HEALTH AND NUTRITION

H&N 1: Lipids and Inflammation

Chair: Eric Murphy, University of North Dakota, USA

Increase in Plasma Ganglioside Content is Associated with Improved Quality of Life in Inflammatory Bowel Disease John J. Miklavcic*[1], Glen Shoemaker[2], Kareena L. Schnabl[3], Alan BR Thomson[4], Vera Mazurak[2], and M Tom Clandinin[5],[1]*Chapman University, USA;* [2]*University of Alberta, Canada;* [3]*Laboratory Medicine and Pathology, University of Alberta, Canada;* [4]*Gastroenterology, Western University, Canada;* [5]*Centre for Health and Nutrition, University of Alberta, Canada*

Dietary ganglioside has been shown to decrease inflammation, improve gut-barrier function, and prevent infection by pathogenic micro-organisms.

Objective. To determine if dietary ganglioside is bioavailable in human participants and whether ganglioside consumption improves clinical outcomes in inflammatory bowel disease (IBD).

Methods Used. Healthy control participants and patients with IBD were recruited for a double-blind, randomized, placebo-controlled study. Participants were allocated to receive either a 1.0 g milk fat fraction with or without ganglioside (80% GD3; 20% GM3) daily for 8 weeks. Blood was drawn to measure plasma ganglioside content at weeks 0, 2, 4, 6 and 8. Participants had bowel symptoms, emotional health, systemic symptoms, and social function assessed by the Quality of Life in IBD Questionnaire.

Results. Level of plasma ganglioside GD3 rapidly increased by 42% after two weeks and by 49% after eight weeks ($P<0.05$). Level of ganglioside GM3 also increased by 8% after eight weeks (not significant). Plasma levels of GM3 and GD3 did not change between baseline and study conclusion in subjects that consumed placebo. Dietary ganglioside improved emotional health by 39% ($P<0.01$) and systemic symptoms by 36% ($P<0.02$) over the course of study in patients with IBD. There was no significant effect of ganglioside on bowel symptoms or social function.

Conclusions. Daily consumption of ganglioside increases plasma ganglioside concentration and constitutes a viable agent for treatment of IBD or for potential treatment of other disorders in which altered ganglioside content has been implicated.

FABP1 and Its Emerging Role in Systemic and Brain Endocannabinoid Systems. Eric Murphy, *University of North Dakota, USA*

Pending.

Are Oxidized Linoleic Acid Metabolites Bioavailable? Ameer Taha*, *University of California, Davis, USA*

Background: Omega-6 linoleic acid (LA, 18:2n-6) is a precursor to oxidized linoleic acid metabolites (OXLAMs) which regulate multiple biological processes. In vivo, OXLAMs can be synthesized enzymatically or non-enzymatically from LA. Recently, our group characterized their abundance in plant oils. However, little is known about their bioavailability.
Objective: The goal of this study is to determine the bioavailability of dietary OXLAMs.
Methods: Two rodent experiments were performed to address the question of whether OXLAMs are bioavailable or not. In Experiment 1, mice were fed for 8 weeks a low LA diet (4% energy), high LA diet (17% energy) or a low LA diet (4% energy) supplemented with thermally oxidized corn oil containing OXLAMs. Plasma and brain OXLAM concentrations were quantified by UPLC-MS/MS. In Experiment 2, rats were gavaged with deuterated 13-Hydroxyoctadecadienoic acid (13-HODE) and tracer incorporation in plasma measured by UPLC-MS/MS at 90 minutes.
Results: In Experiment 1, OXLAM consumption (through the low LA + OXLAM diet) did not alter

plasma or brain OXLAM concentrations compared to the low LA diet. The high LA diet increased plasma and brain OXLAM concentrations compared to the low LA diet. In Experiment 2, labeled 13-HODE was detected in plasma one hour post-gavage (n=1), suggesting absorption. Ongoing analysis will seek to confirm this finding in two other gavaged rats. Conclusion: Dietary OXLAMs may be absorbed through the GI tract but accumulation in plasma and brain appear to be minimal. Thus, in vivo OXLAM concentrations are regulated by dietary LA.

Oxylipins in Kidney Inflammation Harold M. Aukema*, *University of Manitoba, Canada*

Inflammation plays an important role in the progression of kidney diseases. Oxylipins are lipid mediators that are involved in normal kidney physiology and response to physiological challenges, but when present during kidney disease they can contribute to inflammation and worsening of disease. Cystic kidney diseases are the major cause of inherited kidney diseases world-wide, but treatment options for this group of kidney disorders is very limited. Our early studies with forms of pediatric cystic kidney diseases revealed increased levels of lysophospholipid and phospholipase A2, and increased activities of cyclooxygenases (COX). COXs are one of 3 major enzyme pathways that result in the production of oxylipins. Subsequent studies showed that drugs and dietary interventions that reduced disease progression also reduced the production of select COX derived oxylipins in these pediatric forms of the disease. With the advent of technologies that enabled the lipidomic analysis of >100 oxylipins by HPLC/MS/MS, we then showed that the COX derived oxylipins were consistently elevated in both pediatric and adult forms of cystic kidney diseases. Further, these studies showed that these bioactive lipids were elevated early in the disease, and when the disease had only progressed minimally. We then showed that selective COX2 inhibition reduced disease progression by blocking the production of oxylipins in a mouse model of the most common form of cystic kidney disease. Selectively blocking COX2 derived oxylipins therefore provides a potential therapeutic intervention that reduces inflammation and disease progression in these kidney diseases.

EAT 1.1 / H&N 1.1: Structural Determinates of the Metabolic Response for Lipids

Chairs: Michael Rogers, University of Guelph, Canada; and Pamela Hutton, Bunge Loders Croklaan, USA

Replacement of Saturated Fat with Unsaturated Fats from Different Food Sources: Implications for Cardiovascular Risk Kristina S. Petersen*, *The Pennsylvania State University, USA*

Current dietary guidance for prevention and management of cardiovascular disease includes recommendations to replace saturated fats with polyunsaturated fatty acids (PUFA) or monounsaturated fatty acids (MUFA). This presentation will provide an overview of current research focusing on how replacement of saturated fat with MUFA and PUFA from different food sources modulates lipids and lipoproteins, blood pressure, and vascular health. Our lab has shown that replacement of saturated fat with walnuts, or vegetable oils rich in MUFA and PUFA improves lipids and lipoproteins, and blood pressure. Furthermore, data from a randomized, crossover controlled feeding study showed canola oil and high-oleic acid canola oil improved lipids and lipoproteins compared to an oil blend higher in saturated fat. Based on these data and other recent work the magnitude of lipid and lipoprotein lowering expected with different dietary replacement of saturated fat will be explored. In summary, the research presented will describe the effect of different fatty acids within the food matrix on cardiovascular risk and how we can assist consumers to choose foods and dietary patterns that are heart healthy.

Foodomics Insights in the Health Effects of Vegetable Oil YongJiang Xu*[1], Chen Cao[1], Zhaojun Zheng[1], and Yuanfa Liu[2], *[1]Jiangnan University, China; [2]School of Food Science and Technology, State Key Laboratory of Food Science and Technology, Jiangnan University, China*

Vegetable oil plays an important role in daily diet. The differences of fatty acid composition and micronutrient levels result in the varying health effects of different vegetable oils. However, the mechanisms underlying this phenomenon have largely remained elusive. Foodomics is a discipline that studies the food and nutrition domains through the application of advanced omics technologies to improve consumer's well-being, health, and confidence. The objective of this study was to investigate the health effects of different vegetable oils by foodomics analysis. The physicochemical properties and oxidative stability of seven commercial vegetable oils were evaluated, including soybean oil, rapeseed oil, corn oil, sunflower seed oil, rice bran oil, peanut oil and tea seed oil. And then SD rat were fed with high fat diet contained different vegetable oil for 12 weeks. Metabolites in serum and liver tissue were measured using liquid chromatography–and gas chromatography–mass spectrometry, and multivariate statistical analysis was performed by orthogonal projections to latent structures discriminant analysis. The finding showed vegetable oils, especially tea seed oil and rapeseed oil, decrease the liver lipid metabolism disorder in the high fat diet treated mice. This study provided the foundation for quality, nutrition and safety evaluation of vegetable oils and reasonable reference for consumers.

Musseling-up Program: Review of Greenshell Mussel Bioactive Lipids and Role in Inflammation Management and Joint Health Matthew R. Miller*[1], Marlena C. Kruger[2], Fran M. Wolber[2], Hong Tian Hong Tian[3], Parkpoon Siriarchavatana[2], and Saima Rizwan[4], *[1]Cawthron, New Zealand; [2]Massey University, New Zealand; [3]Sanford Ltd, New Zealand; [4]Riddet Institute, New Zealand*

Lipid extracts from New Zealand's iconic Greenshell mussel (GSM) are the world's most

expensive nutritional oil (~$2000 USD/kg). Traditional use of GSM by coastal Maori has been associated with improved joint health and a number of studies have examined the anti-inflammatory effects of GSM extracts. Objectives This review looks at outputs of Musseling up program a 3-year program under the New Zealand National Science Challenge "High Value Nutrition" by a) identifying, discriminating and verifying GSM active ingredients; b) producing novel GSM food/extract products; c) determining the mechanism and efficacy in pre-clinical and clinical trials. Methods We have determined GSM lipid content over seasons, developed rapid near infrared analytical methods and developed novel food applications. Efficacy of GSM was tested 1) in-vitro analysis in macrophage, cartilage osteoclast and pre-osteoblast cell lines, 2) in-vivo in female Sprague-Dawley rats with obesity-induced osteoarthritis and 3) in a clinical trial on bioavailability of the lipids fraction. Results We have determined the variation in GSM with female spring mussels having the highest lipid content. Novel stable GSM rich food ingredients have been made through emulsification and spray drying techniques. The inclusion of GSM in the rat diet significantly reduced blood levels of a cartilage degradation biomarker in rats In vitro, a non-polar lipid extract of GSM significantly reduced osteoclast differentiation in a dose-dependent manner. Clinical trials have determined the extent of the bioavailability of the lipid fractions in 4 different formats. Conclusions GSM food products, food ingredients and extracts provide exciting opportunities for improving joint health and inflammation management.

Serum β-carotene Concentrations is Inversely Associated with Reported Fatty Acid Intake in U.S. Adults Ambria Crusan*[1], Marla Reicks[1], and Susan K. Raatz[2],[1]*University of Minnesota, USA; [2]USDA, ARS, Grand Forks Human Nutrition Research Center, USA*

Objective: Dietary carotenoids are mainly sourced from fruits and vegetables. The bioavailability of carotenoids is dependent on dose, quantity and dispersion, and presence of other nutrients in the diet, specifically fat. However, there is a gap in research on whether specific fatty acid classes affect serum β-carotene concentrations. Our primary objective was to assess the association between serum β-carotene concentrations and reported intake of specific fatty acid classes, utilizing data the What We Eat in America (WWEIA)/National Health and Nutrition Examination Surveys (NHANES). Methods: Data from 9,182 male and female participants 20-85 years of age in the NHANES 2003-2006 nationally representative, cross-sectional survey were analyzed to estimate the relationships between serum β-carotene concentrations and reported saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid intakes. Due to skewing, we log transformed serum β-carotene. Multiple linear regression estimated log(serum β-carotene) based on total reported fatty acid intakes adjusted for age, sex, and ethnicity. Results: Mean and standard error (SE) was 2.51±0.85µg/dL for log(β-carotene). Mean and SE for SFA were 26.72±16.9g, MUFA were 30.40±18.4g, and PUFA were 17.22±11.6g. β-carotene concentrations were weakly and inversely associated with fatty acid classes: SFA (r= -0.15, p

Oleogelation of Emulsified Oil Delays in vitro Intestinal Lipid Digestion Dérick Rousseau*, *Ryerson University, Canada*

The influence of oleogelation on the *in vitro* digestion of oil-in-water (O/W) emulsions was investigated. Rice bran wax (RBX) was used as an oleogelator at concentrations of 0, 0.25, 0.5 1 and 4 wt% of the emulsions, respectively. O/W emulsions containing 1 wt% whey protein

and 20 wt% oil were prepared via hot homogenization, and characterized by rheology, static light scattering, confocal/polarized light microscopy, X-ray diffraction and differential scanning calorimetry. The evolution in particle size distribution, structural changes during oral, gastric and intestinal digestion, and free fatty acid (FFA) release during intestinal digestion were all investigated. All emulsions were generally kinetically stable during the experimental timeframe (4 weeks), with the exception of the 4 wt% emulsion, given the eventual appearance of large RBX crystals (3-5 µm) in the droplets. The rheology and thermal behaviour of the emulsions, as well as the SBO-RBX blends used for each emulsion, demonstrated that addition of RBX led to the formation of rigid oil droplets. During both gastric and intestinal digestion, oil droplet coalescence occurred in all emulsions, and intestinal digestion led to oil droplet aggregation during the early stages of intestinal digestion. The FFA release profiles showed that oleogelation of emulsified oil delayed intestinal lipid digestion. This effect was enhanced with increasing RBX concentration up to 1 wt% due to the increased oil droplet rigidity. With a further increase to 4 wt%, the rate of lipid digestion increased, which was ascribed to the instability of the emulsions containing 4 wt% RBX during digestion caused by large RBX crystals.

Encapsulation, Protection and Controlled Relea se of Nutraceuticals using Biopolymer Microgel Zipei Zhang*, and D. Julian J. McClements,*University of Massachusetts Amherst, USA*

Microgels are one promising colloidal delivery systems widely used for the encapsulation and release of bioactive ingredients. In this study, a novel self-regulating hydrogel microgels were fabricated that contained acid sensitive compounds and a buffer agent (e.g., Mg(OH)2 or CaCO3). This buffer was selected because it is insoluble under alkaline conditions, but soluble under acidic conditions, and can therefore maintain a neutral pH inside the microgels when they are dispersed in a low pH solution (such as acidic gastric fluids). A quantitative ratiometric method based on laser scanning confocal microscopic imaging was also developed to map the microclimate pH inside microgels. The image method was used to detected the pH change of the microgels with or without the encapsulation of buffer agent during digestion process. It was indicated that the initial pH distribution was relatively uniform within the buffer-free microgels, being in the range from pH 6.8-7. For the buffer-loaded microgels, the center pH was slightly higher than the edge with the range from 7.2-7.6. After simulated stomach digestion, the microclimate inside the microgels changed to acid condition. Conversely, the pH value within microgels containing buffer agents was fairly similar to that of the initial value. The activity of loaded bioactives (i.e., enzymes, insulin or probiotics) were successfully reserved after encapsulation within the buffer-loaded microgels during the digestion process. Our results could provide valuable information for the design of microclimate pH mapping technique and development of nutraceutical delivery systems for acid sensitive bioactive ingredients.

The Role of Emulsifiers in Lipid Digestion of Oil-in-Water Emulsions Michael Rogers*, Natalie Ng, Saeed M. Ghazani, Peter Chen, Alejandro G. Marangoni, Amanda Wright, and Douglas Goff,*University of Guelph, Canada*

Fat digestion significantly influences health. Both food composition and food structure influence lipid digestion kinetics and the bioavailability of components they contain. Thus, we need to understand how ALL levels of food structure (i.e., nano-, micro-, macro- and

mesoscales) control the lipemic index of foods. Since surfactants are ubiquitously found in processed foods, there is potential benefit to design food emulsions with tailored lipid digestion profiles. Ultimately our aim is to limit the lipemic index of processed foods however, with the emergence of personalized nutrition there are applications where rapid digestion kinetics could be highly beneficial. 200 ± 20 nm, oil-in-water emulsions, structured using common chain-length (i.e., C18:0) surfactants (e.g. sn-1, and sn-2 monostearin, Span 60, and Tween 60) had their digestion kinetics studied using the TIM-1 simulated gastrointestinal tract. Lipid digestion rate constants, induction times and bioaccessibility were assessed over 5 hr simulated digestions. All parameters are significantly influenced by emulsifier structure, creating exciting new opportunities in structuring infant formulas, energy-dense meal replacements, etc.

Bioavailability of Pesticide Residue in Agricultural Products: Impact of Food Emulsions with Different Surface Properties Ruojie Zhang*, and D. Julian J. McClements, *University of Massachusetts Amherst, USA*

The residue of pesticides in agricultural products has been a big concern for human health since high exposure to pesticide could cause numbers of diseases, such as diabetes, neurological disorders, even cancer. The Bioavailability of pesticides residue in human body are highly impacted by the food components that consumed together. Food emulsions, such as dressings, dips, sauces, and creams, are commonly co-ingested with fruits and vegetables. The purpose of the current study was therefore to examine the potential impact of co-ingestion of emulsions with natural produce on the bioavailability of a hydrophobic pesticide. In current study, the influence of co-ingestion of food emulsions with tomatoes on the bioaccessibility of a model pesticide (chlorpyrifos) was studied. The results indicated that the bioaccessibility of chlorpyrifos (a highly lipophilic pesticide) was shown to depend on the emulsifier types of co-ingested excipient emulsion. Highest pesticide bioaccessibility can be observed for the excipient emulsion formed with phosphate lipid, followed by Tween 80 and WPI. Polysaccharide additions (chitosan, xanthan, β-glucan) can significant impact the bioaccessibility of pesticides that it also depended on the emulsifier type of excipient emulsions. Overall, these results suggest that the bioavailability of undesirable pesticides can be controlled by specificity design excipient emulsion.

Impact of Indigestible Oils on the Bioaccessibility of Vitamin D3 in Nanoemulsion-based Delivery Systems Yunbing Tan*[1], and D. Julian J. McClements[2],[1]*Dept. of Food Science, University of Massachusetts, Amherst, USA;* [2]*University of Massachusetts Amherst, USA*

These is interest in replacing digestible fats with indigestible ones to reduce the calorie content of foods. However, utilization of indigestible oils may have unforeseen nutritional consequences. In this study, the impact of an indigestible oil on the bioaccessibility of vitamin D3 (VD) encapsulated within nanoemulsion-based delivery systems was examined. Four different nanoemulsions were prepared using different combinations of a digestible oil (corn oil, CO) and indigestible oil (mineral oil, MO): CO only; MO only; 1:1 CO:MO system prepared by mixing oils before homogenization (oil mixture); 1:1 CO:MO system prepared by mixing MO and CO nanoemulsions after homogenization (emulsion mixture). A simulated gastrointestinal tract (GIT) was used to study the interaction between pancreatic lipase and the emulsions. The rate of free fatty acid release and the VD

bioaccessibility during 2 h intestine digestion decreased in the same order: CO > oil mixture ≈ emulsion mixture > MO. The digestion of the corn oil occurred primarily during the first 30 minutes, then gradually increased. Except for the MO nanoemulsion, the bioaccessibility of VD increased to a maximum value around 30 minutes of digestion but then decreased during the following 24 h. This effect might be attributed to solubilization of the VD in the mixed micelles, followed by their precipitation. These results show that lipid digestion, micelle solubilization, and micelle aggregation all impact vitamin bioaccessibility. The presence of indigestible oil in the nanoemulsion-based delivery systems reduced vitamin bioaccessibility, but may be useful for giving prolonged release in the colon.

H&N 2: Brain Lipid Biochemistry

Chairs: Eric Murphy, University of North Dakota, USA; and Charles Nider, Abitec Corp., USA

How Docosahexaenoic Acid Enters the Brain: Consensus, Controversies and Updates Richard P. Bazinet*, *University of Toronto, Canada*

The brain requires a constant supply of docosahexaenoic acid from blood to maintain its levels within the brain. Several plasma pools have been proposed to supply the brain with docosahexaenoic acid, including plasma lipoproteins lysophosphatidylcholine and unesterified fatty acids. In this lecture, I will review the evidence for each plasma pool supplying the brain highlighting consensus, controversies and remaining questions. While circulating lysophosphatidylcholine has a higher brain/body partition coefficient than unesterified docosahexaenoic acid, unesterified docosahexaenoic acid entry into the brain is more rapid. The implications of how plasma docosahexaenoic acid-containing lysophosphatidylcholine and unesterified docosahexaenoic acid contribute to maintaining brain docosahexaenoic acid concentrations will also be discussed as well as several recent findings.

Lipidomic analysis of post-mortem Alzheimer's Disease pre-frontal cortex reveals changes in pro-repair lipid pathways Ameer Taha*, *University of California, Davis, USA*

Background: Alzheimer's Disease (AD) is a progressive brain disorder characterized by extensive inflammation and neuronal damage. Specialized lipid mediators known to resolve neuroinflammation and repair damaged neurons have been reported to be reduced in post-mortem brains of AD patients. However, the underlying cause of this reduction is not known. Hypothesis and objective: The present study tested the hypothesis that pro-repair lipid mediators are reduced in post-mortem brains of AD patients because they become trapped within phospholipid and neutral lipid pools via enzymatic esterification. Methods: Post-mortem pre-frontal cortex from pathologically confirmed AD subjects (n=21) and unaffected controls (n=20) was subjected to lipidomic analysis with ultra-high pressure liquid chromatography coupled to tandem mass-spectrometry following separation of brain esterified and unesterified lipid pools with solid phase extraction. Results: Compared to controls, concentrations of several pro-repair lipid mediators esterified to neutral lipids were significantly reduced by ~50% in AD patients ($P<0.05$). No significant changes were observed in free or phospholipid-bound pro-repair lipid mediators.

Conclusion: This study provides novel evidence of reduced esterified pro-repair lipid mediators in post-mortem AD pre-frontal cortex. Targeting pro-repair lipid mediator turnover within neutral lipids may stimulate neuronal repair and resolution of inflammation in AD.

Lipid Binding Proteins and Brain Fatty Acid Uptake and Metabolism: So What is the Role of Liver- Fatty Acid Binding Protein (FABP1)? Eric J. Murphy*, *University of North Dakota, USA*

Over the past 15 years, my laboratory has explored the role of traditional fatty acid binding proteins (FABP) and non-traditional fatty acid binding proteins in facilitating fatty acid uptake and trafficking in the brain. We demonstrated that heart FABP (FABP3), which is localized in neurons, differentially influences brain fatty acid uptake, facilitating the uptake and trafficking of arachidonic acid (ARA). Our observation in brain is a bit different from what we observed for heart fatty acid uptake, suggesting greater selectivity in the brain. We have an extensive body of work on the role of alpha-synuclein (Snca), a non-traditional fatty

acid binding protein that also binds other lipids, in brain fatty acid uptake and metabolism. While Snca influence ARA uptake, it does to by modulating its metabolism by long chain acyl-CoA synthetases. Further, this leads to an important role in brain prostaglandins and 2-arachidonyl glycerol (2-AG) production. Our recent work, in collaboration with Dr. Fred Schroeder, demonstrates that liver FABP (FABP1) also has a role in brain ARA levels. In Fabp1-/- mice, the level of free and total ARA is elevated in the gene-ablated male mice. This increase is similar to the increase observed in the serum of these mice and the levels of the major brain endocannabinoid, 2-AG is increased over 2-fold. This is observation is important as the T94A mutation in FABP1 disrupts its lipid binding in the liver, suggesting a potential to modulate brain ARA level. Further, this is the first observation that a non-brain localized FABP influences brain fatty acids levels, suggesting that there may be a poorly understood interplay between liver lipid metabolism and brain fatty acid uptake and metabolism.

Shotgun Lipidomics Sheds Light on Diabetic Neuropathy Xianlin Han*, *University of Texas Health Science Center at San Antonio, USA*

Diabetic neuropathy is the most common diabetic complication and the greatest source of morbidity and mortality in diabetes. The molecular mechanisms underlying diabetic neuropathy of type 2 diabetes mellitus (T2DM) are still not completely understood although hyperglycemia largely attributes to T1DM diabetic neuropathy. In order to uncover the molecular mechanism(s) leading to T2DM neuropathy, we have recently determined the changes of lipids in brain and nerve tissue samples from db/db mice in a spatiotemporal manner by using our enabling shotgun lipidomics technology platform. We have revealed substantial accumulation of triglyceride and non-esterified fatty acids, and profound reduction of acylcarnitine and myelin lipids in a gradient from sciatic nerve, then spinal cord, to brain cortex, occurred as early as one month of age when the blood glucose level of mice is still normal. We have expanded the findings from lipidomics studies on db/db mice to other T2DM models, such as ob/ob and high fat diet-induced mice, as well as post-mortem brain tissues of T2DM patients. Our mechanistic and functional studies have further demonstrated that abnormal nerve lipid metabolism present in T2DM mice and human tissue is associated with hyperinsulinemia, but independently of hyperglycemia as evidenced with the results obtained from streptozotocin-treated mice (a T1DM model), and precedes nerve structure/function damage. Taken together, our results challenge the current hypothesis that diabetic neuropathy is caused by long-term hyperglycemia placing hyperinsulinemia as a novel driver of nerve damage, and have important implications for the diagnosis and treatment of neuropathy in T2DM.

Cytochrome c is a Plasmalogenase that Oxidatively Cleaves the sn-1 vinyl Ether Linkage of Plasmalogens Christopher M. Jenkins*[1], Kui Yang[2], Gaoyuan Liu[3], Sung Ho Moon[3], Beverly G. Dilthey[3], and Richard W. Gross[3],[1]*Washington University School of Medicine, USA;* [2]*U.S. Food and Drug Administration, United States;* [3]*Washington University School of Medicine, United States*

Plasmalogens are phospholipids critical for cell function and signaling that contain a vinyl ether linkage at the *sn*-1 position and are highly enriched in arachidonic acid (AA) at the *sn*-2 position. However, the enzyme(s) responsible for the cleavage of the plasmalogen vinyl ether linkage have remained elusive. Based upon the susceptibility of the vinyl ether bond to oxidation, we hypothesized that the peroxidase activity of cardiolipin (CL)-activated cytochrome

c could oxidatively cleave the plasmalogen vinyl ether linkage. Accordingly, we found that cytochrome c, in the presence of CL, O_2 and H_2O_2 , or oxidized CL and O_2 , catalyzed the oxidation of the plasmalogen vinyl ether bond, promoting its hydrolytic cleavage and the resultant production of 2-AA-lysolipids and α-hydroxy fatty aldehydes. Using stable isotope labeling in synergy with strategic chemical derivatizations and high-mass-accuracy MS, we deduced the chemical mechanism of this reaction. Specifically, labeling with either $^{18}O_2$ or H_2 ^{18}O resulted in M + 2 isotopologues of the α-hydroxyaldehyde, whereas reactions with both $^{18}O_2$ and H_2 ^{18}O identified the M + 4 isotopologue. Furthermore, incorporation of ^{18}O from $^{18}O_2$ was predominantly located at the α-carbon. In contrast, reactions with H_2 ^{18}O yielded ^{18}O linked to the aldehyde carbon. Importantly, cytochrome c released from myocardial mitochondria subjected to oxidative stress cleaved plasmenylcholine in membrane bilayers, and this reaction was blocked by a specific monoclonal antibody against cytochrome c. Collectively, these results identify the first plasmalogenase in biology, reveal the production of signaling lipids by cytochrome c, and present new perspectives on cellular signaling during oxidative stress.

Homozygous Expression of Mutant ELOVL4 Leads to Seizures and Death in a Novel Animal Model of Very Long-Chain Fatty Acid Deficiency Blake R. Hopiavuori[1,2], Ferenc Deák[1,5,6], Richard S. Brush[2,3], David M. Sherry[1,4], Robert E. Anderson*[1,2,3,4,6], Martin-Paul Agbaga[1,2,3,4,5], [1]Oklahoma Center for Neurosciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA, [2]Dean McGee Eye Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, [3]Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, [4]Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA, [5]Harold Hamm Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, [6]Reynolds Oklahoma Center on Aging, Department of Geriatric Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

Purpose: ELOngation of Very Long chain fatty acids-4 (ELOVL4) is an elongase responsible for biosynthesis of very long chain (VLC; ≥C28) fatty acids; it makes VLC polyunsaturated fatty acids (VLC-PUFA) in retina and testes, and VLC saturated fatty acids (VLC-SFA) in skin and brain. A 2011 case study linked homozygous inheritance of the Stargardt's (STGD3) mutation in ELOVL4 with a central nervous system (CNS) phenotype in humans, including seizures and death. We demonstrate that ELOVL4-synthesized VLC-SFA play an essential regulatory role in pre-synaptic transmission. Methods: We generated an animal model for STGD3/STGD3 inheritance (mut/mut). ELOVL4 localization within the CNS was determined in wild type mice (wt/wt) using immunofluorescence (IF). Synaptic membrane fractionation was performed on baboon hippocampus for lipid analysis. Hippocampal slices from (wt/wt) and (mut/mut) were recorded using a multi-electrode array (MEA). Primary neuronal cultures from hippocampus of (wt/wt) and (mut/mut) mice were subjected to FM1-43 assessment of synaptic vesicle exocytosis rates in the presence or absence of VLC-SFA supplementation. Results: Our STGD3/STGD3 mice recapitulate the human phenotype, developing seizures at P19 followed by death at P21. Membrane fractionation of baboon hippocampus revealed enrichment of 28:0/30:0 in synaptic vesicle membranes. MEA recordings showed a significant dysregulation of both spontaneous and evoked responses in (mut/mut) vs (wt/wt) hippocampal slices. FM1-43 studies showed a significant increase in

synaptic vesicle exocytosis rates in (mut/mut) vs (wt/wt) primary hippocampal neurons and supplementation of 28:0 + 30:0 VLC-SFA to the medium rescued release rates back to wild-type levels, while the LC-SFA, 24:0, did not have a significant effect. . Conclusions: This is the first study to demonstrate mutations in *Elovl4* causing a CNS phenotype in an animal model. The described studies suggest a neuron-specific role for VLC-SFA in the regulation of pre-synaptic synaptic function by impacting the rate of synaptic vesicle release.

H&N 3: Dairy Fatty Acids and Health

Chairs: Moises Torres-Gonzalez, National Dairy Council, USA; and Ignacio Vieitez, UdelaR, Uruguay

Saturated Fat and Cardiometabolic Health Moises Torres-Gonzalez*, *National Dairy Council, USA*

Saturated fat has historically been discouraged by health authorities because its LDL-cholesterol (LDL-C) rising effects. It is still thought that elevated LDL-C levels increase the risk of cardiovascular disease (CVD). However, recent scientific evidence indicates that LDL-C is just one of many mediators of the atherosclerosis process and therefore, the prediction of CVD risk is not necessarily reflected by the measurements of blood LDL-C levels. In this sense, emerging scientific evidence that indicates that saturated fat consumption may not be associated with increased risk of CVD, suggesting that the relationship between saturated fat, LDL-C and CVD is more complex than once thought. The term “saturated fat” has been used to describe mainly the major saturated fatty acids (SFAs) in our diet, e.g. palmitic (C16:0) and stearic (C18:0) acids, however, it results inaccurate because oversimplifies the diversity of saturated fatty acids and may mislead consumers to avoid foods containing “saturated fat” that otherwise may be more beneficial than detrimental. Thus, saturated fat is not a single compound but a family of diverse fatty acids with different physiologic, metabolic and health-promoting properties. During this presentation, a summary of the emerging scientific evidence on saturated fat and its relationship with cardiometabolic health will be presented.

Dairy Fat and Cardiometabolic Health Mario Kratz*, *Fred Hutchinson Cancer Research Center, USA*

Objective: In this presentation, we will provide an overview of the current state of knowledge about the effect of dairy fat on cardiometabolic disease risk, based on both observational studies and randomized controlled trials. Methods Used: Using literature searches on PubMed and Google Scholar, we identified observational studies reporting associations between cardiometabolic disease endpoints (type 2 diabetes, cardiovascular disease, coronary heart disease, stroke) and (a) both full-fat and low-fat dairy products, and (b) dairy fat intake; and randomized controlled trials investigating the effect of interventions rich in full-fat dairy or dairy fat on glucose homeostasis or cardiovascular disease risk factors. Results: Observational studies assessing the relationship between both low-fat and full-fat dairy food consumption and incident type 2 diabetes or cardiovascular disease are widely inconsistent. Plasma phospholipid biomarkers of dairy fat intake are consistently inversely associated with incident type 2 diabetes, but not with cardiovascular disease. Randomized controlled trials testing the effect of full-fat dairy on glucose homeostasis are scarce and inconsistent. Intervention trials assessing effects of full-fat dairy intake on cardiovascular disease risk factors show complex effects on the serum lipid profile that are hard to interpret, and that likely depend on the food matrix in which dairy fat is consumed. Conclusions: Overall, the existing knowledge does not suggest consistent negative effects of dairy fat or full-fat dairy product consumption on cardiometabolic disease risk.

Effects of Medium- and Long-chain Triacylglycerols on Lipid Metabolism and Host Faecal Microbiota Composition in C57BL/6J Mice Shengmin Zhou*[1], Yuan Rong Jiang[2], and Liangli Yu[3],[1]*Wilmar Global R&D Center, China;* [2]*Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China;* [3]*Dept. of Nutrition and Food*

Science, University of Maryland, USA

Objective: In this study, the effects of medium- and long-chain triacylglycerols (MLCT) on lipid metabolism and fecal gut microbiota composition of C57BL/6J mice were investigated. Methods: Three MLCT with different contents of medium-chain fatty acids (MCFA) (10%-30%, w/w) were prepared, and fed to C57BL/6J mice. Comprehensive analysis was used to evaluate the effects of MLCT on the lipid metabolism and gut microbiota composition in C57BL/6J mice. Results: MLCT with 30% (w/w) MCFA showed the best performance in decreasing body weight gain as well as optimizing serum lipid parameters and liver triacylglycerol content. The expression levels of genes encoding enzymes for fatty acid degradation increased markedly and expression levels of genes encoding enzymes for de novo fatty acid biosynthesis decreased significantly in the liver of mice treated with MLCT containing 30% (w/w) MCFA. Gut microbiota composition showed that the dietary intake of a high fat diet containing MLCT did significantly decrease the ratio of Firmicutes to Bacteroidetes and down-regulate the relative abundance of Proteobacteria. In addition, the intake of MLCT can effectively down-regulate the abundance of Helicobacter. Furthermore, a notable increase was found in the total short-chain fatty acid content in feces of mice on a MLCT containing diet. Conclusions: This study shows that MLCT not only have a direct effect on lipid metabolism by influencing gene expression and decreasing triacylglycerol content, but also have an effect on gut microbiota composition, which may be responsible for the anti-obesity effect of MLCT with relatively high contents of MCFA.

Dairy Fatty Acids: Potential Benefits Jana Kraft*, and Allison Unger, *University of Vermont, USA*

Dairy fat is undeniably the most complex and diverse dietary fat source in the human diet, comprising hundreds of different fatty acids and fatty acid derivatives that fulfill structural, metabolic, and functional roles within the human body. Moreover, the fatty acid composition of dairy fat is distinct because approximately 70% of the total fatty acids are saturated, which arise from the effects of ruminal biohydrogenation of dietary unsaturated fatty acids. Over the past two decades, most scientific and public attention has focused on this fatty acid class. Yet, dairy fat contains a large variety of unique minor fatty acid classes or individual fatty acids that are generated by rumen microbes and the mammary gland. These fatty acids consist of short- and medium-chain fatty acids, odd- and branched-chain fatty acids, positional and geometric isomers of octadecenoate, and conjugated linoleic acids. Short-and medium-chain fatty acids are de novo synthesized in the mammary gland utilizing the rumen microbe-derived fermentation products acetate and beta-hydroxybutyrate. Branched-chain fatty acids arise directly from rumen bacteria via de novo lipogenesis, whereas isomers of octadecenoate and conjugated linoleic acids originate from incomplete microbial biohydrogenation processes of feed-derived polyunsaturated fatty acids in the rumen and desaturase activities in the mammary gland. Recent research has indicated that these unique dairy-derived fatty acids possess unusual biological properties and health benefits beyond their basic nutritional value. The presentation will summarize the current knowledge in the research of dairy-derived fatty acids with emphasis on potential health benefits.

Other Dairy Fat Components with Potential Health Benefits: Milk Phospholipids Christopher N. Blesso*, *University of Connecticut, USA*

Milk fat is encased in a tri-layer milk fat

globule membrane (MFGM). Phospholipids (PLs), like sphingomyelin (SM), are important components of MFGMs, accounting for about 1% of total milk lipids. The surfactant properties of PLs are important for dairy products; however, dairy products vary considerably in their PL to total lipid content due to processing. This presentation will focus on the potential of milk PLs in protecting against dysfunctional lipid metabolism, inflammation, and gut dysbiosis. Dietary SM has been reported to dose-dependently reduce the intestinal absorption of cholesterol, triglyceride, and fatty acids in cell culture and rodent studies. Feeding milk PLs has been shown to attenuate some aspects of Western diet-induced dyslipidemia, fatty liver, gut dysbiosis, and inflammation in rodent studies. Additionally, feeding milk PLs attenuates Western diet-induced hyperlipidemia and atherosclerosis in LDL-receptor knockout mice. These hypolipidemic effects of milk PLs observed in rodents are also seen in some human studies, although the extent of reductions in serum cholesterol is typically smaller. This may be explained by the more effective digestion of SM in humans and due to most clinical studies being conducted in healthy populations. Little is known about the impact of milk PLs on inflammation markers and the gut microbiota of humans. Milk PLs should be considered as food matrix factors that may confer health benefits and/or impact effects of other dietary lipids, with implications for full-fat vs. reduced-fat dairy.

A Novel Dietary Oil Rich in Pentadecanoic (C15:0) and Heptadecanoic (C17:0) Odd-chain Fatty Acids Eneko Ganuza*, and Magdalena Amezquita, *Heliae Development LLC, USA*

Plasma levels of pentadecanoic (C15:0) and heptadecanoic acid (C17:0) were associated with lower risk of diabetes type-2 and cardiovascular disease. Dairy consumption, which is the main dietary source of odd-chain fatty acid (OCFAs), was also associated with lower risks of cardiovascular disease in the prospective cohort study “PURE”. These results contrast with the prevailing dietary guidelines against saturated fat intake because roughly 45 % of it comes from dairy. Milk is predominantly composed even-chain saturated fatty acids (~70% total fatty acids, TFA) and contains trace amounts of C15:0 and C17:0 (*Aurantiochytrium acetophilum* strain-HS399, that is capable of accumulating lipids of up to 85% of its weight when grown under heterotrophic conditions. This strain naturally produces palmitic (C16:0) (45%) and docosahexaenoic acid (DHA, C22:6 n-3) (40% TFA), but we have modified our fermentation process to synthesize C15:0 (40%), C16:0 (15%), C17:0 (8%) and DHA (28% TFA). Hence, we now have the capacity to produce, at industrial scale, a new nutritional oil that will help us for the first time to experimentally isolate the effect of dietary odd-chain from even-chain saturated fatty acids. To our knowledge, this is the only natural oil containing high concentrations of OCFAs (>49% TFA), a product that can be used to expand research on anaplerotic metabolism and biomarkers in epidemiology, and ultimately become an important nutritional supplement.

H&N 4: Health and Nutrition Awards and General Topics

Chairs: Matthew Picklo, USDA, ARS, Grand Forks Human Nutrition Research Center, USA; and Elisa Di Stefano, University of Ottawa, Canada

Oxidized dietary fat: a novel risk factor of inflammatory bowel disease and colon cancer via altering gut microbiota Guodong Zhang*, *UMass-Amherst, USA*

Vegetable oils, such as corn, soybean, safflower, and canola oils, consist a substantial part of our diet. Rich in polyunsaturated fats, the vegetable oils are highly prone to oxidation and these oxidation products are commonly found in the diet. However, the effects of oxidized oils on human health are not well understood. Here we show that dietary administration of oxidized corn or soybean oil, even with low concentrations of lipid oxidation products (within the recommended industrial limit of oxidative status of fresh vegetable oil), exaggerates dextran sodium sulfate (DSS)-induced colitis and exacerbates azoxymethane (AOM)/DSS-induced colon tumorigenesis in mice. Oxidized oil reduces the diversity and alters the composition of gut microbiome, and fails to promote colitis in antibiotic cocktail-treated mice. These results suggest that oxidized vegetable oil, even at low oxidative status, could be a novel risk factor of inflammatory bowel disease (IBD) and IBD-associated colon cancer.

4-HNE, an Endogenous Lipid Peroxidation Product, Exacerbates Colonic Inflammation through Activation of Toll-like Receptor 4 Signaling Yuxin Wang*[1], Weicang Wang[2], and Guodong Zhang[3],*[1]UMASS, USA; [2]UMass-Amherst, USA; [3]UMass-Amherst, USA*

Objective: Human and animal studies have shown that the colonic concentrations of lipid peroxidation products, such as 4-hydroxynonenal (4-HNE), are elevated in inflammatory bowel disease (IBD). However, the actions and mechanisms of these compounds on the development of IBD are unknown. A better understanding of the pathophysiological components involved in IBD could help to develop novel strategies to reduce its risk. Methods and Results: We show that systemic treatment with low-dose 4-HNE exacerbates dextran sulfate sodium (DSS)-induced IBD in C57BL/6 mice, suggesting its pro-IBD actions in vivo. Treatment with 4-HNE suppressed colonic expressions of tight junction protein occludin, impaired intestinal barrier function, and enhanced translocation of lipopolysaccharide (LPS) and bacterial products from the gut into systemic circulation, leading to increased activation of Toll-like receptor 4 (TLR4) signaling in vivo. Furthermore, 4-HNE failed to promote DSS-induced IBD in Tlr4-/- mice, supporting that TLR4 signaling contributes to the pro-IBD effects of 4-HNE. Conclusions:4-HNE exacerbate progression of IBD through activation of TLR4 signaling and therefore could contribute to the pathogenesis of IBD.

Anti-inflammatory Effect of a Novel Metabolite from Marine Carotenoid Fucoxanthin Masashi Hosokawa*, Naoki Takatani, Daisuke Taya, Fumiaki Beppu, and Kazuo Miyashita, *Hokkaido University, Japan*

Fucoxanthin is a major marine carotenoid containing in edible brown seaweeds. It has unique structures such as allenic bond and epoxy group in the molecule. We previously reported that dietary fucoxanthin exhibited anti-obesity and anti-diabetic effects through prevention of chronic inflammation via adipokine regulation in white adipose tissue (WAT) in diabetic/obese mice. Dietary fucoxanthin is known to be metabolized to fucoxanthinol and amarouciaxanthin A in the body. Furthermore, we identified an apo-carotenoid paracentrone as a novel metabolite of fucoxanthin in the mice. Paracentrone

significantly down-regurated interleukin-6 and monocyte chemoattractant protein-1 mRNA expressions in macrophage-like RAW264.7 cells activated by LPS. Other apo-fucoxanthinoids such as apo-10'-fucoxanthinal also down-regulated mRNA expression of proinflammatory cytokines in activated RAW264.7 cells. These results suggest that apo-fucoxanthinoids derived from dietary fucoxanthin have anti-inflammatory effect.

Oxidized Triacylglycerols from Grape Seed Oil Modulate Phospholipid Pool in Gastric Cells
Sarah Frühwirth[1], Sofie Zehetner[1], Mohammed Salim[1], Sonja Sterneder[1], Barbara Lieder[1], Luca Nicolotti[2], Martin Zehl[3], Markus Herderich[2], Veronika Somoza[1], and Marc Pignitter*[1],[1]*Department of Physiological Chemistry, Faculty of Chemistry, University of Vienna, Austria;* [2]*Metabolomics South Australia, Australian Wine Research Institute, Adelaide, Australia;* [3]*Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Austria*

Consumption of vegetable oils rich in polyunsaturated fatty acids is considered healthy. However, these oils are characterized by a lack of oxidative stability, leading to the accumulation of lipid oxidation products, which are supposed to have detrimental effects on health. Studies investigating the metabolic response of gastric cells exposed to oxidized lipids are scarce. We aimed at identifying the effects of oxidized triacylglycerols from differently oxidized and in-vitro digested grape seed oils on the cellular metabolism in human gastric tumor cells (HGT-1). Moderately (peroxide value: 18 meq O2/kg) and highly (peroxide value: 28 meq O2/kg) oxidized grape seed oil was in-vitro digested and analyzed for their antioxidant constituents as well as oxidized triacylglycerol profile by LC-MS/MS. HGT-1 cells were treated with isolated fractions containing oxidized triacylglycerols for up to six hours. Changes in the abundances of cellular metabolites were identified by high-resolution MS and MS/MS fragmentation by applying non-targeted metabolomics. In moderately and highly oxidized grape seed oil, epoxidized and hydroperoxidized triacylglycerols, such as TG(54:4)OOH, TG(54:5)OOH, TG(54:6)OOH, TG(54:0)O, TG(54:1)O, TG(54:2)O, TG(54:3)O, TG(54:4)O, TG(54:5)O could be identified, whereas the abundances of the epoxidized lipids were markedly diminished in in-vitro digested oil samples. Incubation of HGT-1 cells with oxidized triacylglycerols from non-digested, oxidized grape seed oil induced the formation of phospholipids, such as PC(18:2), PC(40:7) and PI(20:4). Polar lipids from in-vitro digested oil did not affect the phospholipid metabolism. In-vitro digestion-induced decrease of epoxidized triacylglycerols might be responsible for the loss of the effect of dietary oxidized lipids on intracellular phospholipid pool.

Comparison of Postprandial Triacylglycerols and Satiety Ratings by Healthy Men Following Ingestion of Tempered Palm Stearin-based Emulsions Containing Droplets in Different Physical States
Samar Hamad*[1], Surangi KPH Thilakarathna[2], Amanda Cuncins[3], Melissa Brown[2], and Amanda Wright[2],[1]*University of Gueloh, Canada;* [2]*University of Guelph, Canada;* [3]*University of Guelphph, Canada*

With the prevalence of obesity and the relationship between postprandial metabolism and chronic disease risk, there is growing need to better understand how dietary lipid properties, including physical state, relate to their digestion and ability to impact satiety. Emulsions containing solid fat droplets versus compositionally equivalent undercooled liquid droplets experienced acid-induced partial coalescence and resisted lipolysis under in-vitro digestion conditions. To test the hypothesis that this would translate into differences in lipemic

response and satiety inductions, a double-blinded randomized crossover study was performed. Fifteen fasting healthy male (age = 27.5 ± 5.7 y; BMI = 24.1 ± 2.5; fasting triacylglycerols (TAG) = 0.9 mmol/L) non-restrained eaters consumed 50 g of palm stearin in a 500 mL emulsion beverage which contained droplets tempered in either the partially crystalline (i.e. SE) or undercooled (i.e. LE) state, one at each of two visits. Throughout the 6-hour postprandial period, participants provided blood samples and completed visual analogue satiety scales. Preliminary results reveal that LE had a higher AUC lipemic response and earlier rise from baseline compared to SE. Change from baseline values for Hunger, Appetite and overall Average Appetite were consistently lower, and Fullness was consistently higher, for LE compared to SE, but no significant differences were observed (P>0.05). Therefore, emulsion droplet solid fat was associated with small differences in postprandial TAG, although these did not correspond with differences in participant ratings of satiety.

Analysis of Plasmalogen Species in Serum and Post-mortem Brain Tissue of Alzheimer's Disease Patients Yurika Otoki*[1], Shunji Kato[1], Kiyotaka Nakagawa[1], Di Yu[2], Danielle J. Harvey[3], Lee-Way Jin[3], Britany N. Dugger[3], Walter Swardfager[2], and Ameer Taha[4],[1]*Tohoku University, Japan;* [2]*Sunnybrook Research Institute, Canada;* [3]*University of California, Davis, USA;* [4]*University of California, Davis, USA*

Background and hypothesis: Alzheimer's disease (AD) is a progressive brain disorder characterized by the deposition of abnormal protein aggregates and loss of neurons. In AD brains and blood, marked changes in ether-linked phospholipids known as plasmalogens, have been reported. The complement of molecular plasmalogen species have been difficult to measure, especially choline plasmaglogen (PlsCho), due to little fragmentation in MS/MS and co-elution with other phospholipids during column chromatography separations. Recently, we overcame those limitations by developing a sensitive and selective method for the quantitative determination of plasmalogen molecular species using LC-MS/MS in the presence of sodium ion. Methods: In this study, we quantified plasmalogens including PlsCho and ethanolamine (PlsEtn) species, in serum (n=25-29 per group) and postmortem pre-frontal cortex (n=20-21 per group) of AD patients and age-matched healthy controls. We hypothesized that peripheral and central disturbances in plasmalogen species would be observed in AD patients. Results: In serum, PlsCho and PlsEtn species bearing docosahexaenoic acid (DHA) were significantly increased in AD patients compared to controls. In post-mortem AD brains, PlsCho bearing DHA and PlsEtn bearing arachidonic acid (ARA) were reduced, whereas alkyl phosphatidylcholine bearing DHA was increased. The results suggest dysregulated metabolism of DHA and ARA bearing plasmalogens in serum and brain of AD patients.

Applying Carbon-13 Natural Abundance in Human Plasma as a Tool for the Assessment of n-3 Polyunsaturated Fatty Acid Metabolism Adam H. Metherel*[1], Maha Irfan[1], Shannon L. Klingel[2], David M. Mutch[2], and Richard P. Bazinet[1],[1]*University of Toronto, Canada;* [2]*University of Guelph, Canada*

Carbon-13 isotopic abundance ($\delta^{13}C$) of tissue and blood n-3 polyunsaturated fatty acids (PUFA) can answer complex metabolic questions in rodent models; however, to date, these methods have not been extensively applied in humans. Our objective was to track the changes in $\delta^{13}C$ in human plasma following eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) supplementation.

Participants (aged 21.6 ± 2.2 y) were randomly allocated into one of three supplement groups for 12 weeks: 1) olive oil control, 2) ~3 g/d EPA or 3) ~3 g/d DHA. Blood was collected before and after the supplementation period, and plasma fatty acid concentrations and $\delta^{13}C$ of n-3 PUFA were determined. EPA supplementation increased ($p < 0.05$) plasma docosapentaenoic acid (DPAn-3) concentrations by 9-fold and 2-fold, respectively, but did not affect DHA concentrations. However, both plasma $\delta^{13}C$-DPAn-3 (-28.9 ± 0.3 to -25.0 ± 0.1, milliUrey ± SEM) and $\delta^{13}C$-DHA increased (-27.9 ± 0.2 to -25.6 ± 0.1, $p < 0.05$) to values close to $\delta^{13}C$-EPA in the supplement (-23.5 ± 0.22). DHA supplementation increased ($p < 0.05$) plasma EPA by 130%, but plasma $\delta^{13}C$-EPA was unaffected by supplementation. Our study is the first to assess changes in human $\delta^{13}C$-PUFA following either EPA or DHA supplementation. We provide clear evidence for significant metabolic conversion of EPA to DHA with EPA supplementation, and the absence of retroconversion of DHA to EPA with DHA supplementation. $\delta^{13}C$ of plasma n-3 PUFA in humans is a powerful tool that can track dietary n-3 PUFA to elucidate complex metabolic questions.

Lipidomic Profiling Reveals Soluble Epoxide Hydrolase as a Therapeutic Target of Obesity-induced Colonic Inflammation Weicang Wang*[1], Jun Yang, Jianan Zhang , Yuxin Wang[1], Katherine Z. Sanidad[2], Zhenhua Liu[2], Bruce D. Hammock, and Guodong Zhang[3],*[1]UMass-Amherst, USA; [2]University of Massachusetts-Amherst, USA; [3]UMass-Amherst, USA*

Background: Obesity is associated with enhanced colonic inflammation, which is a major risk factor of colorectal cancer. Considering the obesity epidemic in the United States, there is an urgent need to identify novel therapeutic targets for obesity and its related disease. Method: In LC-MS/MS-based lipidomics approach, ~100 mg of colon tissues from 8 mice per group were mixed with an antioxidant solution (0.2 mg/mL butylated hydroxytoluene and 0.2 mg/mL triphenylphosphine in methanol), 10 µL of deuterated internal standards (500 nM of d4-6-keto PGF1a, d4-TXB2, d4-PGE2, d4-LTB4, d11-14,15-DHET, d4-9-HODE, d8-5-HETE, d11-11,12-EET), and 400 µL extract solution (0.1% acetic acid with 0.2 mg/mL butylated hydroxytoluene in methanol), and then homogenized. The lipid metabolites in the combined solutions were loaded onto pre-washed Waters® Oasis solid phase extraction HLB cartridges, washed with 95:5 v/v water/methanol with 0.1% acetic acid, the analytes were eluted with methanol and ethyl acetate, dried using a centrifugal vacuum evaporator, then reconstituted in methanol for LC-MS/MS analysis. The LC-MS/MS analysis was carried out on an Agilent 1200SL HPLC system (Agilent, Santa Clara, CA) coupled to a 4000 QTRAP MS/MS (AB Sciex, Foster City, CA). The peaks were identified according to the retention time and specific multiple reaction monitoring transitions of the standards of lipid metabolite. The concentrations of the lipid metabolites were calculated against the calibration curve with standards. To study the functional role of sEH in obesity-induced colonic inflammation, we treated mice with sEH inhibitors, TPPU or t-TUCB, in a high-fat diet (HFD)-induce obesity model. Result: Using an LC-MS/MS-based lipidomics approach, we found that obesity-induced colonic inflammation is associated with increased expression of soluble epoxide hydrolase (sEH) and its eicosanoid metabolites, termed fatty acid diols, in colon tissue. Pharmacological inhibition of sEH attenuated obesity-induced colonic inflammation by diminishing the infiltration of inflammatory cells and decreasing the gene expressions of the pro-inflammatory cytokines in colon tissues in obese mice. Furthermore, we examined the sEH genetic

knockout mice in obesity model, and found genetic ablation of sEH reduced colonic concentrations of fatty acid diols and decreased the gene expressions of pro-inflammatory cytokines. Finally, HFD treatment increased expression of phosphorylated GSK3β in colon tissue, while such effect was attenuated in sEH genetic knockout mice, or by treatment with sEH inhibitors, suggesting that pharmacological inhibition and genetic ablation of sEH attenuated HFD-induced activation of Wnt signaling in the colon. Conclusion: Together, these results support that sEH could be a novel therapeutic target for obesity-induced colonic inflammation and associated diseases. Moreover, our study would lead to rapid human translation, as pharmacological inhibitors of sEH are being evaluated in human clinical trials targeting multiple disorders.

Why Fat Was the Dietary Devil … and Then it Wasn't David M. Klurfeld*, *USDA Agricultural Research Service, USA*

Epidemiologic and animal studies beginning in the 1960's suggested that dietary fat increased the risk of several types of cancer. For the last four decades, the number one dietary advice was to eat less fat. Our lab began publishing in the 1980's a series of studies that caloric intake was far more important than fat intake for promotion of tumor growth around the time the low-fat message became widely accepted. Both colon and breast cancers were inhibited by caloric restriction (CR) no matter how much fat was fed. Other labs demonstrated similar effects of CR in a variety of tumor models. We went on to determine that approximately 25% CR was required to significantly inhibit tumor promotion, that genetic obesity markedly promoted tumor growth, and that body fat was also not a significant factor in tumor promotion. These results helped set the stage for the Women's Health Initiative that tested low-fat diet against the average American diet in 50,000 people for reductions in multiple chronic diseases and found no significant benefit on any primary endpoint. Collectively, animal studies and human diet interventions did not support the observational associations of high dietary fat and cancer. The same is true today for claims about meat and cancer. This example of experimental data as a catalyst for overturning accepted dogma in nutrition demonstrates that the field must embrace higher standards for reaching conclusions about causality.

H&N-P: Health and Nutrition Poster Session

Chair: Matthew Picklo, USDA, ARS, Grand Forks Human Nutrition Research Center, USA

1. **Effect of Whey Peptides on Metabolism and Insulin Signaling in Muscle and Fat Cells.** Kenneth D'Souza*[1], Angella Mercer[1], Hannah Mawhinney[2], Thomas Pulinilkunnil[1], Chibuike C. Udenigwe[2], and Petra Kienesberger[1], *[1]Dalhousie Medicine New Brunswick, Canada; [2]University of Ottawa, Canada*

RATIONALE: Adipose tissue and skeletal muscle dysfunction are hallmarks of obesity and insulin resistance. Bioactive peptides derived from food sources including milk and dairy products have gained interest for their roles in obesity and insulin resistance. However, it remains unclear whether and how whey impacts adipose and muscle metabolism and insulin function. HYPOTHESIS: Bioactive whey peptides have an insulin-sensitizing effect on adipocytes and muscle cells. METHODS: Whey peptide mixture was generated via the hydrolysis of whey protein with pepsin and pancreatin. 3T3-L1 pre-adipocytes were incubated with 2.5 mg/ml bovine serum albumin (BSA) or whey peptides during differentiation. Lipid accumulation and the expression of proteins involved in lipid metabolism was analyzed. Insulin resistant C2C12 myotubes were incubated with BSA or whey peptides for 16 h followed by insulin signaling analysis. RESULTS: In 3T3-L1 cells, whey peptides increased expression of the master-regulators of adipogenesis, C/EBPα and PPARγ. This was associated with upregulation of perilipin and adiponectin, markers of enhanced lipid storage and insulin sensitivity, and increased triacylglycerol accumulation. In C2C12 myotubes, whey peptides protected from palmitate-induced insulin resistance, as determined by improved AKT phosphorylation and Glut4 expression. Insulin sensitization of C2C12 myotubes was accompanied by decreased inflammation and ER stress following whey peptide treatment. CONCLUSIONS: Whey peptides promote differentiation and PPARγ activation in adipocytes. In myotubes exposed to an obese-diabetic milieu, whey peptides ameliorate insulin resistance, potentially by reducing inflammation and ER stress. Taken together, our data suggest that whey peptides directly enhance lipid metabolism and insulin function in adipocytes and muscle cells.

2. **Ursolic Acid Attenuates 6-Hydroxydopamine Induced-Apoptosis through Up-Regulation of OPA1 in SH-SY5Y Cells.** Fang-Ting Chang, Chia-Yuan Lin, and Chia-Wen Tsai*, *China Medical University, Taiwan*

Ursolic acid attenuates 6-hydroxydopamine induced-apoptosis through up- regulation of OPA1 in SH-SY5Y cells Fang-Ting Chang, Chia-Yuan Lin and Chia-Wen Tsai* Department of Nutrition, China Medical University, Taiwan *cwtsai@mail.cmu.edu.tw Hypothesis: Mitochondrial dynamic imbalance is related to the pathogenesis of Parkinson's disease (PD). Optic atrophy 1 (OPA1), a mitochondrial fusion protein, is responsible for maintenance of mitochondrial integrity and protection from cell death. In this study, we explored whether ursolic acid (UA) obtained from herbs and fruits could protect SH-SY5Y neuronal cells against 6-OHDA induced apoptosis by up-regulation of OPA1. Methods: Western blotting was used to detect the protein expression. The method of small interfering RNA transfection was used to explore the effect of knockdown of parkin and OPA1. Immunoprecipitation was used to explore whether IκB kinase (IKKγ) interacted with ubiquitin by UA. Results: Cells pretreated with UA attenuated the cell death, nuclear condensation, and cleavage of caspase 3 induced by 6-OHDA. Moreover, UA reversed 6-OHDA-reduced the protein expression of parkin and OPA1. UA alone increased the parkin protein, enhanced the interaction of ubiquitin

and IKKγ, activated the p65 pathway and induced the OPA1 protein. Suppression of parkin reduced the ability of CA on p65 activation and OPA1 induction. In cells-treated with OPA1 siRNA, UA could no longer significantly reverse the apoptosis and nuclear condensation by 6-OHDA. Conclusions: UA increases the protein expression of OPA1 through parkin regulated NFκB pathway, leading to prevent the apoptosis by 6-OHDA.

3. **Incorporation of Antioxidants and Omega-3 Fatty Acids in Fresh Lambs by Feeding Rumen-protected Dietary Supplements.** Chelsea Jeanjulien[1], Jung Hoon Lee*[1], and Stephan Wildues[2], *[1]Fort Valley State University, USA; [2]Virginia State University, USA*

Incorporation of antioxidants and ω-3 fatty acids in fresh meat can provide healthier meat for human and extend the shelf life of fresh meat. Twenty-four lambs were used to assess the effects of rumen-protected dietary supplements (RPSD) containing ω-3 fish oil (140 g/kg) and DL-α-tocopheryl acetate (TOA: 759 mg/kg) with or without L-ascorbic acid (ASC: 784 mg/kg), prepared with a GRAS chemical on fresh lambs. Lambs were assigned to one of three dietary treatments, which consisted of corn basal diet (CBD) with TOA and ASC, and 86.7% CBD plus either RPSD containing TOA only or TOA and ASC. Lambs were harvested after a 49-d of feeding trial, and muscle tissues were obtained from each carcass to analyze α-tocopherol and ascorbic acid contents, and fatty acid compositions. Compared with lambs fed a CBD diet, lambs fed both RPDS containing diets had higher ($P < 0.05$) concentrations of α-tocopherol (LD muscle, 1.15 vs 2.73 and 3.84 μg/g; psoas major, 1.36 vs 3.57 and 4.34 μg/g; gluteus medius, 1.51 vs 4.18 and 4.50 μg/g) in muscle samples. Compared with lambs fed CBD, lambs fed either RPDS containing TOA or TOA + ASC had higher ($P < 0.05$) concentrations of eicosapentaenoic (C20:5ω3, 0.24 vs 0.48 or 0.58%) and docosahexaenoic (C22:6ω3, 0.49 vs 0.85 or 1.13%) acids in LD muscle. The results indicate that feeding lambs with the protecting dietary supplements prepared with GRAS chemicals can increase the deposition of α-tocopherols and ω-3 fatty acids in their muscle.

4. **Identification of Angiotensin-converting Enzyme Inhibitory Peptides Resisting Gastrointestinal Digestion from Thermolysin-digested Egg White Hydrolysate.** Hongbing Fan*, Jiapei Wang, and Jianping Wu, *University of Alberta, Canada*

As a high quality protein, the hen's egg proteins are recognized as excellent sources of bioactive peptides, such as angiotensin-converting enzyme inhibitory (ACEi) peptides. Our previous research showed that egg white hydrolysate (EWH), prepared by thermolysin and then pepsin, decreased blood pressure significantly in spontaneously hypertensive rats (SHRs); further investigation showed that thermolysin-digested EWH (T-EWH) could also reduce blood pressure significantly in SHRs. In addition, in vitro ACEi assay showed that the ACEi activity of T-EWH could be enhanced by simulated gastrointestinal (GI) digestion. These results indicated that T-EWH might contain ACEi peptides with excellent GI stability. Therefore, the purpose of this study is to identify ACEi peptides in T-EWH that resist GI digestion. After conventionally activity-guided fractionation, all fractions were subjected to simulated GI digestion, before and after which their ACEi activities were both determined. Finally, six new ACEi peptides (LAPYK, LKISQ, LKYAT, INKVVR, LFLIKH, and LGHWVY), resisting gastrointestinal digestion, were identified and validated, with IC50 values < 15 uM, respectively.

5. **Effects of Dietary Scallop Oil Prepared from Scallop By-product on Lipid Metabolism in Type II Diabetic/Obese KK-Ay Mice.** Koki Sugimoto*[1], Junpei Nakamura[1], Ryota Hosomi[1],

Munehiro Yoshida[1], and Kenji Fukunaga[2], *[1]Kansai University, Japan; [1]Faculty of Chemistry, Materials and Bioengineering, Kansai University, Japan*

Internal organs of scallops, such as, the midgut glands are rich in eicosapentaenoic and docosahexaenoic acid. Scallop by-products have not yet been utilized to their full potential because they have high concentrations of toxic substances including cadmium and arsenic. We have successfully prepared scallop oil (SCO) containing low toxic substances from scallop by-products. In this study, we prepared SCO from scallop by-products obtained from two different production areas and referred to them as SCOM (SCO from Mutsu, Aomori), and SCOU (SCO from Uchiura, Hokkaido). The aim of this study was to investigate the mechanism by which dietary SCO influences lipid metabolism in KK-Ay mice. Four-week old male KK-Ay mice were sub-divided into four groups. Mice in the control group were fed a AIN93G modified high-fat (3% soybean oil plus 17% lard) diet, and other groups were fed high-fat diet, where 7% of the lard was substituted with SCOM, SCOU, or tuna oil. After 7 weeks, serum triacylglycerol levels decreased significantly in the SCOM, SCOU, and tuna oil-fed mice compared to the control group. Epididymal adipose tissue weight significantly decreased in the SCOU group compared to the control group. The hepatic mRNA levels of fatty acid synthase, acetyl-CoA carboxylase, and stearoyl-CoA desaturase tended to decrease while expression of carnitine palmitoyltransferase-1, peroxisome proliferator-activated receptor alpha, and acyl-CoA oxidase tended to increase in the SCOM and SCOU groups, respectively, compared to the control group. Thus, dietary SCOM and SCOU may ameliorate lifestyle-related diseases such as hyperlipidemia and obesity.

6. **Dietary n-3 polyunsaturated Fatty Acid Ethyl Ester Influences the Composition of Bacteria and Their Metabolites in Rat Cecal Content.**

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Numerous studies have evaluated the gut microbial flora of high-fat or low-fiber diet fed animals under an experimental set-up. On the other hand, only a few reports have focused on the effect of dietary fatty acids on bacterial flora and their metabolites. Our previous study showed that menhaden oil, a eicosapentaenoic acid (EPA) rich oil, had a different effect on the composition of bacteria and their metabolites in rat cecal content compared to soybean oil (SO), lard, and tuna oil (2018 AOCS Annual Meeting). Therefore, the aim of this study was to investigate the effect of dietary n-3 polyunsaturated fatty acid (PUFA) ethyl ester (EE) on the bacterial composition and their metabolites in rat cecal content. Male Wistar rats (4-weeks old) were divided four groups and fed a high-fat diet containing 15% SO, 10% SO + 5% SO-EE, 10% SO + 5% EPA-EE, or 10% SO + 5% docosahexaenoic acid (DHA)-EE. After 28 days, the bacterial composition of cecal content was assessed by sequencing 16S ribosomal RNA gene, and bacterial metabolites including neutral and acidic steroids were measured. Rats fed DHA-EE diet had a high relative abundance of Bacteroidetes and low proportion of Firmicutes compared to the rats fed SO-EE and EPA-EE diet. Fecal secondary bile acids significantly decreased in EPA-EE and DHA-EE diet compared to SO-EE diet. Interestingly, the EPA-EE diet significantly decreased the fecal deoxycholic acid and lithocholic acid contents compared to the SO-EE diet. Thus, not only the

amount of dietary fat but also the fatty acid composition altered the composition of bacteria and their metabolites.

7. **Evaluate the Efficacy of γ-glutamyl Peptide in Reducing Hypertension-associated Vascular Inflammation.** Snigdha Guha*[1] and Kaustav Majumder[2], *[1]University of Nebraska, Lincoln, USA; [2]University of Nebraska, USA*

Nearly 54% of the 75 million adults in the U.S who are suffering from hypertension lack in appropriate preventative intervention to tackle the associated risk factors such as heart failure and atherosclerosis. It is well-documented that hypertension develops from a complex interaction between genetic and environmental factors and increased levels of vascular inflammation is a significant contributor to the pathogenesis of hypertension. Dry-edible beans derived γ-glutamyl peptide (γ-EV) can reduce gastrointestinal inflammation via allosteric activation of Calcium-Sensing Receptors (CaSR). Since vascular cells also express CaSR, it was hypothesized that the intervention of γ-glutamyl peptide (γ-EV) might modulate inflammatory responses in vascular cells via the activation of CaSR. To determine the anti-inflammatory effects, Human Aortic Endothelial Cells (HAEC) and Human Umbilical Vein Endothelial Cells (HUVEC) were first pre-treated (2h) with different concentrations of the γ-EV (0.01mM, 0.1mM, and 1 mM), followed by the treatment with human TNF-α (5ng/mL, 6h) to induce the inflammation. Western blot and ELISA were performed to measure the expressions of pro-inflammatory cell adhesion molecules and chemokines respectively. It was observed that TNF-α treatment significantly increased the expression of the pro-inflammatory cell adhesion molecules (ICAM-1 and VCAM-1) and chemokine (MCP-1). However, the γ-EV pre-treatment at 1mM significantly down-regulated the expression of ICAM-1, VCAM-1, and MCP-1 in HAECs, but not in HUVECs, due to the abundant presence of CaSR in the cell membrane and cytosol of HAECs. Thus, the study concluded that γ-EV could significantly reduce vascular inflammation in HAECs, but not in HUVECs, through CaSR dependent pathways.

8. **Metabolomics Revealed the Selective Effects of Feeding Oxidized Oil on Amino Acid Metabolism in Nursery Pigs.** Yue Guo*[1], Andrea Hanson[1], Lei Wang[1], Brian Kerr[2], Pedro Urriola[1], Gerald Shurson[1], and Chi Chen[1], *[1]University of Minnesota, USA; [2]USDA-ARS, USA*

Oxidized oils from rendering and recycling are an economic source of energy and lipids in swine feeding. Negative effects of feeding oxidized oils on pigs have been observed, but the underlying metabolic events remain to be defined. In this study, oxidized corn oil (OCO) was produced by heating control corn oil (CCO) at 185 oC for 12 h. Weanling pigs were assigned to 4 isocaloric diets containing 9% CCO, 6% CCO + 3% OCO, 3% CCO + 6% OCO, and 9% OCO for 35 days, respectively. Feeding OCO reduced gain:feed ratio. Serum and liver samples collected on d 35 were analyzed by the liquid chromatography-mass spectrometry (LC-MS)-based metabolomics analysis. In the liver, OCO decreased the levels of hepatic glutamic acid, methionine, and glycine, and also the ratio of glutathione (GSH) / glutathione disulfide (GSSG), while increased the gene expression levels of GSH metabolism enzymes. Similarly, OCO decreased the level of tryptophan in serum as well as the levels of carnosine in both serum and liver, while increased the gene expression levels of the enzymes for tryptophan and carnosine catabolism in the liver. In contract, feeding OCO increased the level of serum threonine and decreased the levels of threonine metabolites in the liver. Overall, these results suggests that consuming oxidized oils can selectively modify amino acid metabolism of pigs, which may negatively affect health and

growth performance.

9. **The Mechanisms of Hypoglycemic Effect of Foxtail Millet Based on Transcriptomics.** Fu Yongxia*, Fan Zhang, Xin Ren, and Qun Shen, *China Agricultural University, China*

Our previous animal experiment have proved that foxtail millet feeding significantly improved the blood glucose metabolism in high fat diet and STZ-induced (HFD/STZ) diabetic rats. To further clarify the mechanisms of the hypoglycemic effect of foxtail millet, the difference on liver transcriptional profiles between diabetic rats, normal rats and foxtail millet feeding rats were investigated. The results shown that 4 weeks of foxtail millet feeding in this study could mitigate negative variations of liver transcriptional profile in HFD/STZ diabetic rats. Specifically, foxtail millet feeding activated the insulin-stimulated PI3K/AKT signaling pathway by up-regulating expression of IRS, PI3K and AKT in liver, which will inhibit gluconeogenesis by down-regulating expression of G6P, FBP and PEPCK, and stimulate glycolysis by up-regulating expression of GK and PK subsequently. Moreover, foxtail millet feeding inhibited NF-κB signaling pathway and reduced expression of inflammatory factors, which will weaken the inhibition of insulin signaling pathway and improve blood glucose metabolism ultimately. Keywords: foxtail millet; blood glucose metabolism; insulin signaling pathway; NF-κB signaling pathway

10. **Improving Glycemic Control with Foxtail Millet Foods and How to Get Them in the Marketplace.** Fan Zhang*, Fu Yongxia, Ren Xin, and Qun Shen, *China Agricultural University, China*

The study was to investigate the effect of foxtail millet on glycemic control in subjects with impaired glucose tolerance (IGT). 82 subjects with IGT were separated into two groups for a 12-week foxtail millet diet intervention test, among 18 subjects was control group and others were experimental group. Subject was asked to take foxtail millet-derived products containing 50 g of foxtail millet each day for 90 days. The blood glucose, blood lipid, blood pressure, kidney function, cytokines and other body indexes were fully investigated at week 0, 6, and 12, respectively. The intake of 50g of foxtail millet per day after 12 weeks significantly decreased fasting blood glucose compare with the very beginning (5.74 ± 0.12 at week 0 vs 5.30 ± 0.09 at week 12), 2h-glucose after oral glucose tolerance test (OGTT, 10.21 ± 0.33 at week 0 vs 9.36 ± 0.28 at week 12), HOMA-IR (4.16 ± 0.33 at week 0 vs 3.34 ± 0.25 at week 12) and obesity degree (110.03 ± 1.90 at week 0 vs 108.33 ± 1.73at week 12) and significantly increased leptin concentration (8.30 ± 0.82at week 0 vs 9.55 ± 0.89 at week 12) in subjects with IGT. The intake of 50 g of foxtail millet also has trend to lower the blood lipid and pressure, and increasing kidney function, bone density and body inflammation. Foxtail millet diet significantly improved the glycemic control, especially the postprandial glucose, by increasing insulin sensitivity and leptin concentration and alleviating inflammatory condition in subjects with IGT.

11. **Time-Dependent Differences in Essential Omega-3 Fatty, during Lactation in the Guatemalan Highlands.** Doug Bibus[1]*, Heike Rolker[2], Alejandra Zamora[2], Rosario Garcia-Meza[2], Claudia Arriaga[2], and Noel W. Solomons[2], [1]*Lipid Technologies, LLC and The Center for Spirituality and Healing, University of Minnesota, USA*, [2]*Center for the Studies of Sensory Impairment, Aging and Metabolism, Guatemala*

Objective: To examine time-dependent differences in the long-chain omega-3 fatty acids, DHA and EPA, in lactating Guatemalan women. Methods: Full-breast milk extractions were obtained from 40 women with healthy

infants. Breast milk was sampled at or near four time-points including 40 days (44±3); 80 days (73±4); 120 days (120±7) and 160 days (160±9). Four spots of breast milk were applied to a stabilized PerkinElmer 226 five-spot RUO spot card, dried at room temperature for 60 min and then frozen-stored until shipping to Austin, MN, USA. Breast milk samples were directly esterified with acidic methanol and analyzed by capillary gas chromatography, reporting 42 fatty acids, from C-14 to C-24 and expressed as weight %. We modeled the daily amount of DHA and EPA consumed by infants using the assumptions of 780ml of milk consumed per day with a milk fat content of 34 g/L equally 26.5g of fat per day. Results: The serial amounts of DHA (wt%) were: 0.14±0.03 (T40); 0.16±0.07 (T80); 0.10±0.03 (T120) and 0.21±0.16 (T160). By Kruskal-Wallis analysis, the value at T120 was significantly lower than the value at T160 . Based on the corresponding daily median intakes, DHA(mg) intake models to: 35.9 (T40); 35.9 (T80); 23.8 (T120) and 35.9 (T160). For EPA, the parallel data are: 0.03±0.01 (T40); 0.04±0.02 (T80); 0.02±0.004 (T120) and 0.03±0.03 (T160), corresponding to daily median intakes in mg of: 7.8 (T40); 10.4(T80); 5.2 (T120) and 5.2 (T160). Conclusions: In the context of living conditions and lactation in Guatemala, there is significant variation in the abundance of milk DHA and EPA. In comparison to populational breast milk DHA data, Guatemalan women have lower levels of DHA and may benefit from increased dietary DHA.

12. **Using Dried Human-milk Spots to Document Time-Dependent Omega-3 Content with Oral DHA and EPA Supplementation of Guatemalan Mothers.** Doug Bibus[1]*, Rosario Garcia-Meza[2], Marta Escobar[2], Debora Fuentes[2], Alejandra Maldonado[2], Alejandra Zamora[2], Claudia Arriaga[2], and Noel W. Solomons[2], *[1]Lipid Technologies, LLC and The Center for Spirituality and Healing, University of Minnesota, USA*, *[2]Center for the Studies of Sensory Impairment, Aging and Metabolism, Guatemala*

Objective: To assess serial values of DHA and EPA in human milk using self-sampling dried milk spots in response to daily administration of these fatty acids. Methods: Ten women between 28 and 56 days of lactation were stratified to receive either 450mg DHA/90 mg EPA (full dose 'FD') or 225 mg DHA/45mg EPA (half dose 'HD') as a formulated supplement over a 4-week period. Subjects self-collected breast milk samples at home on a stabilized PerkinElmer 226 RUO spot card on an alternate-day sampling schedule: baseline; x6 in the first 2 weeks; and x4 in the last 2 weeks for a total of 11 card-based samples. After frozen-shipment to Austin, MN, breast milk samples were directly esterified with acidic methanol and analyzed by capillary gas chromatography, reporting 42 fatty acids from C-14 to C-24. Data are expressed as weight %. Results: Average DHA content at baseline was 0.19 +/-0.08 (median 0.19 and range 0.09-0.34 (n=10)). Corresponding data for EPA was: 0.04+/-0.03(median 0.04 and range 0.01-0.10 (n=10)). Median change from baseline to end for FD supplementation for DHA was Δ0.35, from 0.19 to 0.54 (p=0.0033, n=5), representing an increment from 20th to 90th percentiles. With HD, response was Δ0.16, from 0.19 to 0.35 (p=0.019, n=5), to the 65th percentile. The corresponding median EPA responses were Δ0.06 (p=0.004, n=5) and Δ0.02 (p=0.03, n=5). Conclusion: Empowering Guatemalan women with the dried-milk-spot approach allowed demonstration of the substantial impact of oral EPA and DHA supplementation into upper percentiles of human milk content in only 4 weeks. Improved breast milk DHA status may offer improved neural developmental and cognitive outcomes in Guatemalan children.

13. **Extracellular vesicles isolated from two types of olive coproducts** Jerôme Lecomte[1], Bruno Baréa[2], Marie-Caroline Michalski[3], Claire Bourlieu[4], Pierre Villeneuve[5], Amal Fenaghra*[6], Pascal Colosetti[7], and Anne Mey[7],[1]*CIRAD, Greece;* [2]*CIRAD, France;* [3]*INRA, Francce;* [4]*UMR IATE - INRA/CIRAD/UM2/SupAgro, France;* [5]*UMR IATE, CIRAD, France;* [6]*UMR IATE, France;* [7]*UMR CarMeN, France*

Extracellular vesicles (EVs) are nanoscopic membranous structures which are involved in intercellular communication between cells. Recent works highlighted the existence of EVs secreted by plant cells. The objective of our study, is to characterize the main physico-chemical properties of EVs originated from olive coproducts (pomace -PEVs- and wastewaters -WWEVs-). Isolation was carried out using successive centrifugations. The main physical characterizations were size and charge using Dynamic Light Scattering (DLS). The chemical characterization included phenolic compounds characterization using HPLC and lipid classes with TLC. Furthermore, test of their antioxidant effect was conducted using CAT assay. EVs isolated from the two co-products presented distinct physico-chemical properties. WWEVs showed a larger diameter than PEVs that ranged between 20-140 nm versus 20-50nm. On a [2-7] pH range, evolution of charge resulting in different curve shapes for the two VEs but indicated similar isoelectric point [2-3]. WWEVs were more concentrated with phenolic compounds (0.5mM eq HT) and contained more neutral lipids than PEVs. However similar polar lipids classes were found in the two VEs. WWEVs was the fraction with the highest antioxidant activity. EVs originated from wastewaters showed a better profile in lipid and phenolic profile, but due to environmental considerations, working with pomace may be preferable. In perspectives, EVs characteristics will be mimicked and improved at laboratory scale to use them as antioxidant delivery vesicles.

14. **Characterization of Phenolic Profile in Wheat Co-Products Determined by UPLC-MS** Erwann Durand[1], Claire Bourlieu[2], Véronique Vié[3], Pierre Villeneuve[4], Millena Barros Santos*[5], Cécile Barron[5], Valérie Micard[5], Valérie Lullien-Pellerin[5], L Cameron[6], and Mariana Ferreira[6],[1]*CIRAD, France;* [2]*UMR IATE - INRA/CIRAD/UM2/SupAgro, France;* [3]*Institute of Physics Rennes, University of Rennes 1, France;* [4]*UMR IATE, CIRAD, France;* [5]*UMR IATE, France;* [6]*Federal University of State of Rio de Janeiro, Brazil*

Wheat co-products are rich in secondary metabolites that show a wide spectrum of bioactivities, such as phenolic compounds (PC). PC have a moderate to low solubility in apolar media that restricts their application in oil-based products. The choice of the extraction solvent is essential to maximize the yield, recovery and subsequent application of PC. Among green solvents, deep eutectic solvents (DES) have emerged as effective media to extract PC because of their ability to improve their solubility, stability and compatibility with enzymatic reactions, especially when used as a media of lipase functionalization. In this work, PC were extracted by three conventional solvent mixtures (ethanol:water, isopronanol:water and acetone:water) and two formulations of DES (choline chloride:2-propanediol – DES1 and choline chloride:latic acid – DES3) and characterized by UPLC-ESI-QTOF-MSE in negative mode. Data were processed using Progenesis QI with a customized databank of polyphenols applying exact mass error (<10 ppm) and isotope similarity (>80%). A total of 75 compounds were tentatively identified. The flavonoid apigenin 7-O-apiosyl glucoside was the most abundant compound, present in all extracts. Wheat bran showed a higher number of PC identifications

than aleurone extracts. Conventional solvents showed a higher number of PC identifications, probably due to some sort of incompatibility of DES based extracts in MS approaches. However and remarkably, DES3 extracted 9 unique PC, demonstrating that it is a good media to extract specifics wheat PC and to add value to this abundant low-cost by-product of the milling industry.