

## H&N 1: N-6 PUFA: They Are Not as Bad as You Think

*Chairs: Martha Belury, Ohio State University, USA; and Matthew Picklo, USDA, ARS, Grand Forks Human Nutrition Research Center, USA*

### **Are All Fatty Acids Created Equal?** David W.L Ma\*, *University of Guelph, Canada*

In general, n-6 and n-3 polyunsaturated fatty acids (PUFA) have been classified as pro and anti-inflammatory fatty acids, respectively. The convenience of this classification may have been an oversimplification given that several members exist within each family of fatty acids. N-6 PUFA includes linoleic (LA, 18:2n-6) and arachidonic acid (AA, 20:4n-6). The major n-3 PUFA includes alpha-linolenic acid (ALA, 18:3n-3, ALA), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Given such diversity, do all fatty acids within a family exert similar effects? A major challenge of answering this question has been to isolate the effects of individual fatty acids given that they can be further metabolized. Using the delta 6 desaturase knock out mouse model we show that the essential fatty acids, LA and ALA have biological effects independent of their conversion to AA, EPA and DHA, respectively in models of fatty liver disease, immunity and cancer.

### **The Relation Between Omega-6 Fatty Acids and Inflammation** Philip Calder\*, *University of Southampton, UK*

Arachidonic acid (ARA) is the precursor for lipid mediators involved in inflammation. Many anti-inflammatory pharmaceuticals prevent ARA metabolism or block the action of the ARA-derived mediators. Cells involved in the inflammatory response have high amounts of ARA in their membrane phospholipids. Animal and human experiments show that increasing the ARA content of inflammatory cell membranes through increased dietary intake enhances production of pro-

inflammatory lipid mediators. Conversely, lowering the ARA content of inflammatory cell membranes through increased intake of the omega-3 fatty acids EPA and DHA results in decreased production of pro-inflammatory lipid mediators. ARA and EPA seem to counter one another's effects and some human studies have demonstrated that lowering ARA intake at the same time as increasing intake of EPA and DHA is more effective at controlling inflammation than increasing intake of EPA and DHA alone. Linoleic acid (LA) is the metabolic precursor of ARA and LA itself can be metabolised to pro-inflammatory mediators. However, human trials increasing LA intake have generally failed to show an effect of LA on inflammation; they also do not usually show increased ARA content of cell membranes. This may be because LA conversion to ARA is already saturated at current intakes and giving more LA can have no effect. It is important to investigate the effect of a significant lowering of LA intake on ARA content and inflammatory markers. High intake of LA prevents conversion of alpha-linolenic acid to EPA, which could hinder the beneficial effects of omega-3 fatty acids on inflammation.

### **N-6 Polyunsaturated Fatty Acid Intake and Health Outcomes in Epidemiological Studies** Dong Wang\*, *Harvard T.H. Chan School of Public Health, USA*

Objective: To review the association between n-6 polyunsaturated fatty acid (PUFA) intake and health outcomes in epidemiological studies. Methods Used: Narrative review. Results: A recent meta-analysis based on 13 cohort studies found a 15% lower risk of coronary heart disease (CHD)

events (risk ratio (RR)=0.85; 95% CI, 0.78-0.92) and a 21% lower risk of CHD deaths (RR=0.79; 95% CI, 0.71-0.89) comparing the highest to the lowest intake levels of LA [Farvid et al. *Circulation*. 2014]. In a meta-analysis summarizing evidence from the randomized controlled trials, each 5% increase in energy from n-6 PUFA in place of SFA was related to a 10% reduction in CHD risk (RR =0.90, 95% CI, 0.83–0.97) [Mozaffarian et al. *PLoS Med*. 2010]. In a recent prospective cohort study [Wang et al. *JAMA Intern Med*], higher consumption of LA, when compared to total carbohydrate, was associated with an 18% lower total mortality (RR=0.82, 95% CI, 0.79-0.86); higher LA intake was also associated with lower risk of death due to cardiovascular disease, cancer and respiratory disease. In Wang et al's study, replacing 2% of calories from n-6 PUFA with the same calories from SFA was significantly associated with 7%, 11%, and 4% estimated risk reductions in total, cardiovascular disease and cancer mortality, respectively. Conclusions: In the well-conducted epidemiological studies, n-6 PUFA intake was inversely associated with disease risk. It is important to specify the comparison nutrient and examine the effect of n-PUFA intake in an isocaloric comparison in epidemiological studies.

**Omega-6 and Omega-3 Fatty Acids: Focus on Ratios or Levels?** William S. Harris\*, *University of South Dakota School of Medicine, USA*

The health effects of both the omega-3 and the omega-6 families of dietary polyunsaturated fatty acids (PUFAs) are controversial. Whereas the former, especially those PUFAs of marine origin (eicosapentaenoic and docosahexaenoic acids, EPA and DHA), are still generally viewed as beneficial, the latter (linoleic and arachidonic acids, LA and AA) are considered by some to be harmful. Metrics like the ratio of omega-6/omega-3 PUFAs have been proposed to serve as the best single indicator

of dietary (and in vivo) PUFA status. This presentation will discuss the pros and cons of the ratio perspective as opposed to the alternative view that individual amounts of these PUFAs in both the diet and in circulation are the more important metrics. The context of the discussion will center on risk for coronary heart disease. In addressing these questions, the presentation will consider corollary assumptions that are implicit in "ratio" thinking: 1) that current dietary intakes of omega-6 PUFA are excessive (i.e., they adversely impact cardiac health, 2) that eating less LA will reduce tissue levels of the proposed offending omega-6 FA, AA, and 3) that higher intakes of omega-6 PUFAs will cancel the beneficial effects of omega-3 PUFAs. Convincing evidence to support these three assertions are currently lacking.

**H&N 2: Medium Chain Triglycerides and Health**

*Chairs: Fabiola Dionisi, Nestlé Research Center, Switzerland; and Robert Ward, Utah State University, USA*

**Medium Chain Triglyceride in Food Fats and Their Metabolism** J. Thomas Brenna\*, *Cornell University, USA*

Medium chain triglycerides (MCT) are characterized chemically as saturated medium chain length fatty acids (MCFA), arbitrarily and variously defined from 4-6 to 12-14 carbons. They occur naturally in many food fats, usually at minor levels. The major, naturally occurring rich sources include dairy fat (10%), coconut and palm kernel oils (>50% MCFA), and more exotic oils such as cuphea seed oil of which there are numerous species with a variety of specific compositions. Metabolically, MCT sources of MCFA are absorbed differently than FA of chain length >C16 which are assembled into chylomicrons and routed to the lymphatic system before entering the bloodstream. MCFA enter the portal vein directly because of their significant water solubility, with transport in the bloodstream aided by albumin. Unlike >C16 fatty acids, MCFA are transported across the mitochondrial membrane without carnitine esterification. This latter property enables them to be burned in preference to >C16 fats, though not in preference to glucose. Because of this property MCT are thought to have a role in body weight control, and in sports nutrition. MCT are also ketogenic, thought to generate an alternative source of fuel for the brain when glucose is in short supply or possibly when glucose transport into neurons is impaired. Highly refined or synthetic MCT oils consisting of tricaprilyn (trioctanoin, tri-8:0) has long been used medically for patients suffering from fat malabsorption or those that cannot tolerate conventional fats and oils.

**Brain Fatty Acid Beta-Oxidation. If It Isn't a Source of ATP, What Is It For?** Richard P. Bazinet\*, *University of Toronto, Canada*

The brain is especially enriched with specific fatty acids while being very low in others. Earlier models of brain fatty acid uptake predicted that

low abundance fatty acids, simply, did not enter the brain. However, recent in vivo studies demonstrate that a variety of fatty acids, including those of low abundance, not only enter the brain, but enter at rates comparable to those of high abundance. This presentation will discuss recent work with eicosapentaenoic acid, which is almost not detectable in the brain, but enters at a rate similar to docosahexaenoic acid. While the ATP generated from fatty acid beta-oxidation in the brain is small compared to glucose, fatty acid beta-oxidation appears to be a mechanism to regulate levels of certain fatty acids such as eicosapentaenoic acid. Fatty acid beta-oxidation generates oxidative stress and maintaining low levels could be a protective mechanism for the brain. If we are able to extrapolate results with eicosapentaenoic acid to medium chain fatty acids, this could imply they also readily enter the brain and are metabolized to maintain low levels.

**Dietary Medium Chain Saturated Fat Reduces Obesity-induced Outcomes in Mice** Matthew Picklo<sup>1\*</sup>, Petr Žáček<sup>1,2</sup>, Michael Bukowski<sup>1</sup>, LuAnn Johnson<sup>1</sup>, Joseph Idso<sup>1</sup>, Susan K Raatz<sup>1</sup>, <sup>1</sup>USDA-ARS Grand Forks Human Nutrition Research Center; <sup>2</sup>Institute of Organic Chemistry and Biochemistry Academy of Sciences of the Czech Republic

The contribution of saturated fatty acid (SFA) type to obesity is not clear. We tested the hypotheses that the composition of dietary SFA causes selective changes in obesity outcomes and in the plasma lipidome in obese mice. Mice were fed obesogenic diets of 48% energy (en) fat or a control diet with 16% en fat for 16 weeks. Obesogenic groups were divided by SFA source: medium chain SFA (MCSFA; primarily laurate), long chain SFA (LCSFA; primarily palmitate), and mixed SFA (MxSFA) with similar levels of MCSFA and LCSFA. Mice eating the obesogenic diets became obese compared to the control group. Hepatic lipids were elevated in the LCSFA and MxSFA

groups, but not the MCSFA group. While all obese animals were insulin resistant, MCSFA animals had better insulin sensitivity than other obese groups. We quantified 122 phosphatidylcholine (PC), 53 phosphatidylethanolamine (PE), and 10 ceramide (CER) species in the plasma. Hierarchical clustering analysis demonstrated obesity-dependent and SFA-type dependent changes in PE and PC. Selective CER species were elevated with LCSFA but not MCSFA intake and were strongly correlated with plasma insulin and hepatic lipid levels. Principal component analysis of the PC and PE data discriminated all four different dietary groups. These groups were influenced by three components (one being obesity) that explain > 65% of variation of the data. Our data demonstrate that obesity outcomes are SFA-type dependent and that there are SFA-dependent and obesity-dependent lipidomic changes. Further data are needed to clarify the impact of specific SFA intake on obesity outcomes.

#### **The Ketogenic Effect of MCT: Implications for Attenuating the Impact of Alzheimer's Disease**

Stephen C. Cunnane\*, *Research Center on Aging, Canada*

Ketones (beta-hydroxybutyrate and acetoacetate) are the brain's most important back-up fuel when brain glucose supply or uptake is decreased. We propose that the chronic brain energy deficit in Alzheimer's disease (AD) can be partially overcome by a ketogenic supplement thereby helping delay cognitive decline associated with AD. Our rationale is fourfold: (i) Glucose uptake is lower in the frontal cortex of people >65 years old despite cognitive scores that are normal for age. (ii) The deficit in brain glucose uptake is present in young adults with genetic or lifestyle risk factors for AD but in whom cognitive decline has not yet started. (iii) Regional brain glucose uptake is impaired but brain uptake of ketones remains the same in AD and mild cognitive impairment (MCI) as in cognitively healthy age-matched controls. (iv) Interventions that raise ketone

availability to the brain improve cognitive outcomes in both MCI and AD as well as in acute experimental hypoglycemia. These observations point to a brain fuel deficit which appears to be specific to glucose, precedes cognitive decline associated with AD, and which becomes more severe as MCI progresses towards AD. Since glucose is the brain's main fuel, we suggest that gradual brain glucose exhaustion is contributing significantly to the onset or progression of AD. We propose that the brain energy deficit needs to be overcome in order to successfully develop more effective therapeutics for AD. At present, oral ketogenic supplements based on MCT are the most promising means of achieving this goal.

#### **Octanoic Acid and Ghrelin Octanoylation: Origin of C8:0, Mechanisms, and Physiological Effects**

Vincent Rioux\*, *Agrocampus Ouest, France*

Octanoic acid (caprylic acid, C8:0) belongs to the class of medium-chain saturated fatty acids (MCFAs), which also includes caproic acid (C6:0) and capric acid (C10:0). MCFAs are characteristic nutrients present in dairy products and specific oils like palm kernel and coconut oils. They display metabolic properties that are distinct from those of long-chain saturated fatty acids (LCFAs  $\geq 12$  carbons), leading to their rapid gastro-intestinal hydrolysis and absorption, specific transport through the portal vein and rapid beta-oxidation in the liver. These metabolic specificities have been associated with beneficial or neutral physiological effects of dietary MCFAs, compared with LCFAs. Recently, octanoic acid was shown to specifically acylate ghrelin, the only known peptide hormone with an orexigenic effect. Ghrelin is expressed mainly in the stomach. During its maturation, part of the proghrelin is subjected to this unique octanoylation, catalyzed by the Ghrelin O-Acyltransferase (GOAT). Plasma ghrelin exists therefore in both unacylated and octanoylated forms, but only the latter can bind its growth hormone secretagogue receptor 1a (GHSR-1a) located in the pituitary gland. Octanoylated ghrelin

is described to regulate the secretion of GH, the stimulation of appetite and food intake, the modulation of gastric acid secretion and motility, and the regulation of glucose homeostasis and adiposity. Recent data trying to define the origin of octanoic acid used to acylate ghrelin, to study the effect of increasing dietary octanoic acid levels on its own tissue availability, on plasma octanoylated and non-acylated ghrelin concentration, and on the whole physiological effects attributed to ghrelin will be presented.

**MCT Oil for Weight Management: Can Coconut Oil Do the Same?** Marie-Pierre St-Onge\*, *Columbia University, USA*

MCT oil influences energy balance, mostly via enhanced energy expenditure, but also possibly via improvements in satiety and reductions in food intake. Doses of MCT oil of ~20 g increases thermogenesis at a meal and 10 g of MCT oil at a pre-meal snack reduces food intake at a meal served 30 min later. Some metabolites were also favorably altered by MCT oil consumption compared to the control, corn oil: glucose, triglycerides, leptin, and peptide YY. In a 16-wk intervention study, participants consuming MCT oil lost more body fat than participants consuming olive oil. Evidence supports the use of MCT oil for weight management. However, MCT oil is not readily available to consumers and coconut oil has emerged as a new “healthy fat”, with its proponents extrapolating data from MCT oil studies to promote its weight loss potential. Although coconut oil is the richest source of naturally occurring MCT, it contains a large amount of lauric acid, a fatty acid whose inclusion in the medium chain category is often debated. We have developed a new baking fat from coconut oil and tested its thermogenic potential in 15 overweight adolescents in a double-blind, randomized, cross-over study. Participants consumed a breakfast containing 20 g of fat from either corn oil or coconut oil-enriched baking fat. There was no difference in postprandial thermogenesis or satiety

ratings between test fats. Additional research on the effects of coconut oil on energy balance are warranted to determine effectiveness and appropriate dosing.

**H&N 3: Brain, Behavior, and Omega-3s**

*Chairs: Alex Kitson, Pepsico Foods, Canada; and Richard Bazinet, University of Toronto, Canada*

**Long-chain Polyunsaturated Fatty Acids and Infant Formula: A Case Study in Bench to Cradle**

**Translation** J. Thomas Brenna\*, *Cornell University, USA*

The vast majority of infant formulas in the United States contain the long-chain polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6), which were first permitted by the US Food and Drug Administration in 2001. Roughly speaking, n-3 DHA grows brain and n-6 ARA grows bodies. As a scientific case study, preclinical animal studies of these nutrients definitively influenced the design and interpretation of human clinical studies. Early studies were tied to the availability of test substances, and focused on the most obvious of symptoms, namely in skin function, while recognition of neural function was recognized much later. Research in the 1950s established the essentiality of n-6 PUFAs for skin integrity; however, widespread recognition of the essentiality of n-3 PUFAs came decades later despite compelling evidence of their significance. Barriers to an understanding of the essentiality of n-3 PUFAs were as follows: 1) their role is in neural function, which is measured only with difficulty compared with skin lesions and growth faltering that are apparent for n-6 PUFAs; 2) the experimental use of vegetable oils as PUFA sources that contain the inefficiently used C18 PUFAs rather than the operative C20 and C22 PUFAs; 3) the shift from reliance on high-quality animal studies to define mechanisms that established the required nutrients in the first part of the 20th century to inherently challenging human studies. Advances in nutrition require the best practices and opinions available, taking into account the totality of preclinical and clinical evidence.

**How Does Docosahexaenoic Acid Enter the Brain? Updates and Implications for Adults and Infants**

Richard P. Bazinet\*, *University of Toronto, Canada*

The brain is especially enriched with the polyunsaturated fatty acids (PUFA) docosahexaenoic acid (DHA) and arachidonic acid. It has been suggested that the plasma supply to the brain regulates brain PUFA levels by replacing PUFA consumed in the brain. The presentation will review how candidate plasma pools including plasma unesterified DHA, lysophosphatidylcholine containing DHA and DHA-containing lipoproteins may enter the brain. The presentation will focus on our recent kinetic studies in adult rodents and work in rodent pups that have an increased demand for DHA. The implications of knowing how PUFA enter the brain and their rate of uptake will be discussed in the context of nutritional requirements for adults and infants.

**Linoleic Acid Regulates Neurotransmission Through its Oxidized Metabolites**

Ameer Taha\*, *University of California, Davis, USA*

*Abstract not available*

**Omega-3 Fatty Acids Decrease the Neuroinflammatory Response to Amyloid- $\beta$  in a Mouse Model of Alzheimer's Disease.**

Kathryn E. Hopperton, *University of Toronto, Canada*

*Abstract not available*

## H&N 4: Infant Formula Optimization

*Chairs: Merritt Drewery, Louisiana State University, USA; and Carol Lammi-Keefe, Louisiana State University, USA*

### **Evolution of the Infant Formula Industry: A Historical Perspective** Carol Lammi-Keefe and Merritt Drewery\*, Louisiana State University, USA

Infant formula feeding is widely practiced in developed countries. Today, a handful of manufacturers are responsible for a vast majority of the products marketed in the United States, all of whom must meet rigorous standards set forth by the United States Food and Drug Administration and have the common goal of formulating their product(s) to optimize infant health and development. However, the first formula products were developed in the mid-1800s and significant evolution, including advancements in chemistry and food preservation, have since occurred. This presentation will offer a historical perspective of the infant formula industry and set the stage for the remainder of presentations in the “Infant Formula Optimization” session, which are focused on current research and industry advancements.

### **Importance of the Regiospecific Distribution of Long Chain Saturated Fatty Acids on Gut Comfort, Fat, and Calcium Absorption in Infants** Valerie Petit\*, Laurence Sandoz, and Clara Lucia Garcia-Rodenas, Nestlé, Switzerland

Differences in gastrointestinal tolerance as well as in fat and calcium (Ca) absorption are well-recognized between breast-fed (BF) and formula-fed (FF) infants. The different stereospecific distribution of fatty acids on triacylglycerols (TAG) between HM fat and infant formula (IF) fat appears to contribute to some unfavorable outcomes in FF infants. Up to now, only palmitic acid and its sn-1, 3/sn-2 positions in the TAGs were considered as key elements for fat and mineral bioavailability and therefore for stool hardness in FF infants. Today, there is strong evidence for an improved palmitic acid absorption with an IF enriched in sn-2 palmitic acid, whereas the evidence for decreased total fat and Ca stool excretion as well as benefits on stool consistency is less consistent. The myristic and stearic

acids in sn-1(3) positions, in addition to palmitic acid, may also contribute to a decreased fat and Ca absorption as well as stool hardness to FF infants. Analysis of the available literature revealed that a fat blend containing less than  $13 \pm 0.5\%$  of TAGs as sum of sn-1(3) LCSFAs (i.e. myristic, palmitic and stearic acids) may consistently improve the total fat and Ca absorption, as well as stool patterns in healthy, term infants who cannot be breast-fed.

### **Protein Source as a Way to Optimize Sphingomyelin Levels in Infant Formula Closer to Breastmilk** Gisella Mutungi\*<sup>1</sup>, Nora Schneider<sup>2</sup>, and Cian Moloney<sup>3</sup>, <sup>1</sup>Nestlé, USA; <sup>2</sup>Nestlé, Switzerland; <sup>3</sup>Nestlé, Ireland

Myelination is a neuro-development process whereby a fatty membrane wraps around the axons and enables efficient transmission of nerve impulses through neurons. This process is critical in establishing well-organized brain networks. A single pilot study in low-birth-weight infants suggests that dietary Sphingomyelin (SM), a polar lipid, is important in brain development with preclinical research pointing toward its role in myelination process. Mature breastmilk contains SM at levels between 31 - 153 mg/L compared to low levels in IF. SM is a structural component of myelination. The objective was to identify protein sources that can optimize levels of SM in infant formulas (IF) to be compositionally closer to breastmilk. Method: Quantification of SM (mg/100g) by HPLC was performed in alpha-lactalbumin-enriched whey protein concentrate (WPC) (alpha-lac WPC), regular WPC 35% protein (WPC-35) and skimmed milk powder (SMP), some of the protein sources used in IF manufacturing. Results: SM levels were higher in the alpha-lac WPC at  $1220 \pm 67$  mg (Mean  $\pm$ SD), as compared to regular WPC-35 at  $200 \pm 10$  mg or SMP at  $45 \pm 3$  mg. Conclusions: Opting to use this specific alpha-lac WPC protein source that undergoes a unique protein enrichment process that retains higher levels of polar lipids as compared to SMP or regular WPC can increase SM levels in the final

product. This approach is a way to optimize SM composition of IF to be compositionally closer to breastmilk. However, more data is needed to understand the potential impact of dietary SM on myelination and brain development.

#### **Long Chain Polyunsaturated Fatty Acids in Infant Formula: Essential Nutrients for Optimal Development**

Eric L. Lien\*, *University of Illinois, USA*

Infants and young children accrete substantial amounts of long chain polyunsaturated fatty acids (lcPUFAs) during the first several years of life. The predominant lcPUFA of the retina is DHA while the predominant lcPUFAs of brain include both DHA and ARA. Although breast milk contains DHA and ARA, traditional infant formulas contain only their C18 precursors. Attempts to match breast-fed plasma/RBC levels of lcPUFAs by manipulation of C18 amounts and ratios proved unsuccessful due to the limited conversion of precursors to DHA and ARA, indicating the importance of lcPUFA addition to formula. Initial clinical assessment centered on visual and cognitive development. Results demonstrated improvements in visual function, achieving the goal of formula-fed infants more closely matching the developmental milestones of breast-fed infants. Some, but not all, cognitive development studies demonstrated that infants fed lcPUFA-fortified formula had responses closer to breast-fed infants than infants fed unsupplemented formulas. Cognitive development is an exceptionally complex process and negative results may be due to the test utilized, duration of feeding and the levels of DHA and ARA added to the formula. More recent studies have evaluated immune function and allergy development. During the 25 years of lcPUFA formula assessment some studies have evaluated DHA alone, but numerous lines of evidence demonstrate that ARA must be added to formula in addition of DHA. Recent evaluation of FADS1 and FADS2 polymorphisms demonstrate that some mothers and infants may be at higher risk of impaired lcPUFA status than the general population, highlighting an important area of future research.

#### **Structured Triglycerides in Infant Formula: Development of Fat Blends with Numerous Benefits**

Eric L. Lien\*, *University of Illinois, USA*

Human milk (HM) is the gold standard for nutrition of rapidly growing term infants. An appropriate substitute must be provided if an infant cannot be breast-fed. Therefore, the goal of infant formula development is to closely match both the structure and function of HM. Early attempts to mimic the saturated, monounsaturated and polyunsaturated fatty acid profile of HM proved to be unsuccessful due to the generation of high levels of fatty acid-calcium soaps in the stools as well as low levels of both fatty acid and calcium absorption. Subsequent research revealed that HM triglycerides have a unique structure, with high levels of palmitate in the sn-2 position in contrast to commonly used plant-based sources of palmitate (such as palm olein) in which palmitate is located predominantly in the sn-1 and sn-3 positions. The pancreatic lipase-colipase system is selective for triglyceride positions sn-1 and sn-3, generating free fatty acids from these positions. Free palmitate forms insoluble complexes with calcium and these palmitate-calcium soaps are lost in the feces. Structured triglycerides have been developed that more closely mimic the fatty acid distribution of HM triglycerides. Infants fed formulas containing structured lipids have lower levels of stool calcium-fatty acid soaps and improved calcium and fatty acid absorption when compared to infants fed control formulas (similar fatty acid profiles but with palmitate primarily in the sn-1 and sn-3 positions). Specific outcome benefits demonstrated in clinical trials for formulas containing structure lipids include higher bone mineralization and softer stools.

#### **Lipid Characterization in Breast Milk**

Francesca Giuffrida\*, *Nestlé, Switzerland*

Breast milk (BM) is considered the optimal form of nourishment for infants during the first six months of life (WHO) and among its macronutrients, the lipid fraction is crucial, representing almost 50% of the calories supplied to the newborn infant. Lipids occur in milk in the form of fat globules mainly composed of triacylglycerols (~98% of total lipids) surrounded by a

structural membrane composed of phospholipids (PL), cholesterol, enzymes, proteins, glycosphingolipids and glycoproteins. Progress in analytical technologies together with quantitative sampling of BM allows for a better identification and quantification of BM nutrients and thereby providing a deeper understanding of the composition of BM. To improve our knowledge on lipid BM composition, analytical methods to quantify fatty acid (FA) regioisomeric distribution in triacylglycerol (TAG), phospholipid (PL) species, cholesterol, and gangliosides (GD) have been developed and validated. These technologies were applied to quantify lipid classes in BM samples from different mothers. FA regioisomeric distribution in TAG from human milk did not change along the lactation period. PL molecular species distribution did not change over lactation stage, only a decrease in intensity due to the lower concentration of PL at later lactation stages was observed. Major GD class distribution changes during the lactation period, with GD3 decreasing and GM3 increasing over time. In conclusion, our developed methodologies are sensitive and robust for application to large cohorts to gain insights into not only the nutritional intake of breastfed infants, but also the impact of maternal nutrition on lipid output in BM.

**EAT 4 / H&N 4.1: Lipid Structure and Health**

*Chairs: Ignacio Vieitez, UdelaR, Uruguay; and Amanda Wright, University of Guelph, Canada*

**The Role of Food Structure in Lipid Digestibility and Bioavailability** Harjinder Singh\*, *Massey University, New Zealand*

Dietary lipids are derived from plant and animal sources and they are an important source of energy, carrier of lipid-soluble vitamin and constituents of cellular membranes. In natural foods, such as milk, grains, nuts, eggs, meat and fish, lipids are present in the form of complex structures in which triglyceride particles are coated with a stabilising layer of membrane phospholipids and proteins. Ingestion of these foods breaks down the surrounding structures and releases the lipid droplets from the locating matrix, and the droplet interfacial layers are significantly modified during the digestion process. The interfacial structures of the droplets play a key role in the rate of release and subsequent uptake of fatty acids in gastro-intestinal tract. In processed foods, lipid are mostly incorporated within the food matrix in the form of emulsions, where emulsified lipids play a major role in determining the texture, flavour and taste profile of processed foods. In addition, the structural attributes of emulsions and the properties of interfacial droplet layers play a key role in the kinetics of lipid digestion and release of lipid-soluble components. In these systems, the digestion and absorption characteristics of lipid emulsions can be controlled by choosing certain critical parameters, such as the size of the lipid droplets, the type of emulsifier, the type of emulsion and the type and structure of the triglycerides. This kind of knowledge is critical to developing new foods aimed at satiation and subsequent energy regulation and prevention of coronary heart disease.

**Effect of the Interactions Between Sorbitan Monostearate and Candelilla Wax on Soybean Oil Gelation** Carolina M. Teixeira<sup>1</sup>, Thais V. Sarau<sup>1</sup>, Roberta C. Silva<sup>2</sup>, Luiz A. Gioielli<sup>1</sup>, and Juliana N.R Ract\*<sup>1</sup>, *<sup>1</sup>University of Sao Paulo, Brazil; <sup>2</sup>Utah State University, USA*

Oleogels are considered a promising alternative to trans and saturated fats on the formulation of food products. The objective of this study was to evaluate the interactions of sorbitan monostearate (SMS) and candelilla wax (CW) as organogelators (OG) for soybean oil (SO) structuring, aiming at replacing simultaneously saturated and trans fats in tablespread formulations. SMS/CW blends in different proportions were evaluated for their structuring ability in soybean oleogels prepared using different concentrations of those blends. Visual analysis, yield value determination, and thermal behavior of the different oleogels stored for 24 h at 5, 25, and 35 °C were performed. An oleogel prepared with the 40/60 (SMS/CW) blend presented the highest yield value among all the oleogels prepared with OG at 6%, suggesting a synergistic interaction between SMS and CW. A tablespread was prepared using this oleogel as the lipid fraction and compared to a commercial margarine containing 23.6% of saturated fatty acids. The yield value of the commercial margarine was 3.3 times higher than the tablespread formulated with the selected organogel at 5 °C, a ratio that was gradually decreasing with temperature increase, until both spreads did not present any yield value at 25 °C. The tablespread prepared with the oleogel was spreadable, although softer than a commercial reference, presented satisfactory appearance, and contained only 14.5% of saturated fatty acids originally

present in soybean oil.

### **Effect of Palmitic Acid's sn-position and Solid Fat Content on Fasting Lipid Profile in Mice** Tong

Wang\*, *Iowa State University, USA*

Lipid randomization and interesterification change triacylglycerol (TAG) structure and fat's solid fat content profile. This study was designed to investigate how these changes affect lipid metabolism. Kilogram quantity of POP, PPO, and POO were obtained from a natural source or synthesized. The POP and PPO pair of fat was used to study the effect palmitic acid sn-distribution, and the pair of POO and a mixed fat made of PPP, OOO and POP was used to study the effect of solid fat content. Feed intake and body weight of mice were monitored during the trial, and serum lipid profile was determined after 6-week feeding. The pair of fats with different solid fat content did not significantly affect the concentrations of total serum cholesterol, HDL cholesterol, TAG, and non-esterified fatty acid (NEFA). However, the PPO fat significantly reduced feed intake, body weight and serum glucose concentration as compared to POP. These results suggest that the presence of solid fat at the level examined in this study does not affect lipid metabolism and lipemia, however, PPO significantly affects feed intake and NEFA and glucose concentrations. Palmitic acid at the sn-2 position of the TAG may have significant effect on appetite, which may be mediated via the gut receptors.

### **Effects of Liquid Coconut Oil vs. Oleogel on Human Blood Triglycerides, Glucose, Insulin, and Appetite**

Sze-Yen Tan<sup>1</sup>, Elaine W.Y Peh<sup>1</sup>, Alejandro G. Marangoni\*<sup>2</sup>, and Christiani J. Henry<sup>1</sup>, <sup>1</sup>*Singapore Institute for Clinical Sciences, Singapore*; <sup>2</sup>*University of Guelph, Canada*

We aimed to examine if coconut oil in a liquid or an oleogel form, affect blood triglycerides, glucose,

insulin, and appetite when co-ingested with a carbohydrate-rich meal. This was a randomised, controlled crossover study where eligible participants attended a baseline visit where baseline demographics were measured. On test days, participants arrived at the laboratory after an overnight fast of 10 hours. Upon arrival, the cannulation of the antecubital vein was performed and fasting capillary glucose, plasma insulin and triglycerides, and appetite sensations were measured. Following that, orange juice and rice porridge alone (control), or with 22.25g of coconut oil (CO) or 25g of coconut oleogel (CG) (22.25g coconut oil + 2.75g ethylcellulose to form oleogel) was consumed. Subsequently, capillary blood glucose, plasma insulin and triglycerides were measured at fixed intervals for 6 hours. Appetite sensations were also measured using visual analog scales every 30 minutes. Sixteen healthy young adult males completed the study (age=27±6 years, weight = 65.5±5.5kg, BMI=21.9±1.7 kgm<sup>-2</sup>). After test meals, glucose, insulin, triglycerides and appetite sensations changed significantly (time effects, p

### **Structuring Lipids for Possible Infant and Prenatal Maternal Nutrition** Casimir C. Akoh\*, *University of Georgia, USA*

Lipases are used in lipid structuring to add value, produce functional, healthful, and nutraceutical lipids to benefits human health. For infants, lipids contribute most of the energy needed for proper nutrition, growth, and development. To mimic the lipid composition of human milk fat (HMF), physically blended oils have been used in infant formula. Breast feeding has long been accepted as the best for infant feeding. With new interest in the nutritional needs of the infant, it is possible to target these needs using specific structured lipids (SLs). Our objective is to produce SLs as infant formula fat analogs that

mimic breast milk fat in composition using lipases. These SLs contain functional and physiologically important fatty acids for the infant. The HMF analogs were enzymatically prepared by acidolysis and interesterification reactions using different oils and fats substrates catalyzed specific and non-specific lipases. Some of the SL products can serve as infant formula fat analogs or as nutraceutical lipids intended for pregnant women. The SLs have high sn-2 palmitic acid content and comparable fatty acid composition to HMF. Fatty acids such as stearidonic (SDA), eicosapentaenoic (EPA), docosahexaenoic (DHA), gamma-linolenic (GLA), arachidonic (ALA), and oleic acids, were important components of the HMF analogs. Both DHA and ARA are important in brain development and cognitive functions of the infant. The HMF analogs should improve absorption of fatty acids and calcium. These HMF analogs may be incorporated into infant formulas to enhance the development and growth of infants or as nutraceutical lipids by pregnant mothers.

#### **Knowns and Unknowns of Polar Phytosteryl**

**Conjugates.** Laura Nyström\*, Laboratory of Food Biochemistry, Institute of Food, Nutrition and Health, Switzerland.

*Abstract Pending*

#### **Sequential Crystallization of High and Low Melting Waxes to Improve Oil Structuring in Wax-based**

**Oleogels** Iris Tavernier\*<sup>1</sup>, Chi Diem Doan<sup>2</sup>, and Koen Dewettinck<sup>3</sup>, <sup>1</sup>*Ghent University, Belgium*; <sup>2</sup>*Laboratory of Food Technology & Engineering, Ghent University, Belgium*; <sup>3</sup>*University of Gent, Belgium*

Today, a considerable amount of research is focusing on structuring oil-based products with alternatives for trans and/or saturated fats. Several studies have shown the potential of wax-based oleogels. Possibly, combining different waxes could

open up even more opportunities for designing tailor-made wax-based materials with specific properties for various food applications. The main objective of this study was to investigate the effect of combining a high-melting wax (sunflower or rice bran wax) with a low-melting wax (berry wax or BEW) on the crystallization and gelation behavior of the corresponding wax-based oleogels in rice bran oil (RBO). Since sunflower wax (SW) and rice bran wax (RBW) have a similar chemical composition but a very different crystallization behavior, they were also combined in a wax-based oleogel to examine the occurrence of co-crystallization and/or crystal co-existence. Phase diagrams confirmed the sequential melting of the high- and low-melting waxes and the simultaneous melting of RBW and SW. For all three combinations (RBW:SW, SW:BEW and RBW:BEW) two different crystal morphologies could be discerned and powder XRD analysis gave no indication of the presence of co-crystals. Small amplitude oscillatory experiments (amplitude sweeps and frequency sweeps) and textural studies revealed the reinforcement of the high-melting wax crystal network with the addition of the low-melting berry wax, which most probably crystallizes in the voids remaining after crystallization of the high-melting wax. This research provides opportunities for successfully combining high- and low-melting substances in creating semi-solid materials with a wide variety in rheological and textural properties.

#### **Antioxidant Capacity of Different Bioactives in an Oil-like-Structured Heterogeneous Medium**

**Designed for Food Applications** Maria Chatzidaki\*<sup>1</sup>, Maria Zoumpanioti<sup>1</sup>, Giorgos Sotiroudis<sup>1</sup>, Erwann Durand<sup>2</sup>, Jérôme Lecomte<sup>3</sup>, Claire Bourlieu<sup>4</sup>, Aristotelis Xenakis<sup>1</sup>, and Pierre Villeneuve<sup>5</sup>, <sup>1</sup>*NHRF, Greece*; <sup>2</sup>*CIRAD, France*; <sup>3</sup>*CIRAD, Greece*; <sup>4</sup>*UMR IATE - INRA/CIRAD/UM2/SupAgro, France*; <sup>5</sup>*CIRAD/INRA,*

*UMR 1208 IATE, France*

During the last decades there has been an increasing interest from food industry towards edible water-in-oil (W/O) heterogeneous systems similar to vegetable oils' structure and able to solubilize bioactive molecules of different polarities. In this context, effective delivery systems of antioxidants (AO) maximizing their activities in various environments are highly needed. The use of a W/O microemulsion as carrier of AO could be a solution. Such system, based on medium chain triglycerides, was tested to deliver phenolic acids (p-hydroxybenzoic, protocatechuic and gallic), alcohols (tyrosol and hydroxytyrol) and esters. Propionic esters isomers of hydroxyphenyl acetic (HPA) acid were also synthesized using lipase from *Candida antarctica*. All the AO components were tested either delivered in free forms or in W/O microemulsion using complementary antioxidant tests in lipophilic (EPR), heterogeneous (CAT) or hydrophilic (ORACFL) environments. The most efficient bioactives were then evaluated for their ability to reduce the level of reactive oxygen species (ROS) using fibroblast cell lines producing high ROS levels. It was found that the bioactives had a different behaviour in the presence of the oil-like carrier probably due to their partial partition at the interface. In ORACFL for instance micro-emulsion induced higher equivalent Trolox system for most AO. The cellular response of fibroblast was also different when the antioxidants were delivered in the microemulsion instead of DMSO. Altogether, these experiments proposed an integrated approach of the physicochemical and other parameters that modulate the AO activity in various environments that are encountered in food products or along the digestive tract.

**Sonocrystallization of Interesterified and Physical Blends of High Oleic Sunflower Oil (HOSO) and Tristearin** Jeta V. Kadamne\*<sup>1</sup>, Ebenezer A. Ifeduba<sup>2</sup>, Casimir C. Akoh<sup>2</sup>, and Silvana Martini<sup>1</sup>, <sup>1</sup>Utah State University, USA; <sup>2</sup>University of Georgia, USA

The physical (PB) and interesterified (IE) blends of high oleic sunflower oil and tristearin with 20 and 30% stearic acid at the sn-2 position were crystallized without and with application of high intensity ultrasound (HIU). The IE samples were crystallized at supercoolings ( $\Delta T$ ) of 12, 9, 6 and 3 °C while PB were crystallized only at  $\Delta T = 12$  °C due to their slow crystallization characteristics. The PB crystallized faster and had higher SFC than IE at  $\Delta T = 12$  °C and HIU did induce crystallization in the PB samples. Induction in crystallization with HIU was observed at  $\Delta T = 6$  and 3 °C for IE C18:0 20% while at  $\Delta T = 9, 6$  and 3 °C for IE 30%. HIU induced the formation of smaller and more crystals in all samples. The DSC profiles of IE 20% samples showed that HIU induced fractionation in the sample along with crystallization of lower melting TAGs. While in the IE C18:0 30% samples, the high and low melting TAGs crystallized separately and HIU induced their co-crystallization. HIU significantly improved the viscosity,  $G'$  and  $G''$  of the IE 20% samples except at  $\Delta T = 12$  °C. While the  $G'$  and  $G''$  of IE 30% did not increase significantly, the viscosity increased significantly at  $\Delta T = 9, 6$  and 3 °C and it increased from  $1526.5 \pm 880.3$  to  $6818 \pm 901.4$  Pa.s. at  $\Delta T = 3$  °C. These IE fats with improved crystallization properties can be used as trans fat alternatives.

## ANA 5 / H&N 5: Impact of Oil Processing on Health Outcomes

*Chairs: J. Thomas Brenna, Cornell University, USA; and Sean Liu, USDA, ARS, USA*

### **Introduction: Oil Processing or Fatty Acid**

**Composition, What's More Important?** J. Thomas Brenna\*, *Cornell University, USA*

The controversy over health effects of saturated fat and health has raged since at least the 1950s with no signs of resolution. Early data pointed to cholesterol raising properties of saturated fats, initially understood as animal fats (butter, tallow, lard) and later as tropical oils, implying harm to heart health. Unequivocal evidence in experimental animals developed in the 1970s shows that highly refined coconut oil dramatically raises serum cholesterol. The epidemiology of saturated vegetable fats supports low, not high, levels of heart disease in native populations consuming, for instance, coconut as the predominate source of dietary fat. The recent widespread availability of virgin coconut oil prompted head to head studies showing virgin coconut oil does not raise serum cholesterol compared to refined coconut oil. Moreover, studies of the most prominent trans fatty acid in partially hydrogenated vegetable oil, elaidic acid, support lower not high serum cholesterol. Advances in chemical analysis enable sensitive measures of compounds that are created during processing or enter the oils during processing or storage. This symposium will introduce issues about how fats and oils processing may influence the healthfulness of fats and oils independent of fatty acid composition.

**Impact of Industrial Processing and Mitigation on MCPD/Glycidyl Ester Concentrations in Oils and Foods** Jessica K. Leigh\*, and Shaun MacMahon, *US Food and Drug Administration, USA*

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPD), 2-monochloro-1,3-

propanediol (2-MCPD), and glycidol are process-induced chemical contaminants found in refined edible vegetable oils. Formed during the deodorization step of the refining process, these compounds are considered potentially carcinogenic and/or genotoxic, making their presence in edible oils and processed foods containing these oils a potential health risk. For this reason, research efforts over the last several years have focused on developing methodology for the extraction and quantitation of these contaminants in oils, infant formula, and other complex food matrices in an effort to determine levels of exposure. Validated methodology for the quantitative analysis of 3-MPCD and glycidyl esters in oils and various food products will be briefly described in this presentation, followed by a detailed look at the occurrence of these contaminants in a wide array of oils and infant formulas from the United States, Canada, and Europe. In addition, preliminary occurrence data for 3-MCPD and glycidyl esters in other complex food matrices, including chips, cookies, baked goods, and other food items containing refined oils, will be presented. Results from the occurrence studies show a wide range of 3-MPCD and glycidyl ester concentrations across various types of refined oils, as well as varying concentrations among similar infant formula varieties produced by different manufacturers. Finally, an evaluation of the potential impact of processing and mitigation on the concentrations of these contaminants in food products will be discussed.

**A Novel Method to Assess Health Effects of Oils: Virgin and Refined Coconut Oil** Ruijie Liu\*<sup>1</sup>, Can Shi<sup>2</sup>, Elizabeth Mendralla<sup>3</sup>, Kumar S.D. Kothapalli<sup>3</sup>, Xingguo Wang<sup>2</sup>, and J. Thomas Brenna<sup>3</sup>, <sup>1</sup>*Jiangnan*

*University/Cornell University, China; <sup>2</sup>Jiangnan University, China; <sup>3</sup>Cornell University, USA*

Introduction. Head-to-head comparisons of virgin coconut oil and harshly processed copra oil of identical fatty acid profiles show liver cholesterol and triglyceride levels to be dramatically lower in the VCO group, and more similar to PUFA. We hypothesize that the cholesterol raising effect of tropical oils may be due chemical alteration during processing rather than their saturated fats. Our objective was to develop a rapid system to evaluate prompt effects on cholesterol metabolism that recapitulate these effects in vitro. Methods. Coconut oils of various degrees of processing but identical fatty acid profile were used as a test case. Oils were made into a test emulsion and cells were treated with 500  $\mu$ M for 24 h. Gene expression measured by RT-PCR. Results. Cells took up the oils as indicated by changes in their fatty acid profiles. Two genes were responsive to degree of processing: HMGCR, the rate-limiting step for endogenous cholesterol synthesis, and CYP71A that catalyzes the initial step of cholesterol catabolism. HMGCR expression increased from 0.97 to 1.78 fold in a stepwise manner over four processing steps, normalized to untreated cells. At the same time, CYP7A1 expression decreased. The net effect suggest increasing cholesterol levels due to more processed oils. Conclusion. These data are consistent with an increase in cholesterol synthesis and a decrease in cholesterol degradation due to processing, probably attributable to generation of chemical factors specifically influencing cholesterol synthesis. This approach holds promise for rapidly assessing metabolic effects on humans.

**Plasticiser Residues in Edible Oils and Fats– Relevance and Analysis** Jan Kuhlmann\*, *SGS Germany GmbH, Germany*

Plasticisers represent a complex group of world-wide and in large scale applied chemicals.

They ensure important properties to plastic materials but they are also used as auxiliaries in medical and personal care products and in various other household items. Trace contamination of edible oils and fats might occur during harvesting, processing, bottling and storage. Specific plasticisers are suspected to have adverse health effects. In this regard some authorities have started to take action in terms of announcing recommendations or defining TDIs. Also NGOs have picked up the absence of certain plasticisers as quality criteria for foods such as edible oils and fats. Very likely these compounds will raise increasing intention of authorities and consumers in the future which might result in setting MRLs. In order to determine trace amounts of plasticisers for monitoring purpose or to control internal or official limits there is an obvious need for reliable and validated analytical methods. However, analysis of plasticiser residues in foods seems to be challenging as the number and diversity of compounds is increasing while at the same time the ubiquity of plasticiser containing utilities as source of background levels in laboratories raises the issue of cross-contamination. This presentation highlights the relevance of the issue in regard to product quality and market demands. A new analytical approach for the parallel determination of 24 plasticisers in oils and fats by on line coupled LC-MS<sup>2</sup> technique is introduced. Occurrence data for refined and non-refined edible oils and fats will be presented.

**Analysis of Heavy Metals in Rice Bran Oil by Inductively Coupled Plasma (ICP) Spectrometry**

Robert O. Dunn\*<sup>1</sup>, Erica L. Bakota<sup>2</sup>, and Sean Liu<sup>3</sup>, <sup>1</sup>USDA, ARS, NCAUR, USA; <sup>2</sup>Harris County Institute of Forensic Sciences, USA; <sup>3</sup>USDA, ARS, USA

Rice is one of the most important staple crops in the world. Nevertheless, health-conscious consumers have expressed concern regarding the

presence of heavy metals, specifically arsenic, in rice. The United Nations Food and Agriculture Organization (UNFAO) limits the arsenic concentration at 0.2 mg/kg in rice, but has no set limit in rice bran oil. Rice bran oil is known to have good antioxidant activity in foods. The study evaluates the use of inductively coupled plasma (ICP) spectrometry in determining the concentration of arsenic, cadmium, lead, mercury and zinc metals concentrations in crude and refined rice bran oils. Most analytical laboratories digest oil samples into an aqueous matrix before running the analysis for heavy metals. However, digestion of organic samples may increase the experimental error in the analysis. In the present work, the digestion step was omitted and the oil samples mixed with kerosene before ICP analysis. Comparison of the results with data from two independent laboratories indicated large deviations for the arsenic and mercury concentrations.

**Quantifying Trans Fat in Foods: How Low Can We Really Go?** Cynthia Srigley\*, Sanjeewa R. Karunathilaka, and Magdi Mossoba, *US Food and Drug Administration, USA*

The intake of trans fatty acids (TFA) has been associated with numerous potential health risks, leading regulatory authorities, such as the United States Food and Drug Administration (FDA), to issue mandatory labeling regulations for the contents of total trans fat in foods. In June 2015, FDA issued its final determination that partially hydrogenated oils (PHO), the major dietary source of industrial-produced TFA, are no longer generally regarded as safe (GRAS) for any use in human food. However, low concentrations of trans fat (e.g.,

**2016 Monitoring of MCPD Derivatives and Glycidyl Esters in German Foods—Outcome and Applied Methods** Jan Kuhlmann\*, *SGS Germany GmbH, Germany*

Monochloropropanediol (2- & 3-MCPD) and glycidyl esters have raised tremendous attention in the past years as they are world-wide occurring process-induced contaminants which might have adverse effects on health of consumers. Free MCPD can be generated when complex composed foods are heated. By contrast the more complex groups of fatty acid esters of MCPD and glycidol mostly are formed during deodorisation of edible oils and fats. This led to an EU recommendation to monitor free and ester-bound 2- & 3-MCPD and ester bound glycidol in oils and fats but also in a broad variety of oil and fat containing foods. Limits for the Tolerable Daily Intake of free and bound 3-MCPD are set between 0.8 and 4 µg/kg bw d in the EU. Glycidol shows genotoxic properties so that consumers uptake should be As Low As Reasonably Achievable. Anyway, many retailers have set self-defined maximum levels as the issue of MCPD- and glycidol contamination of foods is in public's perception. The release of MRLs recently is discussed by the EU commission. This presentation gives an overview on the occurrence of free 2- and 3-MCPD as well as ester-bound 2- & 3-MCPD and glycidol in different foods. The applied analytical method will be presented as it has been developed newly in order to have a method available that is on the one hand based on the validated approaches for oils and fats but also should be more sensitive and applicable to all different kinds of complex composed foods.

## H&N 5.1: General Health and Nutrition

*Chairs: Susan Ratz, USDA, ARS, Grand Forks Human Nutrition Research Center, USA; and Mathilde Fleith, Nestec Ltd., Switzerland*

**Association of Fatty Acid Intake with Weight Status in the US Population** Susan K. Ratz\*, Zach Conrad, LuAnn K. Johnson, Matthew Picklo, and Lisa Jahns, *USDA, ARS, Grand Forks Human Nutrition Research Center, USA*

The type of fatty acid intake may be related to obesity. Intake of monounsaturated fatty acids (MUFA) is associated with lower rates of obesity in ecological studies and LCn-3 is associated with lower body weight, while intake of saturated fatty acid (SFA) is associated with higher body weight. However, the literature is inconsistent due to the various research methods used. The objective of this study was to describe the association of reported fatty acid intake and weight status using the cross-sectional 2005-2012 National Health and Nutrition Examination Survey (NHANES) and the What We Eat in America (WWEIA) national surveys. A 24-hour dietary recall was performed on 20,434 adults (age 19+). Survey-based linear regression was used to examine the association between total fat, total and individual SFA, MUFA, polyunsaturated fatty acids (PUFA), and BMI categories for normal, overweight, and obesity. Total fat and SFA intake were positively associated with body weight. Contrary to expectations, there was a significant positive trend between weight status and total MUFA. No association was observed between total PUFA, individual PUFAs or LCn-3. Of the individual fatty acids, intake of myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0), as well as oleic acid (18:1) was associated with increasing body weight. The results of this observational research suggest that total fat intake is related to body weight and that intake of individual fatty acids may contribute to weight status.

**Bioactive Lipids from Novel Medicinal Plants of the Tropical Rainforests of East Africa** Fabien Schultz\*<sup>1</sup>, Godwin Anywar<sup>2</sup>, and Leif-Alexander Garbe<sup>3</sup>, <sup>1</sup>*Technical University of Berlin, Neubrandenburg University of Applied Sciences, Germany*; <sup>2</sup>*Makerere University, Uganda*; <sup>3</sup>*Neubrandenburg University of Applied Sciences, Germany*

The majority of plant and insect species of the tropical rainforests in western Uganda and eastern Democratic Republic of Congo have not yet been

discovered; 99% have not yet been screened for bioactivity. Approximately 60% of the world's population relies almost entirely on plants for medication and knowledge of East African plants and their traditional uses is mainly transferred orally from one generation to the next by traditional healers. As a result of resettlement, drift to the cities and deforestation, this ancient knowledge is often lost before any scientific investigation and documentation can take place. The interaction between ethnobotanical hints, modern chemistry, and bioassays offers huge potential for discovery and development of novel drugs or innovative food and cosmetics ingredients. Our study provides the first documentation of 16 different African medicinal plants, which are claimed to have anti-malarial, anti-cancer, anti-biotic, or anti-oxidant properties. One possible methodology for the discovery of novel bioactive lipids is screening selected plant extracts for a broad array of pharmacological activities. We present results of diverse bioassays, e.g. anti-biotic resazurin assay, anti-malarial heme biocrystallization assay, DPPH assay for free radical scavengers, Ames tests for carcinogenic effects, and phytochemical screenings, performed with 50 different plant extracts. Bioassay-guided fractionations combined with NMR and GC/LC-MS techniques enabled identification of bioactive lipids in some medicinal plants, giving rise to future investigations.

**Effects of Stearic Acid on Blood Lipid Levels** Mathilde Fleith\*, *Nestec Ltd., Switzerland*

Current dietary recommendation to limit total saturated fatty acids (SFA) intake to 10%E, based on effects of SFA on cardiovascular diseases (CVD), does not consider potential differences between individual SFA. We reviewed current evidence for the effect of stearic acid (SA), the second most abundant dietary SFA, on CVD to determine whether SA should be included in the SFA to limit. Systematic reviews and meta-analyses of randomized controlled trials (MA-RCT) were searched. Four MA-RCT assessing the effects of SA on blood lipid levels (as risk factor for CVD) were recovered. Despite the different methodologies and regression analyses used, SA was consistently found to

have no effects on blood lipids. However, depending on which macronutrient or FA class is replaced by SA, effects may appear favourable or unfavourable. When SA is used to isocalorically replace SFA, changes in blood lipid levels are overall favourable. When it replaces polyunsaturated fatty acids (PUFA), the changes are unfavourable. Thus SA per se seems having no deleterious effects on blood lipids. If used to replace FA that impact blood lipid levels, then SA may have distinct effects: favourable if used to replace SFA, or unfavourable if used to replace PUFA. Thus when designing fat blends for food product (re)formulation, SA-rich fats may be a good alternative to other SFA rich fats, especially with the goal to limit SFA. In addition, the 10% limit in the recommendation for total SFA may evolve to a more granular recommendation excluding SA, in line with available scientific evidence.

**Dietary Trans-vaccenic Acid Reduces Arthritic Severity in the Collagen-induced Arthritis Model** Mark E. Cook, Jake M. Olson\*, Joni C. Baker, Sarah E. Clifford, and Jennifer Lor, *University of Wisconsin-Madison, USA*

Dairy fat contains trans-vaccenic acid (tVA), which has shown various dietary anti-inflammatory benefits, although dose-specific effects on chronic arthritic inflammation are unknown. Therefore, a study was conducted wherein tVA was varied in the diets of arthritic mice to evaluate long-term anti-inflammatory effects in the murine collagen-induced arthritis (CA) model. Arthritic mice were fed diets nutritionally completed after addition of 3% corn oil (CO) or 0.375%-, 0.75%-, or 1.5% tVA + CO, and assessed for changes in arthritic severity over a 9-week period. Compared to CO-fed arthritic mice, mice fed 0.375-, 0.75-, and 1.5% tVA had a linear reduction in overall arthritic severity (-21%, -42% and -65%, respectively;  $P < 0.01$ ,  $R^2 = 0.31$ ), as evaluated by clinical assessment of limbs. As the endogenous conversion of tVA to cis-9, trans-11 conjugated linoleic acid (c9t11) may explain the observed anti-inflammatory effects of tVA, healthy mice were fed 0, 0.15, or 1.5% tVA diets in a simultaneous study to determine conversion efficiencies of tVA to c9t11 in liver, adipose, and paw tissue at specific time points over 9-weeks. Liver tVA and c9t11 levels saturated by week 5, while adipose tVA and c9t11 did not saturate by week 9, with a conversion efficiency of roughly 50% occurring in both tissues. Alternatively, low-dose tVA (0.15%) reached 100% conversion to

c9t11 by week 9 in paw tissue. Results show dietary tVA dose-dependently reduces chronic inflammation in the CA model, the effects of which may be mediated through its conversion to c9t11 directly in inflamed tissues.

**Encapsulation and Delivery Pancreatic Lipase in Hydrogel Beads with Self-regulating Internal pH Microenvironments** Zipei Zhang\*, Ruojie Zhang, and David J. McClements, *University of Massachusetts Amherst, USA*

The oral delivery of lipase is an important therapy for patients with exocrine pancreatic insufficiency. However, orally ingested lipase loses much of its activity when exposed to the highly acidic environment of the stomach. In this study, pancreatic lipase was encapsulated within hydrogel beads fabricated from food-grade ingredients: alginate (gel former), calcium chloride (cross-linker), and magnesium hydroxide (buffer). A quantitative ratiometric method based on laser scanning fluorescence confocal microscopy imaging was utilized to map the pH microclimate inside the hydrogel beads under simulated gastrointestinal tract (GIT) conditions: mouth, stomach, and small intestine. In the absence of encapsulated buffer, the pH within the beads rapidly decreased when they moved from mouth (pH 6.3) to stomach (pH < 4), leading to a loss of lipase activity in the small intestine. In the presence of encapsulated buffer, the pH inside the beads remained close to neutral in mouth (pH 7.33) and stomach (pH 7.39), leading to retention of lipase activity in the small intestine. Confocal microscopy indicated that lipase was released from the beads under small intestine conditions, while pH-stat analysis showed that the lipase released hydrolyzed emulsified lipids into free fatty acids. The presence of the encapsulated buffer also reduced bead shrinkage under acidic gastric conditions. This study showed that Mg(OH)<sub>2</sub>-loaded alginate beads maintain a neutral internal microclimate throughout a simulated GIT, which preserved lipase activity in the small intestine.

### **Novel Nano Delivery Vehicles for Solubilization and Enhanced Delivery of Cannabinoids**

Nissim Garti\*, Sharon Garti-Levy, and Rotem Edri, *Casali Institute of Applied Chemistry The Hebrew University of Jerusalem, Israel*

The CBD is the most studied component is presently extracted, in most cases, non-selectively, from the plant, by CO<sub>2</sub> or various solvents including ethanol. The purified material is thereafter formulated mainly in vegetable oil and delivered to human by variety of techniques and mainly by oral intake. The activity of the bioactive is dictated in the first stage of reaching the blood stream by its bioavailability (permeation through the guts or skin). In our lab we have developed two new technologies termed NSSL (Nano-sized, Self-assembled liquid fully dilutable delivery vehicles) and MLLC (modified lyotropic liquid crystals (condensed reverse swollen micelles in a gel form) The novel nano droplets are self-assembled, thermodynamically stable, and transparent with mono dispersed droplets with very high surface area and very high solubilization capacities to CBD. The nano droplets are "tailor-made" liquid mesophases designed in their composition and method of preparation (after Monte Carlo Simulations) capable to "solubilize" the CBD (or mixtures of CBD and other cannabinoids including THC) and to deliver the bioactives "on demand" and only after bypassing the acidic conditions of the stomach. Delivery profiles of the release in comparison to other delivery pathways will be given. The chemical, physical and biological stability will be demonstrated.

### **Whey Protein Can Modulate Body Fat-reducing Potential of Conjugated Linoleic Acid in Rats**

Kazunori Koba\*<sup>1</sup>, Yoshimi Arimoto<sup>1</sup>, Koji Kawabeta<sup>2</sup>, Nozomi Tateiwa<sup>1</sup>, Shun-ichi Matsuda<sup>3</sup>, Toshio Iwata<sup>4</sup>, and Michihiro Sugano<sup>5</sup>, <sup>1</sup>*University of Nagasaki, Japan*; <sup>2</sup>*Graduate School of Human Health Science, University of Nagasaki Siebold, Japan*; <sup>3</sup>*Fonterra (Japan) Limited, Japan*; <sup>4</sup>*The Nisshin OilliO Group, Ltd., Japan*; <sup>5</sup>*Professor Emeritus, Kyushu University, Japan*

We previously suggested that the body fat-reducing potential of conjugated linoleic acid (CLA) could be exalted by a combination with soy protein (SOY) instead of casein (CAS) in rats. In the present study, we focused on whey protein (WHY), known as a lipid modulator, and examined how it affects body fat-reducing potential of CLA. Male Sprague-Dawley rats were fed the diets

containing 20% protein (CAS, SOY or WHY) with 1% linoleic acid or CLA (c9, t11-18:2, 39%; t10, c12-18:2, 37%) for 4 weeks. At week 4, abdominal fat was measured with X-ray computed tomography imaging method. As a result, dietary WHY compared with CAS and SOY tended to decrease visceral fat mass, and it was marked in combination with CLA. These observations were associated with the weights of epididymal and perirenal adipose tissues. Dietary CLA lowered serum triglyceride concentration when protein source was WHY and SOY but not CAS. Liver triglyceride concentration was low in the order of CAS, WHY and SOY, and CLA lowered it further to the level of SOY. SOY as compared with CAS significantly decreased hepatic fatty acid synthesis, while it was less evident in the WHY diet. In a separate study measuring respiratory quotient, energy consumption from fat was higher in rats fed WHY or SOY in combination with CLA than the others. Results in the present study suggested that body fat-reducing potential of CLA was more evident in combination with WHY or SOY, but underlying mechanism could be different between WHY and SOY.

### **Whole Blood, Plasma, and Erythrocyte Acyl-lipids are Remodeled at Different Rates with Fish Oil**

**Supplementation** Juan J. Aristizabal Henao\*<sup>1</sup>, Ashley C. Patterson<sup>2</sup>, Richard W. Smith<sup>1</sup>, and Ken D. Stark<sup>1</sup>, <sup>1</sup>*University of Waterloo, Canada*; <sup>2</sup>*Mead Johnson Nutrition, USA*

Changes in blood fatty acid composition with fish oil supplementation is well documented, but comparisons of the lipidomic profiles of whole blood, plasma and erythrocytes with fish oil use have not yet been studied. Briefly, participants were provided with fish oil providing 250, 500 and 1000mg/day EPA+DHA in step-wise phases of 4 weeks per diet level. Blood was collected at baseline and at weeks 4, 8 and 12 and samples were analyzed using UHPLC-MS/MS (Q-Exactive Quadrupole-Orbitrap hybrid). Ten EPA- or DHA-containing complex lipids that increased significantly with increased fish oil intake were identified in whole blood. This included three lipids which responded in a dose-dependent manner to EPA+DHA intakes, which were 16:0/DHA phosphatidylcholine (PC), 16:0/EPA PC, and P-16:0/EPA plasmeyl-phosphatidylethanolamine (PE). Blood fraction analysis revealed that these three lipids changed in both plasma and erythrocytes. Lipidomic analysis also revealed that different EPA or

DHA containing lipids changed along different timelines. For example, P-16:0/DHA plasmalogen-PE and 18:0/DHA phosphatidylserine increases in erythrocytes were not detected until 12 weeks. Unlike fatty acid profiling, lipidomic profiling has the potential to identify unique EPA- or DHA-containing acyl-lipid species that respond differently over time. Characterising these responses in detail and across blood fractions has the potential to identify blood biomarkers of acute, sporadic intake vs. long term, habitual intake which would be a useful tool for nutritional epidemiology.

**Effect of Positional Saturated Fatty Acids of Triacylglycerols on Fat Accretion in C57BL/6 Mice** Shiou Wah Gouk<sup>\*1</sup>, Soek Sin Teh<sup>1</sup>, Phooi Tee Voon<sup>1</sup>, Tony Kock Wai Ng<sup>2</sup>, Augustine Soon Hock Ong<sup>3</sup>, and Yuen May Choo<sup>1</sup>, <sup>1</sup>*Malaysian Palm Oil Board, Malaysia*; <sup>2</sup>*International Medical University, Malaysia*; <sup>3</sup>*Academy Science of Malaysia, Malaysia*

**Background:** We investigated whether dietary fats with major triacylglycerol species of either palmitic acid or stearic acid at the sn-1,3 positions, reduce body-fat accretion in the C57BL/6 mice model.

**Methods:** Four experimental fats- native soybean oil (L-L-L major triacylglycerols), enzymatic-interesterified soybean oil (P-L-P major triacylglycerols), native palm olein (P-O-O major triacylglycerols), and high-oleic sunflower oil (O-O-O major triacylglycerols) were incorporated at 15% w/w into mice chow and fed separately to different groups of 10 individually-housed mice for 15 weeks.

**Results:** The mice in all 4 groups had comparable ( $p > 0.05$ ) body mass gain, body mass gain/g feed and liver mass at the end of the study. However, mice fed with enzymatic-interesterified soybean oil showed significantly lower total body fat ( $6.18 \pm 0.20$  vs.  $7.10 \pm 0.13$ ,  $p < 0.05$ ) and total body fat/g feed ( $1.55 \pm 0.06$  vs.  $1.82 \pm 0.06$ ,  $p < 0.05$ ) compared with mice fed with native soybean oil. Mice on the high-oleic sunflower oil diet had significantly higher subcutaneous fat ( $3.29 \pm 0.14$ g) and visceral fat ( $4.15 \pm 0.24$ g) compared with mice on the palm olein-diet ( $2.75 \pm 0.13$  and  $3.49 \pm 0.15$ g respectively,  $p < 0.05$ ). The visceral fat content of the high-oleic sunflower oil-diet mice was also significantly higher ( $p < 0.05$ ) than that of animals on the enzymatic-interesterified soybean oil diet ( $3.33 \pm 0.12$ g).

**Conclusion:** We conclude that the extent of fat deposition in the mice model used is significantly reduced when the fatty acids at the sn-1,3 positions of triacylglycerols in highly unsaturated oils are replaced by long-chain saturated fatty acids such as palmitic acid, or stearic acid.

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

*Chairs: Mathilde Fleith, Nestec Ltd., Switzerland; and Michelle Judge, University of Connecticut, USA*

### 1. Associations Between Red Blood Cell Fatty Acids and Cardiometabolic Risk Markers Differ in White vs. South Asian Canadian Adults Living in Ottawa

Isabelle Demonty<sup>\*1</sup>, Cunye Qiao<sup>2</sup>, Chao-Wu Xiao<sup>1</sup>, Eleonora Swist<sup>1</sup>, Reiko Nagasaka<sup>3</sup>, Carla Wood<sup>1</sup>, and Walisundera M.N

Ratnayake<sup>1,1</sup> *Nutrition Research Division, Bureau of Nutritional Sciences, Health Canada, Canada;*

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<sup>3</sup>*Food Chemistry and Functional Nutrition, Dept. of Food Science and Technology, Graduate School of Marine Science and Technology, Japan*

**Objective:** Evaluate whether red blood cell (RBC) fatty acids (FA) other than omega-3 index (previously reported) are differently associated with cardiometabolic risk markers in South Asian Canadians (SAC) vs. White Canadians (WC).

**Methods:** Fasting blood samples were taken from SAC and WC adults (20-79y) living in Ottawa and taking no blood lipid or glucose lowering medication (n=235 SAC and 279 WC). Associations between RBC FA and risk markers were evaluated using multiple linear regression models including FA and ethnicity, and, when significant, BMI, age, gender and interactions (ethnicity x FA, ethnicity x covariables). **Results:** HOMA-IR, TC:HDL-C, CRP, and VEGF were higher ( $P < 0.05$ ) whereas HDL-C was lower ( $P < 0.05$ ) in SAC vs. WC. 14:0 was positively associated ( $P \leq 0.01$ ) with blood triglycerides (beta=1.1), LDL-C (beta=1.1), CRP (beta=1.9), HOMA-IR (beta=1.0), and VEGF (beta=0.9) in both SAC and WC, whereas it was positively related to HDL-C only in WC (beta=0.47,  $P=0.005$ ). 22:0 was positively associated ( $P=0.001$ ) with insulin (beta=1.38) and HOMA-IR (beta=1.51) only in WC.

18:3n-3 was positively associated with triglycerides in both WC and SAC (beta=1.2,  $P < 0.0001$ ), but was positively associated with TC/HDL-C only in SAC (beta=0.51,  $P=0.046$ ). Other associations were either similar between SAC and WC or were weaker (lower betas). The models explained 25-35% of the variation in risk markers. **Conclusions:** The relationship between RBC FA and cardiometabolic risk markers differ in SAC and WC, suggesting that differences in dietary intake and/or FA metabolism may possibly play a role in the previously reported differences in cardiometabolic disease risk between SAC and WC.

### 2. Effects of Oil Prepared from a Scallop By-product on Liver Lipid Peroxidation in Mice

Ryota Hosomi<sup>\*1</sup>, Toshifumi Tanizaki<sup>2</sup>, Kenji Fukunaga<sup>2</sup>, Syohei Mori<sup>3</sup>, Shingo Inoue<sup>3</sup>, Takuma Kawanami<sup>3</sup>, Koretaro Takahashi<sup>3</sup>, and Takeya Yoshioka<sup>4,1</sup> *Kansai University, Japan;* <sup>2</sup>*Faculty of Chemistry, Materials and Bioengineering, Kansai University, Japan;* <sup>3</sup>*Faculty of Fisheries Sciences, Hokkaido University, Japan;* <sup>4</sup>*Hokkaido Industrial Technology Center, Japan*

The internal organs other than the edible portion generated during the scallop processing are treated as waste. Waste disposal and by-product management by the food processing industry for environmental protection and sustainability are mandatory. However, by-product of scallop has not progressed effective usage because it has a high content of cadmium (Cd). We have successfully prepared the oil (SBO) with a low Cd content (0.2 mg/kg) from by-product of scallop. To clarify the potential of SBO as a health-maintaining and -promoting food material or supplement, we investigated the effects of SBO on liver lipid

contents and peroxidation in mice. SBO contained high levels of EPA (30%) and DHA (10%). Four-week-old male or female ICR mice were fed a basal diet containing 7% soybean oil, and basal diet supplement with 1 % or 5% SBO or tuna oil. No significant differences were observed in the liver thiobarbituric acid reactive substances values among the experimental groups. In addition, mice fed 5% SBO diet were significantly decreased the liver  $\alpha$ -tocopherol contents compared with mice fed a basal diet. However, mice fed 5% SBO diet was not affected the index of serum alanine amino transferase, aspartate amino transferase, and urea nitrogen. This study demonstrated that SBO has a possibility of an effectively usage as EPA high content oils.

### 3. Enhancing Omega-3 Fatty Acids and Alpha-tocopherols in Caprine Milk by Feeding Rumen-protected Fish Oil Supplements Jung Hoon Lee\*, Beruk Lemma, and Christina Alfred, Fort Valley State University, USA

Nine lactating goats were used to assess the effects of rumen-protected fish oil (RPFO) and tocopherol, prepared with a GRAS chemical on caprine milk composition. Lactating goats were offered three different diets consisting of 95% basal diet with 5% lipid from either poultry fat (PF; control), fish oil (FO), or RPFO using a 3x3 Latin Square design with three 14-d periods. Both FO and RPFO contained  $\alpha$ -tocopherol (0.075%), eicosapentaenoic (EPA; C20:5 $\omega$ 3, 14.1%) and docosahexaenoic (DHA; C22:6 $\omega$ 3, 12.3%) acids. Collected blood and milk samples from individual goats at the end of each feeding period were analyzed for  $\alpha$ -tocopherol and fatty acid composition. The blood serum from goats fed RPFO diet tended ( $P = 0.08$ ) to have a higher amount of  $\alpha$ -tocopherol than did those from goats fed either PF or FO diet. However, no significant differences were found in the amount of  $\alpha$ -tocopherol (0.87–

0.90  $\mu\text{g}/\text{mL}$ ) in the milk samples from the 3 different diets. Compared with goats fed PF-diet, goats fed either FO- or RPFO- diet had significantly higher concentrations of EPA (1.76 vs 10.57 or 13.23%) and DHA (1.95 vs 4.58 or 5.38%) acids in blood serums. Furthermore, goats fed either FO- or RPFO- diet had significantly higher concentrations of EPA and DHA in milk and goats fed RPFO had the highest concentrations of EPA (1.66 vs 0.81%) and DHA (1.86 vs 1.21%) in their milk. Feeding lactating goats with rumen-protected fish oils and tocopherols might increase the deposition of omega-3 fatty acids in caprine milk, but not tocopherols.

### 4. Maternal Fatty Acid and Inflammatory Status Affects Infant Heart Rate and Heart Rate Variability

Merritt Drewery\*<sup>1</sup>, Adriana V. Gaitan<sup>2</sup>, Derek Miketinas<sup>1</sup>, Steve B. Spedale<sup>3</sup>, Ericka Seidemann<sup>4</sup>, Karen Elkind-Hirsch<sup>4</sup>, and Carol Lammi-Keefe<sup>1,1</sup>, <sup>1</sup>Louisiana State University, USA; <sup>2</sup>Louisiana State University, United States; <sup>3</sup>InfaMedics, Woman's Hospital, USA; <sup>4</sup>Woman's Hospital, USA

Heart rate (HR) and heart rate variability (HRV) are valuable markers of health in various populations. In early life, specifically, HR and HRV reflect maturation of the autonomic nervous system and degree of cardiac-autonomic integration. Previous studies have demonstrated that intervention with n-3 long chain polyunsaturated fatty acid (LCPUFA) during pregnancy favorably affects fetal HR and HRV; similar observations have been reported with an n-3 LCPUFA intervention in infants. However, infant HR and HRV have not been assessed in relation to maternal fatty acid status during pregnancy. Further, previous studies have not considered the role of maternal inflammation in programming infant HR and HRV. Accordingly, we examined the relationship between maternal fatty

acid/inflammatory status during the third trimester and infant HR/HRV during the first 6 months of life. Fatty acids of particular interest were those of the n-6 and n-3 series. Inflammatory factors assessed included endocannabinoids, interleukin-6, adiponectin, C-reactive protein, and tumor necrosis factor.

#### 5. **Cardiometabolic Risk Markers, Red Blood Cell Fatty Acids, and Their Associations Differ in White vs. South Asian Canadian Children and**

**Adolescents Living in Ottawa** Isabelle Demonty\*<sup>1</sup>, Rong Huang<sup>1</sup>, Eleonora Swist<sup>1</sup>, Isabelle Massarelli<sup>2</sup>, Isabelle Rondeau<sup>2</sup>, Winnie Cheung<sup>1</sup>, Lois Fernandez<sup>1</sup>, and Walisundera M.N Ratnayake<sup>1</sup>, <sup>1</sup>*Nutrition Research Division, Bureau of Nutritional Sciences, Health Canada, Canada;* <sup>2</sup>*Food Surveillance Integration Division, Bureau of Food Surveillance and Science Integration, Health Canada, Canada*

**Objective:** Evaluate whether cardiometabolic risk markers, red blood cell (RBC) fatty acids (FA), and the associations between RBC FA and risk markers vary between White Canadian (WC) and South Asian Canadian (SAC) children and adolescents. **Methods:** Fasting blood samples were taken from 67 SAC and 79 WC aged 6-19y and living in Ottawa. EPA and DHA intake was assessed by a food and supplement questionnaire. Associations between RBC FA and cardiometabolic risk markers were evaluated using multiple linear regression models. Final models included FA and ethnicity, and, when significant, BMI, age, gender and interactions. **Results:** Fewer SAC (58%) than WC (72%) children/adolescents had a healthy weight (WHO-defined). SAC had higher blood glucose, insulin, HOMA-IR, apoB/apoA1 ratio, and leptin, and lower HDL-C than WC (all  $P < 0.05$ ). SAC consumed more EPA (20:5n-3) + DHA (22:6n-3) from food (0.38 mg/day) than WC (0.23 mg/day) ( $P=0.05$ ) and had higher omega-3 index and levels

of 22:6n-3, 18:3n-3, total omega-3s and 18:2n-6, but lower 22:0, 24:0, 16:1c9, 22:4n-6, and 22:5n-3 than WC (all  $P < 0.05$ ). Positive associations (18:3n-3 with triglycerides ( $\beta=4.6$ ,  $P=0.0002$ ) and 22:0 with total cholesterol ( $\beta=1.1$ ,  $P=0.04$ )) were observed in SAC but not in WC. 16:1c-9 was positively associated with leptin in WC ( $\beta=4.1$ ,  $P=0.007$ ) but not in SAC. **Conclusions:** SAC children/adolescents have a less healthy cardiometabolic profile than WC despite higher intake and RBC levels of long-chain omega-3s. Different relationships between RBC FA and risk markers in SAC vs. WC suggest that differences in FA intake/metabolism may possibly contribute to different cardiometabolic risk.

#### 6. **Dietary Exposure to Conjugated Linoleic Acid *cis*-9, *trans*-11 Prevents Collagen-Induced Arthritis**

Jessica A. Muhlenbeck\*, Daniel E. Bütz, Jake M. Olson, Daniela Uribe-Cano, and Mark E. Cook, *University of Wisconsin-Madison, USA*

Two conjugated linoleic acid (CLA) isomers, *cis*-9, *trans*-11 (CLAc9t11) and *trans*-10, *cis*-12 (CLAt10c12), reduce inflammation in a number of animal models, including collagen-induced arthritis (CA). However, little is known about the ability of individual CLA isomers to prevent autoimmune disease onset. Evidence that mixed isomer CLA drives T helper cell (Th) 1 responses suggests that CLA, or a specific isomer, exacerbates onset of Th1 autoimmune diseases. In two experiments, we examined if prior dietary exposure to CLAt10c12 (experiment 1) or CLAc9t11 (experiment 2) affected the incidence or severity of CA. DBA/1 mice were fed a semi purified diet with either 6 % corn oil (CO, w/w), 5.75 % CO plus 0.25 % CLAt10c12, or 5.5 % CO plus 0.5 % CLAc9t11 prior to arthritis development. Arthritis incidence and severity, anti-collagen antibodies, paw cytokines, and hepatic fatty acids were measured. CLAt10c12 had no effect on arthritis incidence but increased

arthritic severity (42 %,  $P = 0.02$ ); however, CLAc9t11 decreased arthritis incidence 39 % compared to CO fed mice ( $P = 0.01$ ), but had no effect on disease severity. CLAt10c12-induced increase in anti-collagen type II IgG antibodies may be a mechanism by which this isomer increased arthritic severity, and CLAc9t11-induced increase in Th2 paw cytokines (IL-4 and IL-10,  $P \leq 0.04$ ) may explain how CLAc9t11 reduced the arthritis incidence. While both isomers are well known to reduce inflammation in arthritic mice, this new data suggests isomer differences when fed prior to autoimmune disease. .

#### 7. Effects of Dietary $\beta$ -conglycinin on Insulin Sensitivity and Liver Lipid Concentration in OLETF Rats

Koji Kawabeta\*<sup>1</sup>, Shizuka Hase-Tamaru<sup>1</sup>, Kazuhito Suruga<sup>1</sup>, and Kazunori Koba<sup>2</sup>, <sup>1</sup>*Graduate School of Human Health Science, University of Nagasaki Siebold, Japan*; <sup>2</sup>*University of Nagasaki, Japan*

The effects of dietary  $\beta$ -conglycinin ( $\beta$ -CON) on insulin sensitivity, adipose tissue weights, and plasma and liver triglyceride concentrations were examined in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are an animal model for obesity and type 2 diabetes. The rats are obese on account of overeating and late-onset of a type 2 diabetes (25 wk-old). Male OLETF (6 wk-old) rats were fed with the diets containing either 20% casein (CAS) or CAS replaced 50% with soy protein (SOY) or  $\beta$ -CON for 13 weeks. During the feeding period, fasting blood glucose levels were measured every 3 weeks, and insulin tolerance test (ITT, 0.75 IU/kg) was performed on week 12. Compared with CAS, SOY and more clearly  $\beta$ -CON decreased the blood glucose level at 30 min after insulin injection. The finding was accompanied with an increase of adiponectin levels in plasma and gene expressions of adiponectin receptor 1 and insulin receptor substrate-1 in muscle. The results suggested that  $\beta$ -

CON could increase insulin sensitivity in presymptomatic obese phase of OLETF rats. Also  $\beta$ -CON decreased mesenteric adipose tissue weights and liver triglyceride concentrations. The results could be partly due to fatty acid synthesis in the liver. Dietary  $\beta$ -CON compared with CAS decreased gene expression and enzyme activity of fatty acid synthase in the liver, associated with by down-regulation of sterol regulatory element-binding protein 1c. Therefore, the results in the present study suggested that  $\beta$ -CON could improve the progression of diabetes in OLETF rats.

#### 8. Nanovehicles for Inhibition of the Formation of Advanced Glycation End Products (AGEs)

Karina Latorre and Alejandra Medrano\*, *UdelaR, Uruguay*

Nanotechnology is a rapidly expanding technology with a lot of potential in a greatly amount of areas including pharmaceutical and food industries. Liposome are an example of this nanovehicle. This nanoscopic structures consist of one or multiple concentric phospholipidic bilayers membranes that encase aqueous compartments in which bioactive compounds could be protected during digestion such as phytochemicals present in blueberry extract and inhibiting the formation of advance glycation end products (AGEs). Accumulation of AGEs is a causing factor for diabetic complications, aging, and several types of cancers. The objective of this work was to evaluate the viability of the antiglycating blueberry extract incorporation into liposomes. Liposomes were elaborated using hand shaken method with phosphatidilcolina and cholesterol in chlorophorm-methanol. To minimize their size, liposomes were extruded with polycarbonate filters. Liposomes characterization was done by determination of temperature and transition enthalpy through differential scanning calorimetry. Liposomes formation and stability were analysed by droplet size profile using a Coulter Counter Multisizer

particle size analyzer. The content of blueberry extract in the liposomes was determined for quantification of phenolic acid by UHPLC. The encapsulation efficiency (EE) was defined as the ratio of encapsulated blueberry extract (EE) to total blueberry extract (TE) expressed as a percentage. Antiglycation property of blueberry extract compounds was evaluated by the method described by Lunceford and Gugliucci (2005) Liposomes presented stability and retained in a 60 % of the blueberry extract. The results of BSA–methylglyoxal assay indicated blueberry extract encapsulated had significant ( $p \leq 0.05$ ) effect on inhibiting AGE formation. To conclude, liposomes were effective to the incorporation of antiglycation blueberry extract and their future incorporation in foods.

**9. Bioactivity Assessment of Novel Non-conjugated Non-methylene Interrupted Dienoic Acids Isolated from Beef Fat** Payam Vahmani\*<sup>1</sup>, William J. Meadus<sup>2</sup>, Pascale Duff<sup>1</sup>, David C. Rolland<sup>1</sup>, and Michael E.R Dugan<sup>1,1</sup>*Lacombe R&D Centre, Agriculture and Agri-Food Canada, Canada;* <sup>2</sup>*Agriculture and Agri-Food Canada, Canada*

During ruminal biohydrogenation of  $\alpha$ -linolenic acid, non-conjugated non-methylene interrupted dienoic acids are formed, namely trans(t)10,cis(c)15-18:2 and t11,c15-18:2. We have isolated these isomers from beef fat using a combination of Ag+SPE and Ag+HPLC, and sought to test their effects on lipid metabolism in 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes were treated with 35 $\mu$ M or 70 $\mu$ M of t10,c15-18:2,t11,c15-18:2, t10, c12- conjugated linoleic acid (t10,c12-CLA; positive control),c9,c12-18:2 (linoleic acid; negative control) or bovine serum albumin control (BSA control). After a five day treatment period, cells were analyzed for fatty acid composition and gene expression using gas chromatography and quantitative PCR respectively.

Cellular triacylglycerol and protein were quantified using colorimetric kits. T10,c12-CLA substantially decreased ( $P < 0.05$ ) adipocyte triacylglycerol (TAG) content which was mainly related to a reduction in 16:0, 15:0 and c9-16:1 and c9-18:1. T10,cis12 also decreased ( $P < 0.05$ ) the expression of genes related to fatty acid synthesis,  $\Delta 9$ desaturation, elongation and uptake. Other fatty acid treatments did not affect the expression of any genes tested or cell TAG and fatty acid content. Culturing cells with t10,c15-18:2, t11,c15-18:2 resulted in tentative detection of their novel chain shortening,  $\Delta 6$  desaturation and elongation products. However, no  $\Delta 9$  desaturation was found for t10,c15-18:2 or t11,c15-18:2. Despite being a diene and having a t10 double bond, t10,c15-18:2 does not exert the same anti-adipogenic effects as t10,c12-CLA. T11,c15-18:2 is likely not the substrate for c9,t11,c15-conjugated linolenic acid (c9, t11, c15-CLnA), the major CLnA found in ruminant fats.

**10. Incorporation and Stability of Vitamin A as a Functional Component in Self-assembled Supramolecular Oleogels** Cecilia B. Arnaud<sup>1</sup>, and Nuria C. Acevedo\*<sup>2,1</sup>*Oniris Nantes Atlantic National College of Veterinary Medicine, Food Science and Engineering, France;* <sup>2</sup>*Iowa State University, USA*

Blends of  $\gamma$ -oryzanol and  $\beta$ -sitosterol can self-assemble into fibrillar networks in a liquid oil phase to form organogels. These supramolecular gels can be potentially used to protect and deliver bioactive components such as vitamin A which is an essential nutrient for human physiological functions regarding epidermal cell growth and more widely for vision. Its derivative, retinyl palmitate (RP), is photosensitive thus it loses its biological activity when exposed to UV irradiation. In this study, 5% w/w sterol organogels were prepared at three different  $\gamma$ -oryzanol: $\beta$ -sitosterol ratios (1:2, 1:1 and 2:1) using three cooling rates during preparation (11 °C/min, 3 °C/min and 0.9 °C/min). Retinyl

palmitate (RP) was incorporated into the matrices in proportions of 0.05%. The results show that RP entrapped in the gel matrices was significantly protected from degradation. For all gels, RP activity remained relatively high; at least 90% of RP activity remained after 80 min UVA irradiation of the gels, while 75% RP activity persisted in liquid oil under similar conditions. Supramolecular gels prepared at low cooling rates exhibited a significantly higher protection on RP photostability. These results are indicative that RP protection may be associated to a tighter assembly achieved by a slow cooling and thus a more developed network; which correlated well with optical microscopic observations and rheological properties of the gels. From the results obtained, it can be concluded that  $\gamma$ -oryzanol/ $\beta$ -sitosterol organogels proved their efficiency as a matrix designed to stabilize RP and a potential delivery system of bioactive components subject to degradation by oxidation.

**11. Effect of Resveratrol or Red Wine on Oxidative Stress Biomarkers Associated with Atherosclerosis in LDLr-KO Mice** Livia N. Chassot, Gabriela G. Roschel, and Inar A. Castro\*, *University of Sao Paulo, Brazil*

Supplements containing isolated resveratrol are widely consumed, although there is no information about its efficiency compared with the consumption of red wine aiming cardiovascular protection. Thus, our objective was to evaluate moderate consumption of red wine compared to the isolated resveratrol using a prevention and reversion model for fatty streaks development. In PREVENTION protocol, the groups were fed a regular diet for eight weeks and then they had the fatty streaks development induced by an atherogenic diet (HIGH FAT) for another eight weeks. In the REVERSAL protocol, diets were inverted and the supplementation began at the end of the first eight weeks. Preliminary results indicated that the weight gain resulting from the

consumption of atherogenic diet of animals belonging to the PREVENTION protocol ( $40.94 \pm 0.89$ g) was higher ( $p < 0.001$ ) than the weight gain of the animals in the REVERSAL protocol ( $32.61 \pm 0.61$  g), suggesting an interaction between dietary transition and the age of the animals on weight gain modulation. In the PREVENTION protocol, animals supplemented with wine showed lower feed intake, increased water consumption, and a trend ( $p = 0.060$ ) to present lower glucose concentration than the control animals, while all other parameters were similar between the two groups. In the REVERSAL protocol, no difference was observed among animals supplemented with wine and animal control. However, both groups differed from baseline (CONT HF), due to the replacement of atherogenic by regular diet. Results from isolated resveratrol trial and biomarkers associated with atherosclerosis are still being analyzed.

**12. Microbial Lipase for Reducing Serum Triglycerides** Kelly Gregory\*<sup>1</sup>, Duc Tran Do<sup>2</sup>, Caroline Best<sup>1</sup>, Fanbin Kong<sup>2</sup>, Deborah Winetzky<sup>1</sup>, and Chris Penet<sup>1,1</sup>*BIO-CAT, USA; <sup>2</sup>University of Georgia, USA*

Hypertriglyceridemia is implicated in conditions such as pancreatitis, diabetes, atherosclerosis and the development of cardiovascular disease. Pancreatin is known to reduce triacylglycerides (TAG) to monoacylglycerides, which can be converted back to triacylglycerides via the Kennedy pathway thus they may have a limited effect in alleviating hypertriglyceridemia. Unlike pancreatin, a lipase from *Candida rugosa* hydrolyzes all three fatty acids from the glycerol backbone, and removal of all three fatty acids prevents the reformation of triacylglycerides, which may lower serum TAG levels. Studies were conducted to compare pancreatin to the *Candida rugosa* lipase for the extent of TAG hydrolysis under simulated gastric conditions followed by verification in a

dynamic gastric simulator. Soybean oil was used to evaluate oil hydrolysis, as measured by the increase in glycerol concentration, in digestion experiments using simulated gastric conditions (pH 4.0, 37°C, 2 hours). A model food matrix containing canola oil, tuna and corn starch was used to evaluate oil hydrolysis, as measured by the increase in glycerol concentration, in experiments conducted in the University of Georgia Dynamic Gastric Simulator (DGS). The *Candida rugosa* lipase released 99.5% more glycerol from soybean oil compared to pancreatin. Digestion studies in the DGS showed greater glycerol release by the *Candida rugosa* lipase and were highly correlated to earlier experiments. The *Candida rugosa* lipase was more effective than pancreatin at removing all three fatty acids from the glycerol backbone as evidenced by the high level of glycerol released in both sets of experiments, and suggests that it may be a good therapeutic candidate for further testing in lowering serum TAG.

#### 14. Antifungal Hydroxy Unsaturated Fatty Acids from Seed Oils and Fermentation as Food

**Preservatives** Nuanyi Liang\*<sup>1</sup>, Pengfei Cai<sup>2</sup>, Datong Wu<sup>2</sup>, Yuanjiang Pan<sup>2</sup>, Jonathan M. Curtis<sup>1</sup>, and Michael G. Gänzle<sup>1,1</sup>*Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Canada;*  
<sup>2</sup>*Dept. of Chemistry, Zhejiang University, China*

Spoilage or phytopathogenic fungi can affect the yields and nutritional quality of agricultural products; additionally, mycotoxins and fungal infections present significant food safety concerns. Some fungi have developed increased resistance toward common antifungal agents, increasing the amounts of fungicides required. Therefore, the development of alternative environmental-friendly antifungal agents is highly desirable. Hydroxy unsaturated fatty acids (HUFA) are antifungal compounds found in active concentrations in fermented foods such as sourdough bread. Here,

we investigated the production methods and in vitro antifungal activity of HUFA extracted from different matrices. Coriolic acid (13-hydroxy-9,11-octadecadienoic acid) was extracted from Coriaria seed oil, 10-hydroxy-12-octadecenoic acid from cultures of *Lactobacillus hammesii* and 13-hydroxy-9-octadecenoic acid from cultures of *Lactobacillus plantarum* TMW1.460Δlah, respectively. HUFA were purified by high-speed counter-current chromatography (HSCCC) and the fractions were characterized by LC/MS. Their antifungal activities were tested against filamentous fungi representing pathogenic and spoilage organisms, and yeasts that are relevant in food fermentations or spoilage. HUFA had a unique antifungal spectrum compared to other unsaturated fatty acids, as HUFA specifically inhibited filamentous fungi, including *Aspergillus niger*, *Penicillium roqueforti*, *Aspergillus brasiliensis* and *Aspergillus clavatus* with minimum inhibitory concentrations (g/L) of 0.29±0.07, 0.33±0.14, 0.50±0.25 and 0.25±0.00 respectively, while all yeasts were resistant to HUFA. Based on their inhibitory spectrum, HUFA were not effective against yeasts involved in food spoilage, however, they can be applied as antifungal agents in fermented foods that require growth and activity of yeast. Future experiments will be designed to explore the potential use of HUFA as food-grade antimold agents.

#### 17. Greening-induced Oxidation of Sunflower Butter Cookies as a Function of Temperature and Vegan Egg Replacers

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Sunflower seed butter is an alternative plant-based butter to the commonly allergenic peanut and tree-nut butters on the market. Sunflower butter can be an additional source of antioxidants, however, 70% of sunflower butter's phenolic compounds are chlorogenic acid (CGA). This CGA covalently binds to sunflower proteins in moist,

alkaline conditions, causing protein denaturation and green pigmentation to occur, limiting the use of sunflower butters, and the protein co-product from sunflower oil processing. To determine the role of moisture, sunflower butter cookie formulation was varied by baking temperatures (325°F and 375°F) and use of egg replacers (chia seeds, flax seeds, and banana). Chia cookies baked at 325°F had the highest moisture (16.7%), an  $a^*$  value of -10.5 after 1.5h, and a CGA content of 25.0  $\mu\text{g/g}$  as determined by HPLC. Banana cookies baked at 375°F had the least moisture (12.8%), an  $a^*$  value of -2.9 after 1.5h, and a CGA content of 35.4  $\mu\text{g/g}$  of cookie. Chia cookies baked at 325°F were 23.8% more moist, 3.6 times more green, and had 29.2% less CGA than banana cookies baked at 375°F. Tryptophan fluorescence measured at  $\lambda_{\text{EX}}$  280nm,  $\lambda_{\text{EM}}$  recorded 300-500nm with  $\lambda_{\text{max}}$  of 356nm for tryptophan residues was normalized to the treatment with the highest fluorescence emission. Banana cookies had the highest tryptophan fluorescence intensity, indicating the least protein oxidation. When moisture is decreased, sunflower cookies undergo less greening, indicating higher free antioxidant CGA and protein content. Ultimately, temperature and cookie dough formulation can be modified to improve sensory and nutritional quality.

#### 18. Main Circulating Endocannabinoids and Their Precursor Long Chain Fatty Acids during Pregnancy

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Endocannabinoids, lipid mediators derived from fatty acids, regulate pregnancy and are related to successful pregnancy outcome. The aim of this exploratory study was to assess how members of the circulating endocannabinoid metabolome and their precursor fatty acids change throughout pregnancy and at delivery (cord vein, representing infant status). Analyses focused on endocannabinoids AEA (anandamide) and DHEA (docosahexaenoyl ethanolamide) and their precursor fatty acids AA (arachidonic acid, 20:4n6) and DHA (docosahexaenoic acid, 22:6n3), respectively, as these fatty acids are preferentially transferred across the placenta. We assessed AEA, DHEA, AA, and DHA in overweight (BMI 25.0-29.9  $\text{kg/m}^2$ ) pregnant women (n=14) at 20, 24, 30, 36 gestational weeks (GW) and at delivery by liquid chromatography-mass spectrometry. Concentrations of AEA were correlated with those of AA at delivery ( $p \leq 0.05$ ). DHA concentrations were correlated with those of DHEA at all time points ( $p \leq 0.05$ ). Concentrations of AEA, DHEA, AA, and DHA were higher ( $p \leq 0.05$ ) at delivery than during pregnancy (20-36 GW). DHEA concentration was higher than the concentration of AEA during pregnancy and at delivery ( $p \leq 0.05$ ), but there were no differences between the concentrations of precursor fatty acids, AA and DHA ( $p > 0.05$ ). The findings of this exploratory study lead to questions regarding synthesis rates, degradation, and receptor-binding of the endocannabinoids, DHEA and AEA, during pregnancy and how these are utilized by the developing infant. The impact of endocannabinoids for infant development remains to be further explored. Funded in part by USDA grant 2012-67017-19293 and the LSU AgCenter.