger reduction.

As a consequence, perception, liking, satiety, and the desire for a follow up meal generally depends on the dynamic interaction between the food and the body, and as a consequence can usually not be related directly to food composition, structure, and rheological properties before consumption.

This presentation will focus on the perception of the dynamic food structures in the mouth and specifically the role fat in this. It will be outlined how this can be approached experimentally and how a correct understanding of the way a food behaves during oral processing can be used in practice to adjust the sensory quality.

## H&N-P: Health and Nutrition Poster Session

Chair: M. Fleith, Nestlé Research Center, Switzerland

### 1. The Effect of Blood Glucose Levels Increase Controlling of Brown Rice Defatted with Supercritical Carbon Dioxide.

M. Matsubara<sup>1</sup>, Y. Nakato<sup>2</sup>, and E. Kondoh<sup>1</sup>, <sup>1</sup>University of Yamanashi, Japan, <sup>2</sup>KOA Electronics Co., Ltd., Japan.

If you have high blood glucose levels for long periods, this can cause a terrible and incurable diabetes type 2 which can pose a serious damage for your body, such as kidney failure, strokes, heart attacks, vision loss, infections, amputation of hand or leg in worst cases.

So, it is very important to keep your blood glucose levels close to normal to prevent these complications by a change of your eating pattern and physical activity program. It is well known brown rice induces the effect of blood glucose lowering in normal and diabetics compared with white rice having higher Glycemic Index more than brown rice.

But, new brown rice we called "Sushi Genmai" in Japan was shown much lower blood glucose levels than brown rice and lowest Glycemic Index.

We studied and developed New brown rice defatted using supercritical carbon dioxide (scCO<sub>2</sub>) without reducing more nutrients such as fiber, minerals, vitamins, and protein. In addition, this new brown rice was resulted in easy cooking with no rinsing, no soaking, short time cooking, and no waxy bad smell more than brown rice.

New brown rice (Sushi Genmai) defatted with scCO<sub>2</sub> is more effective to reduce blood glucose levels and a more health beneficial food for normal and diabetics compared with brown rice and white rice.

### 2. Evaluation of Soybean-navy Bean Emulsions.

S.X. Liu, M. Singh, A. Wayman, and J.A. Kenar, USDA, ARS, NCAUR, Functional Foods Research Unit, USA.

As health-conscious consumers are increasingly looking for plant-based protein-rich beverages for weight management, cancer prevention, and overall cardiovascular health, a lot of soy milk products of all varieties have been brought to the market to meet this need. However, beverages based on pulses are not common in the marketplace despite the fact that pulses, like soybean, are legumes and healthful. This is partially due to difficulty of producing shelf stable pulse milk. In this study, emulsions made from soybean and pulse (navy bean) blend of different proportionalities were made; two methods of processing were used: traditional boiling and jet cooking. The physical properties and storage stability were measured and compared. It was found that emulsion with higher soybean content has higher shelf life stability, smaller particle size, higher reflective index, higher fat, lower starch, and lower viscosity.

### 3. Effects of Lysophosphatidylcholine Derived from Squid and Starfish on Leukotrienes Release from Mast Cells.

M. Takasugi<sup>1,2</sup>, S. Kakoi<sup>1</sup>, S. Yasutake<sup>1</sup>, T. Tsushima<sup>3</sup>, K. Takahashi<sup>3</sup>, and H. Arai<sup>4</sup>, <sup>1</sup>Kyushu Sangyo University, Japan, <sup>2</sup>University of California, Davis, USA, <sup>3</sup>Hokkaido University, Japan, <sup>4</sup>Kitami Inst. of Technology, Japan.

Leukotrienes (LTs) produced from plasma membrane phospholipids of mast cells play crucial roles in allergic inflammation. It has been suggested that lysophosphatidylcholine (lysoPC) functions as a lipid mediator and modifies eicosanoid synthesis such as LTs. In Japanese fishery industry, large amounts of squid integumental skin and starfish are disposed as industrial waste. Thus, exploring their potential for nutraceutical application is important. In this study, we examined the effects of lysoPCs derived from squid integumental skin and starfish viscera on LTs production by mast cells, which activities were compared with palmitoyl-lysoPC.

LysoPCs were obtained by the partial hydrolysis of PCs derived from squid integumental skin and starfish viscera. Mouse mast cells (PB-3c) in Tyrode buffer were stimulated with calcium ionophore at 37°C for 20min in the presence of lysoPC. Then LTB<sub>4</sub> and LTC<sub>4</sub> produced by the cells were determined.

LysoPCs from squid and starfish strongly suppressed LTs production. The suppressive activity of squid and starfish lysoPCs was significantly higher than that of palmitoyl-lysoPC. These data suggest that lysoPC may alleviate allergic symptoms and the difference of fatty acid constituent of lysoPC may affect the activity. Squid integumental skin and starfish viscera might be useful for materials of functional foods.

### 4. Effect of Dietary Lysophosphatidylcholine Containing n-3 PUFAs on Lipid Contents and Fatty Acid Compositions in the Serum and Brain of Rats.

R. Hosomi<sup>1</sup>, K. Miyauchi<sup>1</sup>, K. Fukunaga<sup>1</sup>, T. Nagao<sup>2</sup>, K. Sugimoto<sup>3</sup>, M. Yoshida<sup>1</sup>, and K. Takahashi<sup>4</sup>, <sup>1</sup>Kansai University, Japan, <sup>2</sup>Osaka Municipal Technical Research Inst., Japan, <sup>3</sup>Phytopharma, Inc., Japan, <sup>4</sup>Hokkaido University, Japan.

Lysophospholipids has several attractive characteristics such as better emulsion properties and low molecular weight compared with diacylphospholipids. Currently, there is not a biological information that the effect of dietary lysophosphatidylcholine (LysoPC) containing n-3PUFAs. In a present study, we investigated the effects of LysoPC containing n-3PUFAs on lipid contents and fatty acid compositions in the serum and brain of rats. LysoPC containing n-3PUFAs were extracted the squid meal, which is fisheries byproduct, and were partially hydrolysis by lipase. After the reaction, the mixture was filtered to remove the enzyme and fatty acid. Groups of male Wistar rats were fed

AIN93G diet containing soybean oil (SO, 7%), fish oil (2.5%)+SO (4.5%), phospholipids containing n-3PUFAs (PL, 2.5%)+SO (4.5%), and LysoPC containing n-3PUFAs (2.5%)+SO (4.5%) for 4 weeks. Dietary LysoPC decreased the serum cholesterol and hepatic triacylglycerol (TAG) contents compared with dietary sole SO. The reduction of hepatic TAG contents by dietary LysoPC are partly due to the enhancement of acyl-CoA oxidation activity and the suppression of acetyl-CoA carboxylase activity in liver. In addition, the DHA contents in cortex, cerebellum, hippocampus, and brainstem were not significantly altered.

**5. *In vitro* and *in vivo* Insights into the Digestion of a Unique Natural Emulsion: Human Milk.** S. De Oliveira<sup>1</sup>, A. Deglaire<sup>1</sup>, C. Moustiés<sup>1</sup>, O. Ménard<sup>1</sup>, A. Bellanger<sup>2</sup>, F. Carrière<sup>3</sup>, P. Villeneuve<sup>\*4</sup>, E. Dirson<sup>5</sup>, Y. Legouar<sup>1</sup>, F. Rousseau<sup>1</sup>, D. Dupont<sup>1</sup>, and C. Bourlieu<sup>1</sup>, <sup>1</sup>INRA-AGROCAMPUS, France, <sup>2</sup>Dept. of Pediatrics, CHU Rennes, France, <sup>3</sup>CNRS, Aix Marseille Université, France, <sup>4</sup>CIRAD, UMR IATE, France, <sup>5</sup>Lactarium-Infant Nutrition & Dietetics, CHU Rennes, France.

Human milk is the ideal food for infant nutrition. Understanding the digestive behavior of this natural complex colloidal emulsion is essential for neonatal nutrition and a key step in developing infant formulas with optimized health benefits. However, ethical reasons limit *in vivo* trials. Thus, it is important to develop relevant *in vitro* models. In this aim, a dynamic *in vitro* digestion system (DIDGI®) was applied to human milks or infant formula.

The dynamic digester parameters were based on an exhaustive literature review to mimic closely the digestion of newborns. Raw or pasteurized pooled human milks (HM) or a liquid infant formula (IF) were digested in triplicate. In parallel, *in vivo* study was conducted on preterm newborns at Rennes Hospital (NCT02112331) to validate gastric *in vitro* data. Lipolysis, liberated fatty acids and the structural changes of the matrices were evaluated along digestion.

HMs differed from IF in terms of chemical composition (specifically regiodistribution), prehydrolysis state, and emulsion structure. These initial differences impacted lipolysis kinetics and deconstruction. In comparison, the pasteurization of HM only impacted emulsion disintegration, protein aggregation, and the persistence of native fat globules.

Our model will be useful to the scientist community and food manufacturers who focus on neonatal digestion and infant formulas optimization.

**6. Physicochemical Properties of Goat Milk Fat as Influenced by Feeding Fish Oil Entrapped in Chemically Treated Protein Matrices.** J.H. Lee, B. Lemma, and C. Alfred, Fort Valley State University, USA.

The effect of entrapped fish oil (EFO) containing eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids on milk fat properties of lactating goats was investigated. The

EFO was prepared with a generally recognized as safe (GRAS) chemical, fish oil (FO), and defatted soy flour. Nine lactating goats were assigned to three diets using a 3×3 Latin square design with three 14-d periods. The three diets consisted of 95% basal diet containing alfalfa meal, yellow corn, and soybean meal, plus 5.0% lipid from either poultry fat (PF), FO, or EFO. Each period consisted of a 10-d adjustment to assigned diets followed by 4-d of milking collection. The collected samples from each goat were analyzed for proximate and fatty acid composition, as well as solid fat content (SFC) in milk fat. Compared with lactating goats fed PF-diet, goats fed either FO- or EFO-diet had higher ( $P<0.01$ ) concentrations of EPA (0.30 vs. 0.81 or 1.66%) and DHA (0.32 vs. 1.21 or 1.86%) in milk fat. Furthermore, the EFO-diet goats showed the highest EPA and DHA concentrations. Feeding goats with EFO-diet produced milk fat that had a lower ( $P<0.05$ ) SFC than did the milk fat from FO- or PF-fed goats.

**7. Nascent VLDL as a Major Carrier of Oxo-phospholipids in Rat Plasma.** A. Kuksis, J.J. Agren, and W. Pruzanski, University of Toronto, Canada.

Studies of human plasma lipoproteins have established HDL as major carrier of oxo-phospholipid, including isoprostane esters, while mature VLDL carries very little of it despite comparable polyunsaturation and absence of PAF-acetylhydrolase, an oxo-phospholipid hydrolase. We reexamined the apparent abnormality by taking advantage of the nascent plasma VLDL available from Triton injection in rats. VLDL ( $d=1.006\text{g/ml}$ ) was isolated by conventional ultracentrifugation 2 hours after injection with Triton WR 1339. Major oxo-phospholipids of plasma VLDL and liver were quantified by HPLC interfaced with either positive or negative ESI-MS containing internal standards. Total PtdCho-IPs of mature rat plasma VLDL averaged 2.5nmol/mg VLDL protein, which was doubled following Triton. The PtdEtn-IPs of mature VLDL averaged 0.5nmol/mg protein, which increased to 2-5nmol/mg protein following injection of Triton. The liver levels of PtdCho-IPs and PtdEtn-IPs averaged 10nanomoles/g, and decreased to 5nmoles/g after Triton infusion. The corresponding PtdIns-IP and PtdOH-IP levels ranged from high picomoles to low nanomoles/mg protein or g of liver, as did the hydroperoxide, hydroxide, and epoxide levels of the examined glycerophospholipids. We conclude that nascent VLDL is a major carrier of oxo-glycerophospholipids, which upon VLDL maturation become transferred to other plasma lipoproteins and hydrolyzed.

**8. Regulation of Metabolic Control Factors via PGC-1 Up-regulation by Fucoxanthin.** M. Hosokawa, R. Tateyama, Y. Ohuchi, A. Katsuki, and K. Miyashita, Hokkaido University, Japan.

Fucoxanthin is a marine carotenoid found in edible brown seaweeds. It has unique structure including an allene bond and epoxide in the molecule. We have previously reported that dietary fucoxanthin attenuates white adipose

tissue (WAT) weight gain of diabetic/obese KK-*A<sup>y</sup>* mice. Interestingly, fucoxanthin induced thermogenic factor, uncoupling protein-1 (UCP1) in the WAT as well as brown adipose tissue (BAT) of KK-*A<sup>y</sup>* mice. The UCP1 induction was also observed in the diet-induced obesity (DIO) C57BL/6J mice feeding high fat diet. In the WAT of KK-*A<sup>y</sup>* and DIO C57BL/6J mice fed fucoxanthin, mitochondrial biogenesis factors, including cytochrome C, NRF1, and TFAM were induced through up-regulation of PPAR $\gamma$  coactivator-1 (PGC-1). These findings suggest that dietary fucoxanthin contributes to energy expenditure through the induction of mitochondria factors regulated by PGC-1 in the WAT of obese mice.

**9. The Effect of Oils Rich in Linoleic Acid on Body Composition in Postmenopausal Women with Metabolic Syndrome.** R.M. Cole, S. Puchala, J.Y. Ke, R.R. Andridge, B. O'Donnell, D. Bradley, and M.A. Belury, Ohio State University, USA.

During menopause women may experience increases in fat mass and decreases in lean mass which can increase the risk of sarcopenia, an age-related loss of muscle mass, quality, and function. A previous study demonstrated that supplementing postmenopausal diabetic women with high-linoleic safflower oil decreased abdominal fat and increased lean mass<sup>1</sup>. For this study, we evaluated the effect of consuming linoleic acid-rich oils every day for 16 weeks on body composition in postmenopausal women (n=14) with metabolic syndrome. When adjusting for body mass index (BMI), there was a trend towards an increase in appendicular lean mass (ALM) (p=0.08). There were no significant changes in total lean mass/BMI (p=0.16) or abdominal fat/BMI (p=0.33). However, change in plasma linoleic acid (LA) was negatively correlated with change in abdominal fat/BMI (r=-0.40), though in this small sample the association did not reach statistical significance (p=0.33). The non-significant trends of changes in body composition in this pilot study combined with the positive effects of consuming oils high in LA on fat and lean mass in other populations support the need for a large randomized controlled trial conducted in postmenopausal women with metabolic syndrome.

<sup>1</sup>Norris LS, et al. *Am J Clin Nutr* 2009; 90: 468-476

**10. Evidence for Change in Cardiolipin Remodeling, Induced by the Chemotherapeutic Drug Doxorubicin.** D. Snok<sup>1</sup>, B. Cotten<sup>1</sup>, T. Banh<sup>1</sup>, R.M. Cole<sup>1</sup>, T. Orchard<sup>1</sup>, M.M. Gaudier-Diaz<sup>2</sup>, A. DeVries<sup>2</sup>, and M.A. Belury<sup>1</sup>, <sup>1</sup>Dept. of Human Sciences, Ohio State University, USA, <sup>2</sup>Dept. of Nutrition, Ohio State University, USA.

Cancer treatment with the anthracycline, doxorubicin (DOX), results in muscle loss and fatigue, and increases susceptibility to recurring cancer and cardiometabolic disease. DOX causes oxidative damage to mitochondrial membranes of skeletal muscle. Cardiolipin (CL) is an inner mitochondrial membrane protein that assists in ATP

generation and acts as a structural sensor of energy homeostasis. Lysocardiolipin acyltransferase-1 (ALCAT-1) changes CL structure in a way that alters energy production and may initiate cell death. We hypothesize that DOX treatment will dysregulate CL remodeling by ALCAT-1, resulting in a loss of skeletal and heart muscle function. Young adult BalbC female mice were ovariectomized, injected once or twice with a DOX (13.5mg/kg) and cyclophosphamide (135mg/kg) cocktail or a saline control, and euthanized 6h or 5d after the final injection. Mice treated with a single injection exhibited higher levels of ALCAT-1 mRNA in skeletal muscle both 6h and 5d after injection. After a second injection, muscle, heart, and body mass were significantly lower in comparison with saline-injected controls, correlating with significant changes of ALCAT-1 mRNA in these tissues. These findings suggest damage to muscle tissue from DOX may involve changes of CL synthesis and remodeling ultimately to alter mitochondrial energy metabolism.

**14. A Comparison of Anti-inflammatory Effects of Rumenic Acid to Celecoxib in the Collagen Induced Arthritis Model.** J.M. Olson, A.W. Haas, J. Lor, H.S. McKee, and M.E. Cook, University of Wisconsin-Madison, USA.

Cyclooxygenase-2 (COX-2) inhibitors (e.g. celecoxib) are frequently prescribed analgesics for chronic inflammatory diseases such as arthritis, but disrupt metabolic regulation, increasing risk of myocardial infarction. Dietary rumenic acid (RMN) also reduces arthritic severity, but unlike COX-2 inhibitors, RMN reduces the activity of phospholipases A<sub>2</sub> (PLA<sub>2</sub>) at the apex of the arachidonic acid (AA) cascade without known metabolic dysregulation. Therefore, a study was conducted to test whether 0.5% dietary RMN reduces arthritic severity equivalently to daily 5mg/kg celecoxib (recommended dose) in the murine collagen-induced arthritis model. Arthritic DBA/1 mice received diets containing one of the following supplements: 0.5% corn oil (CO, w/w), 0.5% RMN, or 0.5% CO plus one of three celecoxib doses (0.5, 5 or 50mg/kg bw), over 42 days. RMN reduced arthritic severity significantly compared to CO-fed mice (0.2±0.5 vs. 1.9±0.4, respectively, P=0.01), while celecoxib dose-dependently reduced severity compared to CO. Linear regression of the celecoxib dose-response showed 0.5% RMN (i.e. 600mg/kg dose) reduced arthritis equivalently to 2.6mg/kg celecoxib. Although the RMN dose required for this equivalence may be impractical in the human diet (12.1g/d), lower doses of RMN may be as effective and should be studied further in this context.

**17. Enhancing Shelf Life of Sunflower Oil Usin Bamboo Leafy biomass as Antioxidant Material.** V. Kardam, S. Satya, K.K. Pant, and S.N. Naik, Indian Inst. of Technology Delhi, India.

To overcome the thermo-oxidative stability problem in Sunflower oil (SO), extracts of leafy biomass from four bamboo species (*B.vulgaris*, *B.arundinacea*, *D.strictus*, *B.*

*tulda*) were prepared in methanol and ethanol. The polyphenol content of methanolic extract of *B. vulgaris* was significantly ( $p < 0.05$ ) higher ( $40 \pm 1.1 \mu\text{g/ml}$  Tannic acid equivalent, TAE) than other three bamboo species (range 28–24.5  $\mu\text{g/ml}$  TAE). DPPH radical scavenging activity ( $\text{IC}_{25} = 0.49\text{--}0.72 \text{mg/ml}$  TAE), total antioxidant activity (0.56–0.62  $\text{mg/ml}$  Ascorbic acid equivalent) and reducing assay were determined in all the above extracts. Antioxidant activity Index of SO enriched with 0.2% bamboo leafy extract determined by Rancimat assay ( $110^\circ\text{C}$ ) was found significantly ( $p < 0.05$ ) higher (AAI=2.56–2.07) than 0.02% BHT (AAI=1.69). Isothermal-gravimetric analysis at  $185^\circ\text{C}$  confirmed the stability of bamboo extracts than BHT which decomposed rapidly during initial hour. In view of best characteristics of the methanolic extract of *B. vulgaris*, its efficacy for enhancing shelf life of SO was evaluated. The extract significantly retarded ( $p < 0.05$ ) oxidation and deterioration in SO heated at  $185 \pm 5^\circ\text{C}$  for 24hrs and found comparable to BHT in terms of peroxide value, iodine value, free fatty acid, polar compound. Therefore, bamboo leafy extracts can be considered as a good stable substitute for synthetic antioxidants posing human health hazards.

#### 18. A Sulfated Flaxseed Polysaccharide and Its Macrophage Immunomodulatory and Anti-hepatitis B Virus Activities.

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A novel sulfated flaxseed polysaccharide (FP-1) with an average molecular weight of 2,626kDa, was purified from flaxseed using ion exchange and size exclusion chromatography. FP-1 consists primarily of rhamnose (23.8%), fucose (10.6%), arabinose (4.0%), xylose (35.2%), mannose (0.2%), glucose (2.7%), and galactose (23.4%). The main glycosidic linkages of FP-1 are (1 $\rightarrow$ 4)-linked- $\beta$ -D-xylan, (1 $\rightarrow$ 2,3,4)-linked- $\beta$ -D-xylan, T-linked- $\beta$ -D-xylan, T-linked- $\alpha$ -arabinose and (1 $\rightarrow$ 3)-linked- $\alpha$ -arabinose. Further investigation reveals that FP-1 has a triple-helix conformation and sulfated structures. Such structures are present in polysaccharides with immunomodulating and antiviral activities. FP-1 possessed potent immunomodulatory activity on murine macrophages. FP-1 induces tumor necrosis factor  $\alpha$ , nitric oxide, and interleukin (IL-6 and IL-12) production through activation of the related mRNA expression. In addition, FP-1 inhibited the expression of hepatitis B surface antigen and thereby inhibited hepatitis B virus (HBV) induced proliferation, and interfered with HBV DNA replication. These findings provide a possible molecular basis for observed beneficial therapeutic and nutritive flaxseed properties and supports the use of flaxseed polysaccharides as functional food ingredients, immunopotentiators, and vaccine

adjuvants.

**19. Interfacial Studies on Four Lp(a) Isoforms: Relationship Between Stability and Isoforms?** A. Santonastaso<sup>1</sup>, V. Vié<sup>\*2</sup>, G. Paboef<sup>2</sup>, V. Bolanos-Garcia<sup>3</sup>, C. Scotti<sup>1</sup>, L. Salvaneschi<sup>4</sup>, and S. Beaufilet<sup>2</sup>, <sup>1</sup>Dept. of Molecular Medicine, Unit of Immunology & General Pathology, University of Pavia, Italy, <sup>2</sup>IPR Inst. of Physics, Rennes University, France, <sup>3</sup>Dept. of Biological & Medical Sciences, Oxford Brookes University, UK, <sup>4</sup>Immunohaematology & Transfusion Medicine Dept., IRCCS Policlinico San Matteo Foundation, Italy.

Lipoprotein(a) [Lp(a)] is a novel risk factor for cardiovascular diseases. Lp(a) is composed by an LDL-like particle and a unique molecule of apolipoprotein(a) [apo(a)], that is responsible for the polymorphic nature of Lp(a), as it contains a domain, namely Kringle IV-type2 (KIV-2), which can be tandemly repeated from 3 to >40 times. Although it was demonstrated that smaller Lp(a) isoforms retain stronger atherogenic potential, the molecular mechanisms are still unsolved. Biophysical characterization of different Lp(a) isoforms may offer insights to clarify the role of apo(a) size in atherosclerotic diseases. For the first time, we present interfacial measurement data collected on four purified Lp(a) isoforms called K20, K24, K25, and K29. Two groups had been detected: K20 and K24 came and spread faster than K25 and K29 regarding the ellipsometric data. Moreover, K20 and K24 had a higher final surface pressure that means a higher interfacial molecular packing than the longer isoforms, K25 and K29.

Finally, these results seem to show that the interfacial behaviour can be related to the number of Kringle IV-type2 present in the Lp(a).

**20. Lipase-catalyzed Synthesis of Beta-sitosteryl Esters of Omega-3 Fatty Acids for Incorporation into Milk.** D. Louis<sup>1</sup>, P.M. Tomasula<sup>2</sup>, M. Diao<sup>1</sup>, N. Boyle<sup>1</sup>, and S.E. Lumor<sup>1</sup>, <sup>1</sup>Delaware State University, USA, <sup>2</sup>USDA, ARS, ERRC, USA.

Beta-sitosteryl ester of omega-3 fatty acids were produced using lipase catalysis. Three lipases were originally studied, but *Candida rugosa* lipase was selected for further investigation because of its higher enzymatic activity. A response surface methodology (RSM) study is currently underway for the optimization of the synthesis of the beta-sitosteryl esters. The parameters being studied are temperature, substrate molar ratio, reaction time and enzyme load. The best combination of these experimental parameters that produces the highest yield of the beta-sitosteryl esters will be selected for large-scale synthesis of the steryl esters. The purified esters will be incorporated into milk, followed by rheological and chemical analyses. Our goal is to incorporate an amount of the products that will deliver the recommended levels of omega-3 fatty acids per serving.