

# 2011 Annual Meeting Abstracts

## Health and Nutrition

### MONDAY

#### MORNING

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### **H&N 1: Mark Bieber Memorial Symposium: Childhood Obesity - Understanding and Implications of a National Epidemic**

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Chair(s): E. Bailey-Hall, Martek Biosciences Corp., USA; and M. Craig-Schmidt, Auburn University, USA

#### **The Epidemiology of Childhood Obesity in the US.** C.L. Ogden, National Center for Health Statistics, CDC, USA

The prevalence of childhood obesity in the US is high, currently almost 17% of children and adolescents ages 2-19 years are considered obese. Following a tripling in the prevalence of childhood obesity since 1980, the rapid increase in prevalence has slowed and possibly leveled off during the last 10 years. Childhood obesity often tracks to adulthood and, in the short run, childhood obesity can lead to psychosocial problems and cardiovascular risk factors such as high blood pressure, high cholesterol and abnormal glucose tolerance or diabetes. This session will describe the epidemiology of childhood obesity in the US.

#### **Genetic and Environmental Factors contributing to Childhood Obesity in the Hispanic Population.** Nancy F. Butte, USDA, ARS, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA

Obesity is a complex disease influenced by multiple genetic and environmental factors. Objective: To genetically map childhood obesity and its comorbidities in the Hispanic population. Methods: VIVA LA FAMILIA cohort consisted of 1030 children from 319 families. In-depth phenotyping to characterize the obese children and their siblings included anthropometry, body composition, diet, physical activity, energy expenditure and metabolic risk factors. A genome-wide scan was performed, followed by gene resequencing using 3730XL DNA Sequencers and SNP genotyping using HumanOmni1M-Quad BeadChips. Heritabilities, genetic correlations, chromosomal linkage and measured genotype analysis (MGA) were performed using SOLAR. Results: Quantitative genetic analyses provided strong evidence for both genetic and environmental contributions to obesity and its comorbidities. Heritabilities ( $h^2$ ) ranged from 0.24 to 0.75 for weight and body composition, and from 0.30 to 0.60 for diet and physical activity. Risk factors for metabolic diseases were also heritable ( $h^2=0.25$  to 0.73). Linkage, resequencing and genome-wide association have identified several chromosomal regions that contain SNPs associated with obesity traits. Fine mapping has focused on three significant quantitative trait loci (QTLs) on chromosomes 1p36, 13q and 18q22. Conclusion: The high prevalence of childhood obesity among Hispanic children may be attributed to genetic susceptibility in a highly permissive obesogenic environment.

**How Obesity Went to Our Heads: CNS Regulation of Food Intake and Body Weight.** R.J. Seeley, Cincinnati Diabetes and Obesity Center Metabolic Diseases Institute, Cincinnati, OH, USA

**Neighborhood Greenness and Child Obesity.** G.C. Liu, Indiana University School of Medicine, Indianapolis, IN, USA

Studies of environmental factors and children's body mass index (BMI) suggest that neighborhood greenness and population density are significant correlates of obesity. We tested whether greenness and residential density are independently associated with two-year changes in children's BMI and whether the associations are modified by age, sex, race/ethnicity and socioeconomic status. The sample included children ages 3 - 16 who lived at the same address for 24 consecutive months and received longitudinal health maintenance from an urban clinic network, 1996 - 2002 (n=3,842). Multiple regression was used to model BMI z-scores in year 2, controlling for greenness, residential density and other covariates including socioeconomic status and weight status at baseline. Higher neighborhood greenness was associated with BMI z-scores that were significantly lower at Time 2. Higher density was not associated with changes in children's z- BMI. Children living in neighborhoods with higher greenness had lower odds of increasing their BMI z-scores versus decreasing or maintaining them over time (OR= .87; 95% CI: 0.79, 0.97). Neighborhood greenness may indicate surroundings that promote children's physical activity with lower BMI z-scores over time. Current policy supporting neighborhood design to improve walkability for adults may be less relevant to children in urban environments.

**The 2010 Dietary Guidelines: Evidence-Based Recommendations for an Obesogenic Environment.** R.C. Post, USDA, CNPP, Alexandria, VA, USA

USDA's development and implementation of Federal nutrition guidance-including the Dietary Guidelines for Americans and the MyPyramid Food Guidance System-are critical in helping to stem and eventually reverse the disturbing trends for obesity and diet-related chronic diseases. It is important for children and teens, especially, to adopt healthier eating behaviors that are balanced with physical activity for overall health and fitness that will last their lifetime. The focal point of Federal efforts to stem the trends of obesity and chronic disease is the Dietary Guidelines. The 2010 Dietary Guidelines for Americans policy - an evidence-based, scientific blueprint for promoting good nutrition and health - was released on January 31, 2011. The Guidelines are the basis for Federal policy in government nutrition programs, and serve as the basis for dietary guidance and messages in nutrition education. The Guidelines provide advice for Americans, ages two years and older, and for individuals at risk of chronic disease, about building healthy eating patterns and making food choices that promote health and prevent disease, set standards for the nutrition assistance programs, guide nutrition research and education, and are the basis for USDA nutrition promotion activities. To promote the messages of the Guidelines, the Center for Nutrition Policy and Promotion uses on-line interactive tools, as well as a variety of print materials, to reach the general public and targeted audiences. To effectively implement the 2010 Dietary Guidelines, a socio-ecological framework must be

employed where all sectors of the American society have a role and responsibilities.

**The Food Industry and Its Response to Public Health Issues.** R. Black, Kraft Foods, USA

**Eliminating Childhood Obesity One Step at Time.** Rachel Lindstrom, America On the Move Foundation, Center for Human Nutrition, University of Colorado, USA

According to the Centers for Disease Control and Prevention, 16% of children in the United States, aged 2-17, are considered obese using a cut point of >95th% body mass index-for-age (BMI-for-age) while more than one-third of children, aged 8-19, are considered overweight or obese using a cut point of >85th% BMI-for-age. Interestingly, there is evidence to indicate that children only gain an excess of about 1 pound per year over a ten year period. The mismatch between energy requirements for growth and positive energy balance resulting in excess weight gain (energy gap) has been calculated to be between 100-200 kilocalories (kcal) per day for growing children. Therefore, a small changes approach, in which children increase caloric expenditure by 100 kcal/day and decrease energy intake by 100 kcal/day, would address the energy gap. Putting this small change approach to work in children, America On the Move, an evidence-based nonprofit, developed a family program designed to help families increase daily activity and make healthier eating choices. The family program has been shown to significantly increase daily step activity and positively, significantly impact BMI-for-age. A small changes approach may be a healthy, sustainable way to prevent and reverse childhood overweight and obesity.

**Elevated Palmitoleic Acid Levels Improve Insulin Sensitivity.** Sarojini J.K.A. Ubhayasekera<sup>1</sup>, Johan Staaf<sup>2</sup>, Jonas Bergquist<sup>1</sup>, Anders Forslund<sup>3</sup>, Peter Bergsten<sup>2</sup>, <sup>1</sup>Department of Physical and Analytical Chemistry, Uppsala University, Box 599, 751 24, Sweden, <sup>2</sup>Department of Medical Cell Biology, Uppsala University, Box 571, 751 23, Sweden, <sup>3</sup>Department of Women's and Children's Health, Uppsala University, Box 256, 751 85, Sweden

Childhood obesity is a major cause of surging numbers of young individuals developing type 2 diabetes. Despite similar degree of obesity, variations in how rapidly obesity-related complications develop are observed. The fatty acid palmitoleate has documented 'cytoprotective' effects on the insulin-producing  $\beta$ -cell. We hypothesized that elevated circulating palmitoleate levels in the young obese individuals correlated with improved  $\beta$ -cell function as manifested in lowering of fasting insulin levels. To test the hypothesis fasting insulin and palmitoleate levels were measured in 100 young obese (ISO-BMI >35) subjects. Levels of the free fatty acid were determined by converting the acid into its amide derivative. The derivatization method is only effective towards free fatty acids. Derivative was analyzed by gas chromatography - mass spectrometry (DB5-MS 30m x 0.18mm, 0.18 $\mu$ m). When basal insulin levels were plotted against palmitoleate levels, significantly lower basal insulin levels were observed in individuals with higher palmitoleate levels. We conclude that palmitoleate is a marker for fasting hyperinsulinemia. However, further studies needs to be done to show if it also could prevent the development of insulin resistance and type 2 diabetes.

## AFTERNOON

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### H&N 2: Lipids and Inflammation

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Chair(s): P.J. Huth, PJH Nutritional Sciences, USA; and M.-C. Michalski, INRA, France

#### **JNK Activation, A Matter of Fat.** Anja Jaeschke, University of Cincinnati, Cincinnati, OH, USA

Obesity is associated with chronic inflammation, characterized by macrophage infiltration of adipose tissue and elevated circulating inflammatory cytokines leading to activation of stress-activated pathways, including the cJun-NH<sub>2</sub>-terminal kinase (JNK) pathway. Activation of JNK causes development of insulin resistance, directly, through IRS phosphorylation and indirectly, through regulation of expression of inflammatory cytokines. Obesity is also linked with elevated levels of circulating free fatty acids (FFA) that can accumulate in non-adipose tissues leading to cellular injury and possibly organ failure. In liver FFA can activate the intrinsic apoptotic pathway through JNK activation which promotes progression from simple steatosis to NASH and advanced hepatic fibrosis. Interestingly, recently it was demonstrated that the ratio of unsaturated to saturated FFA, determines the amount of apoptosis, indicating that the quality, rather than the quantity of FFA determines hepatic stress and consequently JNK activation and cell death. These findings suggest an important role for the JNK signaling pathway in metabolic diseases, such as insulin resistance and NAFLD.

#### **The Role of Dietary Fatty Acids in Inflammation.** P.M. Kris-Etherton, M. Flock, Dept. of Nutritional Sciences, Penn State University, University Park, PA, USA

Inflammation is an important underlying cause for many chronic diseases, including cardiovascular disease (CVD). It is becoming increasingly clear that dietary fatty acids modulate inflammation and inflammatory responses, both positively and negatively. However, there are many unresolved questions, especially for saturated fatty acids (SFA). The limited epidemiologic and controlled clinical trial data are inconsistent, with some studies demonstrating adverse results whereas others have reported no associations/effects. A problem with the epidemiologic data is that some studies have not been multivariable-adjusted. A well controlled clinical study showed that lauric, myristic and palmitic acids (63 g/day of these fatty acids) had no effect on fibrinogen, CRP, IL-6 or e-selectin, whereas a very high stearic acid diet (38 g/day) increased fibrinogen compared with the high carbohydrate control diet. Postprandial studies, in contrast, tend to show that SFA are pro-inflammatory, although the data are somewhat variable. However, unsaturated fatty acids, including monounsaturated fatty acids but especially polyunsaturated fatty acids (PUFA - both omega-6 and omega-3 classes) have been shown, for the most part, to have anti-inflammatory effects, especially omega-3 PUFA. Clinical studies of high intakes of omega-6 PUFA show unaltered or lower levels of inflammatory markers. The inflammatory and anti-inflammatory effects observed to date are primarily manifested as changes in concentrations (and gene expression) of CRP, TNF- $\alpha$ , and IL-6 among others.

#### **Anti-inflammatory Effects of the Omega-3 Fatty Acids.** J. Wesley Alexander, University of

Cincinnati, USA

**Dietary Carbohydrate Restriction: Impact on Insulin Resistance, Fatty Acid Composition and Inflammation.** J. Volek, University of Connecticut, Storrs, CT, USA

Saturated fats are often implicated in the worsening of insulin resistance, but the effect is contingent upon the presence of ample carbohydrate. Well formulated low carbohydrate diets in the presence of high saturated fat leads to improvement in insulin sensitivity despite increased lipolytic rates and release of fatty acids into the circulation. In two recent experiments in our laboratory, we have made the striking observation that dietary carbohydrate exerts a significant regulatory role on plasma saturated fat levels. Under weight stable or weight loss conditions, when carbohydrate intake is low we showed that increasing saturated fat intake results in significantly reduced plasma saturated fat. The other remarkable finding in our previous work has been a significant decrease in inflammatory markers despite a marked increase in dietary saturated fat and plasma phospholipid arachidonic acid (ARA). Increasing ARA in membranes, however, does not inevitably lead to greater inflammation and several lines of evidence suggest its presence in membranes is associated with increased insulin sensitivity and decreased inflammation. These findings are consistent with the concept that dietary saturated fat is efficiently metabolized in the presence of low carbohydrate, and that a low carbohydrate diet results in better preservation of plasma ARA and decreased inflammation.

**Conjugated Linoleic Acid's Anti-inflammatory Action in an Animal Model of Rheumatoid Arthritis.** Mark E. Cook<sup>1,2</sup>, Shane M. Huebner<sup>2</sup>, <sup>1</sup>University of Wisconsin, Department of Animal Sciences, Madison, WI 53706, USA, <sup>2</sup>University of Wisconsin, Department of Nutritional Sciences, Madison, WI 53706, USA

Dietary conjugated linoleic acid (CLA) has been shown to have anti-inflammatory properties in animal models of endotoxin-induced cachexia, airway hypersensitivity, inflammatory bowel disease, and lupus. Human trials investigating the effects of supplemental CLA on airway type I hypersensitivity (asthma and allergy) have confirmed findings originally reported in animal models. We now report that dietary CLA has significant anti-inflammatory effects in mice with collagen-induced arthritis when measured as arthritic score, joint swelling, and histopathological changes. Our findings show that both the c9t11- and t10c12-CLA isomers exhibited anti-inflammatory action and the ability to regulate key inflammatory cytokines; however t10c12-CLA has greater anti-inflammatory effects than c9t11-CLA. The anti-inflammatory effects of both dietary CLA isomers on arthritis are likely mediated through their ability to suppress tumor necrosis factor  $\alpha$  and interleukin- $1\beta$  and not through changes in anti-collagen antibody production or class switching. Plasma hypoaminoacidemia, a hallmark of chronic inflammation, was restored toward normalcy (plasma amino acid levels found in non-arthritic controls) in arthritic mice fed either CLA isomer. Our findings in the collagen-induced arthritic model have been supported by a recent human trial and provide strong evidence of the importance of dietary CLA in the control of inflammatory disease.

**Inflammation Induced by Excessive Fat Intake: Role of Endogenous Endotoxin Absorption**

**and Metabolism.** M.C. Michalski<sup>1,5</sup>, F. Laugerette<sup>2,3</sup>, B. Benoit<sup>4,1</sup>, M. Alligier<sup>4,5</sup>, A. Geloën<sup>3</sup>, C. Soulage<sup>2</sup>, S. Lambert-Porcheron<sup>5</sup>, R. Burcelin<sup>6</sup>, M. Laville<sup>4,5</sup>, H. Vidal<sup>3,5</sup>, <sup>1</sup>INRA UMR1235, Villeurbanne, France, <sup>2</sup>INSA-Lyon, Villeurbanne, France, <sup>3</sup>INSERM U870, Oullins, France, <sup>4</sup>Université de Lyon, Lyon, France, <sup>5</sup>CRNH Rhône-Alpes, Oullins, France, <sup>6</sup>I2MR, Toulouse, France

Low-grade inflammation is now recognized as a major metabolic feature in obesity that leads to increased risks of insulin resistance and cardiovascular disease. Among possible causes triggering such inflammation, recent literature and our data revealed the role of gut endotoxin absorption during the digestion of dietary lipids. In our recent studies, healthy humans were subjected to a dietary intervention consisting of increased daily fat intake. Moreover, animal studies were conducted where mice were subjected to fat-rich diets of various compositions. Altogether, our results show that excessive fat intake leads to low-grade inflammation by mechanisms involving endotoxin metabolism.

## TUESDAY

### AFTERNOON

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#### BIO 3 / H&N 3.1: Functional Lipids - Bioactive Properties

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Chair(s): R. Moreau, USDA, ARS, ERRC, USA; and R.J. Ostlund, Washington University in St. Louis, USA

#### **An Overview Of Functional Lipids.** R.A. Moreau, ERRC, ARS, USDA, Wyndmoor, PA, USA

The main functions of lipids in plants, animals, and microbes are: a) to serve as a source of energy and carbon skeletons and b) to serve as structural components in biological membranes. Although there is no authoritative definition of functional lipids, one can informally define them as a subset of functional foods, which are considered to be similar in appearance to conventional foods consumed as part of a usual diet, but they have been demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions. This symposium includes presentations from experts on seven functional lipids: xanthophylls, medium chain triglycerides, phytosterols, structured lipids, diacylglycerol oils, EPA- and DHA-rich oils, and tocotrienols. Each talk will include descriptions of the occurrence and chemical properties of each functional lipid and historical and recent physiological and clinical studies that provide evidence of the unique health-promoting properties of each of these seven functional lipids.

**Medium Chain Triglycerides.** P.J.H. Jones, Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB, Canada

Medium-chain triglycerides (MCTs) are dietary components that have been extensively examined for their role as weight control agents. MCTs, possessing fatty acids of 8-10 carbons in length, possess less energy per gram and are highly ketogenic compared to long chain triglycerides (LCTs). More importantly, however, MCTs compared to LCTs undergo distinct

digestive and metabolic processes that may result in enhanced catabolism. Specifically, MCTs are rapidly oxidized and can be swiftly converted to energy, with less tendency to undergo deposition as body fat. Studies in animals and humans demonstrate that consumption of MCTs in low to moderate quantities may have substantial enhancing actions on thermic effect of food (TEF). For instance, sizeable differences in 24-h EE (energy expenditure) after consumption of 30 g of MCTs versus 30 g of LCTs have been shown; with these increases in EE theoretically resulting in about 0.5 kg of fat loss over a month if effects were to persist over that period. However, even smaller doses of MCTs have been shown to cause larger diet-induced thermogenesis than LCTs. MCTs appear to increase EE as short chain FA are transported from gut directly to liver where they are catabolized immediately for energy which would be beneficial for weight loss. MCTs may also reduce body weight by enhancing satiety. These nutritional properties of MCTs are believed to upregulate energy expenditure, suppressing intake, thereby increasing weight loss, leading to reduced body fat accumulation. In conclusion, consumption of MCT-containing foods may contribute to control of body weight and should be recommended as a part of weight loss strategies.

**Phytosterols.** R.J. Ostlund, Washington University in St. Louis, USA

**Enzymatic Production of Betapol™ and Other Structured Lipids.** C.C. Akoh, University of Georgia, Athens, GA, USA

Betapol™ (Loders Croklaan) was developed to mimic the structure and composition of human milk fat (HMF) for use in infant formula formulations. It was produced by enzymatic modification of vegetable oils by sn-1, 3-specific lipase. Betapol contains high levels of palmitic acid at the sn-2 position and oleic or unsaturated fatty acids at the sn-1, 3-positions. The aim was to improve the absorption of calcium, fat, and to soften infant stools after breast feeding. Various studies have reported the syntheses and fatty acid compositions of Betapol and similar HMF analogs. Infant formula fat analogs can be synthesized to include unsaturated eicosapentaenoic (EPA, 20:5 n-3), docosahexaenoic (DHA, 22:6 n-3),  $\gamma$ -linolenic (GLA, 18:3 n-6), and stearidonic (SDA, 18:4 n-3) acids for their bioactive or physiological functions. Vegetable oils and palmitic acid-containing fats are also used as part of the substrates. These structured fats are referred to as structured lipids (SLs). Amaranth, hazelnut, canola, and modified soybean oils have been used to prepare infant milk fat analogs. SLs lipids can also be prepared to replace trans fats in margarine, shortenings, and spreads formulations for their functionality in foods. Palm and palm kernel oils as well as stearic acid-containing fats are often part of the substrates.

**Nutritional Characteristics of Diacylglycerol Oil.** T. Yanagita, Saga University, Saga, Japan

Dietary lipids have been recognized as contributory factors in the development and the prevention of metabolic syndromes and cardiovascular risk clustering. Therefore, it is important to know what kinds of lipids are adequate for our health. Diacylglycerol (DAG) occur naturally as a minor component, present up to about 10% of various vegetable oils. Dietary DAG, particularly 1,3-DAG, has been reported to have metabolic characteristics distinct from dietary triacylglycerol (TAG). Studies in animals and human demonstrated biological effects of dietary

DAG such as lowered postprandial blood triglyceride level, body weight/fat reductions, and increased fat metabolism. One of the explanations was generated by animal studies showing that lymphatic transport of chylomicron after 1,3-DAG ingestion was significantly delayed and reduced than TAG ingestion, presumably as a result of poor re-esterification of FA onto either 1-monoacylglycerol or glycerol, major digestive products from 1,3-DAG. In addition, repeated ingestion of DAG increased activities of beta-oxidation of liver and small intestine, suggesting the mechanism responsible for the body weight/fat reduction on a DAG diet. Having these clinical evidence and mechanism of actions, DAG may be promising aid for preventing the diseases associated with the obesity and metabolic syndrome when used in place of vegetable oil in regular diet.

**EPA and DHA-rich Oils.** N. Salem, A. Ryan, Martek Biosciences, USA

Algal oil-derived DHA has now been fed to tens of millions of babies in infant formula in the US and throughout the world the source of which is the dinoflagellate *C. Cohnii*. Food and supplement oil derived from the Thraustochytrid *Schizochytrium* sp. are also widely used and contain both DHA and DPA n-6 fatty acids. More recently, a different strain of *Schizochytrium* sp. has been developed that is enriched in both DHA and EPA and may be considered a "vegetarian fish oil". A quick search of the literature indicates over 16000 research reports about EPA or DHA. It is well established now that DHA is critical for normal brain and retinal functions and deficiency leads to a loss of a variety of functions. On the other had, supplementation of the diet with EPA and DHA leads to increased bloodstream and tissue levels of these lipids and provides a health benefit in a growing number of organ systems and diseases. This may be largely the result of the modern diet departing from a higher n-3 and lower n-6 traditional diet in many cultures. Several important mechanisms have been found for DHA involving the modulation of G-protein coupled receptor signaling, decreased apoptosis, modulation of protein expression and synthesis of bioactive molecules similar to anandamides or resolvins.

**$\alpha$ -Tocotrienol: The Natural Vitamin E Against Stroke.** Chandan K. Sen, Ohio State University, OH, USA

Following failed clinical trials testing  $\alpha$ -tocopherol (TCP), interest in other naturally occurring forms of vitamin E is sharply rising. Meta-analyses of clinical trials testing the efficacy of vitamin E in human health suffer from a blind spot because they fail to recognize that  $\alpha$ -TCP, the only form of vitamin E tested in such trials, represent one-eighth of the natural vitamin E family. Our laboratory has demonstrated that neuroprotection by  $\alpha$ -tocotrienol (TCT) at nanomolar concentration represents the most potent functional property of the entire vitamin E family. Neuroprotective as well as hypocholesterolemic properties of  $\alpha$ -TCT make it a good candidate for nutrition-based intervention in people at high risk for stroke. Transient ischemic attack (TIA), or ministroke, serves as a sentinel warning sign for high-risk stroke patients. Prophylactic stroke therapy therefore provides an opportunity for intervention in TIA patients prior to a major stroke event. The current state of evidence warrant clinical assessment of  $\alpha$ -TCT in TIA patients. Furthermore,  $\alpha$ -TCT is a nutrient that is GRAS certified by the US FDA and is not a drug with potential side effects. Thus,  $\alpha$ -TCT may be considered as a preventive nutritional countermeasure for people at high risk for stroke. Supported by NIH NS42617.

**Lutein and Zeaxanthin: Dietary Sources, Bioavailability and Bioactivity.** M.G. Ferruzzi, Purdue University, West Lafayette, IN, USA

Carotenoids, a family of hydrophobic pigments abundant in fruits, vegetables and grains, have drawn significant attention in recent years due to their association with several health benefits. While the provitamin A activity of carotenoids including  $\beta$ -carotene is well documented, non-provitamin A carotenoids including lutein and zeaxanthin have been increasingly studied for their health promoting activities including: antioxidant activity, cardiovascular health, eye health, skin health and neuroprotection. With the potential for a significant role in health promotion, interest in content, bioavailability and biological activities of these pigments from foods has intensified. This lecture will focus on dietary sources of lutein and zeaxanthin and will discuss factors impacting bioavailability of these pigments. Additionally, the impact of food formulation and processing on stability, absorption and metabolism of these bioactive pigments will be described. Finally, tissue distribution and specific bioactivities will be discussed in the context of proposed mechanism of disease prevention. A better understanding of natural sources of lutein and zeaxanthin, as well as factors affecting their bioavailability and activity is critical to development and assessment of products for specific health benefits.

### **H&N 3: Lipid Modulators and Messengers**

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Chair(s): H. Durham, Louisiana State University, USA; and E. Berdyshev, University of Illinois at Chicago, USA

**Peroxisome Proliferator-activated Alpha Nuclear Receptors (PPAR-alpha) Provide a New Molecular Target for Treatment of Cognitive Impairment and Nicotine Dependence.** S.R. Goldberg<sup>1</sup>, S. Yasar<sup>2</sup>, <sup>1</sup>NIDA, IRP, NIH, Baltimore, MD, USA, <sup>2</sup>Johns Hopkins University School of Medicine, Baltimore, MD, USA

Peroxisome proliferator-activated alpha nuclear receptors (PPAR-alpha) regulate genes involved in lipid metabolism and inflammatory responses. The endogenous ligand for PPAR-alpha, oleoylethanolamide (OEA) is degraded by fatty acid amide hydrolase (FAAH). Drugs that inhibit FAAH increase endogenous levels of both the endocannabinoid anandamide and OEA. We and others recently found that directly acting synthetic PPAR-alpha agonists, as well as systemically administered OEA and the FAAH inhibitor URB597, which increases brain OEA levels, enhance memory acquisition in rats and mice and this enhancement is reversed by a PPAR-alpha antagonist. In other experiments with rats and monkeys, we found that PPAR-alpha agonists selectively reduce nicotine-reinforced drug-taking behavior and prevent relapse to drug-seeking. Finally, in in-vivo electrophysiological experiments with rats, PPAR-alpha agonists reversed nicotine-induced excitation of ventral tegmental area dopamine neurons and dopamine release in the nucleus accumbens shell. Thus, PPAR-alpha is a new molecular target for treating tobacco dependence and cognitive impairment. Supported in part by the Intramural Research Program of NIDA, NIH, DHHS.

**Endocannabinoids and Cannabinoid Receptors: The Chicken and Egg Caveats in Nomenclature.** A.C. Howlett, Wake Forest University Health Sciences, Winston-Salem, NC,

USA

The Cannabinoid Receptor subcommittee of the IUPHAR Nomenclature Committee has provided a definition of cannabinoid receptors to mean those G protein coupled receptors that are regulated by cannabinoid agonists. The CB1 receptors in the brain, and CB2 receptors in the immune system, clearly interact with phytocannabinoids, as well as a host of synthetic ligands having the classical and non-classical cannabinoid structures, as well as other non-related hydrophobic compounds. The endogenous ligands for these receptors are eicosanoid compounds arachidonyl-ethanolamide and 2-arachidoylglycerol. With the discovery of a variety of related eicosanoid amides, esters and ethers, it is now a conundrum regarding whether these compounds should be referred to as "endocannabinoid" ligands. This begs the question of whether receptors for these ligands should be called "cannabinoid" receptors. This talk will provide an overview of these lipid mediators and their receptors and targets in the cell.

**Endocannabinoid Signaling: A Promising Strategy for Pain Modulation.** V. Seybold, University of Minnesota, Minneapolis, MN, USA

People in pain have exploited the palliative properties of marijuana for centuries. Discovery of endogenous lipids that bind to cannabinoid receptors and the enzymes that regulate their levels offer new opportunities for the management of pain. Levels of anandamide in the periphery contribute to defining the threshold for sensitivity of somatosensory neurons to noxious stimuli. In a murine model of cancer pain, hyperalgesia is associated with a decrease in the level of the endocannabinoid anandamide in area of tumor development, and treatments that raise local levels of endocannabinoids promote analgesia. Insights into the mechanisms underlying these observations come from studies of the interaction between tumor cells and sensory neurons. Chemical mediators released from cancer cells increase the expression of fatty acid amide hydrolase, the enzyme that degrades anandamide, in sensory neurons as well as the CB1 receptor that underlies the inhibitory effect of anandamide on sensory neurons. Therefore, manipulation of endocannabinoid metabolism in the periphery may be an effective strategy in managing cancer pain.

**Plasma Endocannabinoids and Inflammatory Markers during Pregnancy: Is There a Connection?** H.A. Durham<sup>1</sup>, J.T. Wood<sup>2</sup>, N Lam<sup>1</sup>, A Tipler<sup>1</sup>, A Makriyannis<sup>2</sup>, C.J. Lammi-Keefe<sup>1</sup>, <sup>1</sup>AgCenter, Louisiana State University, Baton Rouge, LA, USA, <sup>2</sup>Center for Drug Discovery, Northeastern University, Boston, MA, USA

Pregnancy accompanied by insulin resistance leads to an inflammatory state which is exacerbated by adiposity. Endocannabinoids (ENDOs), endogenous lipid messengers, play a pivotal role in inflammation and pregnancy outcome. Our objective was to evaluate the relationship between inflammatory markers (IMs) and plasma ENDOs in normal and overweight pregnant women (N=17) at 20-22, 23-26, 32 and 38-40 weeks. We quantified plasma ENDOs (anandamide, palmitoylethanolamine, oleoylethanolamine (OEA), docosahexaenoyl, ethanolamine, 2-arachidonolglycerol (2-AG), 2-palmitoylglycerol, 2-oleoylglycerol) using liquid chromatography-mass spectrometry. IMs, interleukin-6 (IL-6) and leptin were measured using Enzyme-Linked ImmunoSorbent Assay. Canonical correlations were used to evaluate relationships between IMs and ENDOs after adjusting for weeks of pregnancy. The results

showed during the second and third trimesters of pregnancy, IL-6 was positively related to OEA and inversely related to 2-AG ( $p= 0.0206$ ). Leptin was not related to the measured ENDOS in the significant canonical variate. These preliminary findings suggest a relationship exists between IL-6, OEA and 2-AG, which may have implications for maintaining a successful pregnancy.

**Sphingolipid Signaling System in Deciding Survival: Rescue of Sphingosine Kinase-1-Knockout Phenotype in Cardiac Arrest through the Inhibition of Sphingosine-1-phosphate Lyase.** Evgeny Berdyshev, University of Illinois at Chicago, Chicago, IL 60612, USA

Sphingosine-1-phosphate (S1P) is an important regulator of pro-survival signaling. Two sphingosine kinases (SK), SK1 and SK2, form S1P in cells while S1P lyase irreversibly degrades S1P and controls the total pool of cellular sphingolipids. Deletion of the SK1 gene is detrimental in both in vitro and in vivo models of ischemia/reperfusion induced injury. Cardiac arrest and resuscitation are associated with the most severe cases of ischemia/reperfusion induced stress and injury. Using our mouse model of cardiac arrest and return of spontaneous circulation (CA/ROSC), we demonstrate that SK1 knockout (SK1-KO) mice have decreased survival after CA/ROSC, and that the upregulation of S1P levels via pharmacological S1P lyase inhibition rescues the effect of SK1 gene deletion in this model. Inhibition of S1P lyase in SK1-KO mice is associated with profound changes in tissue and circulatory levels of signaling sphingolipids and results in the upregulation of the expression of Bcl-2 protein. Thus, targeting S1P lyase represents a novel strategy leading to the improved survival through the upregulation of S1P- and Bcl-2-mediated signaling.

**Short-term and Long-Term Consequences of Inhibiting Endocannabinoid Catabolic Enzymes to Reduce Pain.** A. Lichtman, Virginia Commonwealth University, Richmond, VA, USA

The endocannabinoid system consists of two receptors (i.e., CB<sub>1</sub> and CB<sub>2</sub>), endocannabinoids (i.e., anandamide and 2-AG), and enzymes regulating endocannabinoid biosynthesis and catabolism. While CB<sub>1</sub> modulates short-term synaptic plasticity and is primarily responsible for the CNS effects of marijuana, CB<sub>2</sub> is associated with the immune system. Inhibition of fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL) leads to increased levels of anandamide and 2-AG, respectively. Inhibition of either enzyme reduces nociceptive responses in a variety of pain models. Chronic treatment with selective inhibitors of FAAH and MAGL reveals dramatically distinct consequences on CB<sub>1</sub> receptor function. Whereas the antinociceptive effects of FAAH inhibitors are sustained following chronic administration, repeated injections of a high dose of a MAGL inhibitor leads to tolerance in acute and chronic pain models and cross-tolerance to cannabinoid agonists. These effects are accompanied with CB<sub>1</sub> receptor down-regulation and desensitization, as well as a loss of short-term synaptic plasticity. In conclusion, inhibition of either FAAH or MAGL produces similar efficacy in reducing pain, but chronic inhibition of MAGL results in tolerance and disturbances in brain CB<sub>1</sub> receptor function.

**Opposing Effects of Cannabinoid-1 and 2-receptors on Inflammation and Oxidative Stress: Implications for Tissue Injury.** Pal Pacher, National Institutes of Health, NIAAA, Bethesda, MD, USA

Accumulating recent evidence suggests that cannabinoid 1 (CB1) receptor activation by endocannabinoids in endothelial cells, cardiomyocytes and inflammatory cells may amplify the reactive oxygen species-MAPK activation-cell death pathway in pathological conditions when the endocannabinoid synthetic or metabolic pathways are dysregulated by excessive inflammation and/or oxidative/nitrosative stress, thereby contributing to the development of pathophysiology of multiple cardiovascular and other diseases. In contrast, emerging studies have provided compelling evidence that CB2 receptor activation is protective against tissue injury by decreasing the endothelial cell activation/inflammatory response (for example, expression of adhesion molecules, secretion of chemokines, and so on), and by attenuating the leukocyte chemotaxis, rolling, adhesion to endothelium, activation and transendothelial migration, and interrelated oxidative/nitrosative damage. This talk is aimed to shed light on the opposing roles of endocannabinoids and CB receptors in various forms of tissue injury, and to delineate the evidence supporting the therapeutic utility of selective CB2 receptor agonists or CB1 receptor antagonists, which are devoid of psychoactive effects, as a promising new approach to limit tissue damage.

**Putting Together the Pieces.** D. Diersen-Schade, Mead Johnson Nutrition, USA

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### **PHO 3 / H&N 3.2: Applications of Phospholipids with n-3 Fatty Acids**

Chair(s): T. Wang, Iowa State University, USA; G. Wang, Cargill, USA; and J. Whittinghill, Solae, LLC, USA

**Nutritional Properties of Phospholipids with n-3 Fatty Acids.** T. Wang, Iowa State University, USA

Phospholipids (PLs) are health-enhancing nutrients. PC and PE maintain and promote the function of central nerve system. In recent years, PLs have also been shown to be good carriers and delivery means for enriching omega-3 fatty acids (FAs) in blood lipids. Evidences are provided in this review from several original research papers. PLs with omega or n-3 FAs are also shown to have anti-obesity and anti-cancer effects.

**Lipase Catalyzed Production of Lysophospholipids Rich in DHA Originating from a Marine Microalga.** L. Poisson, F. Ergon, G. Pencreac'h, IUT de Laval - MMS (EA 2160), Laval, France

Nutritional interest of  $\omega$ 3 polyunsaturated fatty acids (PUFAs) is no longer in question among scientists however the form under which these  $\omega$ 3 PUFAs should be delivered to be as efficient as possible needs further investigations. Nevertheless it seems that phospholipids as well as lysophospholipids are possible good carriers for  $\omega$ 3 PUFAs and it seems that docosahexaenoic acid (DHA,  $\omega$ 3 C22:6) is less subject to peroxidation when linked to phospholipids than to triglycerides or ethyl esters. The use of lipase for the production of lysophospholipids rich in DHA originating from the marine microalga *Isochrysis galbana* has been studied. Lipid analysis

was first carried out to define the content of DHA in the phospholipid fraction and the position of DHA on the phospholipid backbone. A screening of 12 lipase preparations was then implemented with a view to highlight enzymes leading to a discrimination between DHA and others fatty acids within phospholipid deacylation. A lysophospholipid fraction containing 79% of DHA was obtained with a recovery yield of 85%. The phenomena of non enzymatic acyl migration during phospholipid deacylation was also investigated with a view to lower the content of non desirable final products.

**Delivery of Bioactive Compounds through Milk Phospholipids Nanoliposomes.** Bitah Farhang, Yukio Kakuda, Milena Corredig, University of Guelph, Guelph, ON, Canada

Liposome technology is an effective technology for encapsulation of bioactive compounds. This research is focused on liposomes prepared from milk phospholipids which can be considered of great nutritional value per se, because of high content in sphingomyelin. Nanoliposomes were prepared using microfluidization, and  $\beta$ -Carotene and Vitamin C were incorporated as model hydrophobic and hydrophilic compounds. The results show high incorporation efficiencies for  $\beta$ -Carotene and high stability of vitamin C when entrapped in the liposomes. The incorporation efficiency of 26.5% for vitamin C was obtained with 10% milk phospholipids. About 70 % of encapsulated vitamin C remained after storage at 4°C and pH 7 for 7 weeks, without significant increases in peroxide value and liposome size. Incorporation of  $\beta$ -Carotene yielded larger nanoliposomes and by simultaneous incorporation of  $\beta$ -Carotene and Vitamin C, the incorporation efficiency of 92 % was obtained for  $\beta$ -Carotene without any significant change in vitamin C encapsulation. These results would suggest that  $\beta$ -Carotene enhanced the lamellar size of the liposomes, without increasing the lumen volume. As fractions of milk phospholipids derived from the milk fat globule membrane become commercially available, there will be an increased demand for these ingredients for the preparation of liposome material as a delivery system.

**The Antioxidant Effect of Phosphatidylserine in Refined Fish Oil.** A.J. Reid<sup>1</sup>, S.M. Budge<sup>1</sup>, M. St Onge<sup>2</sup>, <sup>1</sup>Dalhousie University, Halifax, NS, Canada, <sup>2</sup>Ascenta Health Ltd., Dartmouth, NS, Canada

Increasing the oxidative stability of fish oils is economically and nutritionally important with the increase in consumer knowledge of the value of long chain omega-3 fatty acids. Phosphatidylserine has been investigated for its antioxidant/synergistic effect on oil stability. Literature indicates that there is a synergistic relationship between tocopherols and phospholipids, including phosphatidylserine. The individual and combined effect of phosphatidylserine and tocopherols on the oxidation stability of refined triacylglycerol (TAG) fish oil was examined. Oil was incubated in the dark at 40°C and oxidation was monitored by following changes in peroxide and p-anisidine values. Tocopherol levels were also measured to determine their decomposition over the oxidation period.

**Extraction of Phospholipids from Egg Yolk: Effect of Solvent and Drying Treatment.** H. Wang<sup>1</sup>, L. Yao<sup>2</sup>, T. Wang<sup>2</sup>, <sup>1</sup>Center for Crops Utilization Research, Iowa State University, Ames, IA 50010, USA, <sup>2</sup>Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50010, USA

Two solvents, hexane and ethanol, were used to extract lipids from two types of pre-dried egg products, drum-dried egg yolk and pelletized spray-dried whole egg powder. The phospholipids were precipitated using acetone at two temperatures. The purity of the phospholipids fraction was evaluated by NMR. The effects of different pre-drying treatment, solvent types, and acetone precipitation temperature on the yield and purity of egg phospholipids will be presented and discussed. This research was designed to develop a commercially feasible method to extract phospholipids from chicken eggs.

**Role of Phospholipids Reverse Micelles on Lipid Oxidation: Impact of Minor Components on Physicochemical Properties of Stripped Soybean Oil.** B.C. Chen, D.J. McClements, E.A. Decker, Department of Food Science, University of Massachusetts, Amherst, MA, USA

Phospholipids self-assemble in bulk oils to form reverse micelles that can alter the microenvironment where chemical degradation reactions occur, such as lipid oxidation. In this study, we examined the influence of phospholipid reverse micelles (1) on the activity of non-polar (alpha-tocopherol) and polar (Trolox) antioxidants ; and (2) on the interaction of minor components, i.e., diacylglycerols, monoacylglycerols and free fatty acids in stripped soybean oil (SSO). Reverse micelles were formed by adding 1000 microM 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) to SSO. The addition of DOPC reverse micelles had a prooxidant effect, shortening the lag phase of SSO at 40, 55 °C. Small angle X-ray scattering (SAXS), fluorescence life time decay, interfacial tension were used to characterize the physical structure changes when SSO incorporated with reverse micelle encountering with minor component individual or combinations. Lipid hydroperoxides and volatile hexanal were measured under different conditions to understand the oxidation kinetics of SSO. These results will improve our understanding and control of lipid oxidation in bulk oils.

**Efficient Enzymatic Synthesis of Phenolic Ester by Increasing Solubility of Phenolic Acids in Ionic Liquids.** Zhiyong Yang, Zheng Guo, Xuebing Xu, Department of Molecular Biology, Aarhus University, Aarhus, Denmark

Compounds from phenolic acid family are well known natural antioxidants, but the application of phenolic acids as antioxidants in industry is limited due to the relatively low solubility in oil-based media. The properties of phenolic acids can be modified through enzymatic lipophilization and modified phenolic acids will have amphiphilic property, therefore they can be localized at oil-water or water-oil phase where oxidation is considered to occur frequently. It had been reported that immobilized *Candida Antarctica* lipase B was the most effective biocatalyst for the various esterification reactions, and it had been widely used for esterification of various phenolic acids with fatty alcohol or triglycerides. However, the conversion of phenolic acids is low due to low solubility in hydrophobic solvents and hindrance effect of unsaturated side chain towards the enzyme. Our studies show these barriers can be overcome by increase the solubility of phenolic acids. Ionic liquid Methyltrioctylammonium Trifluoroacetate can dissolve different phenolic acids in very high concentration. This ionic liquid was therefore applied for esterification of phenolic acids with fatty alcohol or triglyceride.

**Lipid Vesicles with High Entrapment Efficiency Prepared by Using Emulsions.** Sosaku

Ichikawa, University of Tsukuba, Tsukuba, Ibaraki, Japan

Lipid vesicles (also called liposomes) can be utilized as carrier of functional materials. However, the preparation of size-controlled vesicles containing hydrophilic materials with high entrapment efficiency is rather difficult. In this presentation, two types of methods for preparing lipid vesicles using emulsions are presented. One is "Vesicle formation from W/O/W multiple emulsions" and other is "Vesicle formation by lipid-coated ice droplet hydration method". In the former method, W1/O/W2 emulsion was prepared by microchannels emulsification that enables to reduce the leakage of to-be-entrapped materials from W1 phase to W2 phase. The organic solvent in the oil phase (O) was evaporated to form vesicles. With this procedure, vesicles with high entrapment efficiency, namely over 90% for 200 nm-sized, could be formed. The size reflects the water droplet (W1) size. In the latter method, giant vesicles (GVs) could be formed by the hydration of lipid-coated ice droplet containing to-be-entrapped materials. Carboxylesterase as a model enzyme was entrapped in GVs, and the enzymatic reaction inside GVs was conducted. These methods enable the preparation of rather large amount of vesicles with high entrapment efficiency and controlled-size, potentially useful in the research and practical fields of food-, cosmetic- and pharmaceutical- industries.

## WEDNESDAY

### MORNING

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#### H&N 4: General Nutrition I

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Chair(s): R. Ward, Utah State University, USA; and A. Zhou, Utah State University, USA

**Effects of Dietary Milk Fat Globule Membrane on Tissue Lipid Metabolism and Related Gene Expression in Fischer-344 Rats.** A.L. Zhou, R. Ward, K. Hintze, Utah State University, Logan, UT, USA

Effects of dietary MFGM on tissue lipid metabolism and gene expression were compared to dietary anhydrous milk fat (AMF). MFGM group had more PL and TAG in plasma and less FFA, CE and TAG in liver. MFGM group had less TAG and total lipids in visceral adipose tissue (VAT). The diets were high in sucrose, which could promote fatty liver development. MFGM diet had more choline, which may account for less lipids accumulation in the liver. MFGM Suppressed CYP17A1, SCD, DHCR24 and ME1 but up regulated NR1D1, CYP8B1 and HSD17B6 in the liver compared to AMF. Down-regulation of genes controlling fatty acid synthesis (SCD and ME1) may explain the less amount of FFA and TAG in the liver. Down-regulation of genes related to cholesterol synthesis (CYP17A1 and DHCR24) may explain the less amount of CE in the liver. Compared to AMF, MFGM suppressed lipid transporting (SCARB1), phospholipase D activity (PLD2), catabolism of fatty acids (ECHS1 and ACADS), cholesterol synthesis (AACS) in VAT. The less amount of TAG and total lipids in VAT may be due to decreased lipid transport into VAT. In addition, MFGM suppressed the expression of SCD and ACSL5 in muscle. In general, dietary MFGM may prevent lipids accumulation in liver and adipose tissue by influencing lipid transport and metabolism through complex regulation of relevant genes.

**Regulation of Hepatic Fatty Acids and Cholesterol Synthesis by Fucoxanthin.** F. Beppu, T. Tsukui, M. Hosokawa, K. Miyashita, Hokkaido University, Hakodate, Hokkaido, Japan

Fucoxanthin (FX) is a major marine carotenoid which is found in edible brown seaweeds such as *Undaria pinnatifida* and *Laminaria japonica*. In the recent study, we have observed that FX exhibits anti-obesity and anti-diabetic effects on diabetic/obese KK-Ay mice. In this study, we examined the effect of FX on fatty acid and cholesterol metabolism in the liver. After 4 weeks feeding FX diet, the amount of DHA increased in the liver of diabetic/obese KK-Ay. Further, stearic acid (18:0) increased and oleic acid (18:1n-9) decreased. In the liver of the mice, FX increased the expression level of delta 6-desaturase, which is the rate limiting enzyme in biosynthesis of n-3 and n-6 polyunsaturated fatty acids, increased and decreased stearyl CoA-desaturase (delta 9-desaturase). On the other hand, increase in SREBP2 expression level was also observed in the liver of FX-fed mice, although cholesterol level did not increase. These results show that dietary FX modulates fatty acid and cholesterol metabolism in the liver.

**Effects of Dietary Plant Epidermal Wax on Insulin Resistance in KKAY Mice.** Nakamichi Watanabe<sup>1</sup>, Yuri Takeo<sup>1</sup>, Mana Fujimoto<sup>1</sup>, Kenshiro Fujimoto<sup>2</sup>, Yoshio Takamura<sup>3</sup>, Masaru Takumi<sup>4</sup>, <sup>1</sup>Showa Women's University, Tokyo, Japan, <sup>2</sup>Koriyama Women's University, Fukushima, Japan, <sup>3</sup>Okinawa Sugar Canes Research Corporation, Okinawa, Japan, <sup>4</sup>Koyo Sangyo Co., Ltd., Tokyo, Japan

In our previous study, we compared the effects of dietary olive oil, sugarcane wax, and sorghum wax on plasma insulin levels and showed that dietary sorghum wax decreases plasma insulin levels in KKAY mice. One of the differences between sorghum wax and sugarcane wax lies in their relative content of polycosanol and polycosanal; that is, polycosanol is abundant in sugarcane wax, whereas polycosanal is abundant in sorghum wax. Because polycosanol is suggested to metabolize to polycosanal *in vivo*, the effect of dietary sorghum wax on insulin resistance may be stronger than that of sugarcane wax. Therefore, we performed another animal study. We used sorghum wax with high polycosanal content and reduced sorghum wax with high polycosanol content as the experimental diets for KKAY mice. Plasma insulin levels of mice in the sorghum (polycosanol) group decreased, but not of mice in the reduced sorghum (polycosanol) group. We also analyzed the levels of some types of adipocytokines. Plasma level of resistin was significantly lower in the sorghum group than in the control and reduced sorghum groups. Because polycosanoic acid is metabolized from polycosanal, we conducted the present study, which is the third animal study, to validate the differences in the effects between polycosanal and polycosanoic acid on insulin resistance.

**Fish Oil Prevents High Fat Diet-induced Impairments in Adiponectin and Insulin Response in Rodent Skeletal Muscle.** J.M. Tishinsky, R.A. Gulli, K.L. Mullen, D.J. Dyck, L.E. Robinson, University of Guelph, Guelph, ON, Canada

Adiponectin (Ad) is an adipocyte-derived protein that stimulates fatty acid oxidation (FAO) and improves insulin response in skeletal muscle. Fish oil (FO)-derived n-3 fatty acids have been shown to increase Ad secretion, but the impact of FO on Ad response in muscle is unknown. We sought to determine if FO supplementation could prevent high fat diet-induced impairments in

Ad and insulin response and to investigate underlying mechanisms. Rats ( $\geq 12$ /diet) were fed a control diet (10% kcal from fat, CON), a high fat diet (60% kcal from fat, LARD), or a high fat diet with FO (45% lard, 15% menhaden oil, FISH) for 4 wks. Ad-stimulated FAO, insulin-stimulated glucose uptake and expression of Ad signaling and inflammatory proteins were determined in soleus muscle. Ad stimulated FAO in CON and FISH (+36%, +39%, respectively;  $P \leq 0.05$ ) but not LARD animals. Similarly, insulin increased glucose uptake only in CON and FISH animals (+82%, +33%, respectively;  $P \leq 0.05$ ). Expression of TLR4, a key inflammatory protein, was increased in LARD but not FISH animals. There were no differences in expression of Ad signaling (APPL1, AMPK, ACC) or other inflammatory (NF- $\kappa$ B, I $\kappa$ B $\alpha$ ) proteins. Our results suggest that FO prevents high fat diet-induced impairments in Ad and insulin response, possibly through an inflammatory mechanism.

**Pattern Recognition for Discrimination of Dyslipidemic States.** Gerard Dumancas<sup>1</sup>, Lisa Reilly<sup>2</sup>, Neil Purdie<sup>1</sup>, Mary Kimani<sup>1</sup>, <sup>1</sup>Oklahoma State University, Stillwater, OK, USA, <sup>2</sup>Bethany College, Bethany, WV, USA

In the modern era, biomedical research plays a very critical role in human health. Within the biomedical research area, scientists are searching for new biomarkers that would serve to identify the causes of obesity, coronary heart disease, diabetes, hypercholesterolemia, and cancer among others. A mature, patented reagent system based on its selectivity to the  $-\text{CH}=\text{CH}-\text{CH}_2-$  group is exploited for pattern recognition of dyslipidemic individuals. The simple colorimetric assay is rapid, rugged, and inexpensive that produces a characteristic molar absorbance spectra for cholesterol, omega-3 (methyl esters of linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids), and omega-6 (methyl esters of linoleic, conjugated linoleic (CLA), and arachidonic fatty acids). A full factorial design simulation of synthetic serum in chloroform solutions accomplished the discrimination of eight clusters. Each cluster corresponded to specific levels of lipids prepared. Application of the assay to real serum samples revealed ten clusters with each corresponding to a disease state according to Fredrickson's Classification of Dyslipidemias.

**Palm Olein and Olive Oil Cause a Higher Increase in Postprandial Lipemia Compared with Lard but Had No Effect on Plasma Glucose, Insulin, and Adipocytokines.** Teng Kim-Tiu<sup>1,2</sup>, Gowri Nagapan<sup>1</sup>, Cheng Hwee Ming<sup>2</sup>, Kalanithi Nesaretnam<sup>1</sup>, <sup>1</sup>Malaysian Palm Oil Board, Kajang, Selangor, Malaysia, <sup>2</sup>University of Malaya, Kuala Lumpur, Malaysia

Postprandial lipemia impairs insulin sensitivity and triggers the pro-inflammatory state which may lead to the progression of cardiovascular diseases. A randomized, crossover single-blind study ( $n = 10$  healthy men) was designed to compare the effects of a high fat load (50 g fat), rich in palmitic acid from both plant (palm olein) or animal source (lard) versus an oleic acid-rich fat (virgin olive oil) on lipemia, plasma glucose, insulin and adipocytokines. Serum triacylglycerol (TAG) concentrations were significantly lower after the lard meal than after the olive oil and palm olein meals (meal effect:  $P = 0.003$ ; time effect:  $P < 0.001$ ). The greater reduction in the plasma non-esterified free fatty acids (NEFA) levels in the lard group compared to the olive oil meal was mirrored by the changes observed for serum TAG levels ( $P < 0.05$ ). The magnitude of response for plasma glucose, insulin and adipocytokines [interleukin-6 (IL-6), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and leptin] were not altered by the type of dietary fats.

A significant difference in plasma IL-1 $\beta$  was found over time following the three high fat loads (time effect:  $P = 0.036$ ). The physical characteristics and changes in TAG structure of lard may contribute to the smaller increase in postprandial lipemia compared with palm olein. A high fat load but not the type of fats influences concentrations of plasma IL-1 $\beta$  over time but had no effect on other pro-inflammatory markers tested in the postprandial state.

**Anti-inflammatory Effect of Newly Synthesized  $\Delta 7$ -Eicosatrienoic Acid ( $\Delta 7,11,14-20:3$ ) on Murine RAW264.7 cells.** Lu-Te Chuang, Yu-Lung Huang, Wen-Cheng Huang, Jia-Siang Liao, Department of Biotechnology, Yuanpei University, Hsinchu, Taiwan

$\Delta 7$ -eicosatrienoic acid ( $\Delta 7$ -ETrA;  $\Delta 7,11,14-20:3$ ) is a rare polyunsaturated fatty acid (PUFA) originally from pine seed oil. Our earlier report showed that PNA and its metabolites ( $\Delta 7$ -ETrA) significantly substituted arachidonic acid (AA) in cellular phospholipids, resulting in the reduction of pro-inflammatory prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) synthesis. However,  $\Delta 7$ -ETrA is not commercial available. In this study, we chemically synthesized  $\Delta 7$ -ETrA, and determined if  $\Delta 7$ -ETrA modulated PUFA metabolism and relieved inflammatory responses in murine RAW264.7 cells. Results showed that  $\Delta 7$ -ETrA was successfully C2-elongated from enriched PNA extracts, and then separated by argentated column chromatography. The identification of  $\Delta 7$ -ETrA was examined by analyses of gas chromatography (GC)/GC-mass spectrum, and the purity was approximate 96%. When macrophages were incubated with various concentrations of  $\Delta 7$ -ETrA, this fatty acid incorporated into phospholipids and substituted AA in a dose-dependent manner.  $\Delta 7$ -ETrA reduced synthesis of nitric oxide (NO), PGE<sub>2</sub> and interleukin-6 (IL-6), but slightly enhanced tumor necrotic factor- $\alpha$  (TNF- $\alpha$ ) production. Our study showed that  $\Delta 7$ -ETrA modulated the PUFA metabolic pathway and the responsiveness of macrophages to inflammatory stimulators.

**Effect of Modulating both the Ratio and Concentration of Dietary PUFA on Inflammatory Cytokines in Mice Treated with Lipopolysaccharide.** R.E. Ward, K. Hintze, J. Tawzer, M. Lefevre, Utah State University, Logan, UT 84341, USA

The American diet is rich in omega-6 fatty acids, and many have suggested that the high ratio of n6 to n3 may have negative health impacts. Consequently, dietary advice is given to improve this ratio by increasing intake of omega-3 fatty acids. However, a second strategy, in theory, would be to reduce intake of omega-6s. The focus of this work was to create a matrix of nine rodent diets, based on the AIN-93G, with PUFA at 3 ratios (1:1, 10:1 and 20:1) and 3 concentrations (2.5%, 5% and 10% of calories). Mice were fed the diets for 8 weeks and then subjected to an intraperitoneal injection of lipopolysaccharide to stimulate a systemic inflammatory response. Sixteen cytokines were subsequently measured in the mouse plasma 24h after the inflammatory challenge using a multiplexed ELISA technique. Interestingly, the red blood cell PUFA profiles of animals were stable across dietary PUFA ratios, resulting in similar omega-3 indices. However, there were differences in cytokine secretion as a function of both PUFA ratio and concentration, depending on the specific analyte. In summary, higher n6:n3 ratios and higher dietary PUFA are associated with an enhanced inflammatory response to ip injected LPS

**Omega-3 Dietary Supplementation in Alzheimer's Disease.** C. Bascoul-Colombo<sup>1,2</sup>, K. Hall<sup>1</sup>, R. Nair-Roberts<sup>1</sup>, I. Garaiova<sup>2</sup>, S. Plummer<sup>2</sup>, C. Hughes<sup>1</sup>, M. Good<sup>1</sup>, J. Harwood<sup>1</sup>, <sup>1</sup>Cardiff

University, UK, <sup>2</sup>Obsidian Research Limited, UK

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Despite over a hundred years of research, there is no cure for the disease. Thus, ways of preventing its onset and/or slowing its progression are of particular interest. Evidence from epidemiological and animal studies suggests that omega-3 polyunsaturated fatty acids (PUFA) and antioxidants may reduce the incidence of AD and, more specifically, attenuate  $\beta$ -amyloid pathology and improve the cognitive symptoms associated with the disease. However, the results to date are inconclusive and therefore, further research is required. This study assessed whether omega-3 oil-based nutritional supplements could ameliorate  $\beta$ -amyloid pathology and cognitive abnormalities in a transgenic mouse model of AD. Tg2576 transgenic mice, which over-express the human APP<sup>sw</sup> mutation, were fed different diets containing an omega-3 oil and/or a natural antioxidant, curcumin. Spatial memory, anxiety and executive functioning were assessed using behavioral tests. Biochemical analyses were carried out to measure molecular changes associated with the  $\beta$ -amyloid pathology and inflammation in the brain. Preliminary results show that the brain levels of the omega-3 PUFA increased significantly and some AD-related symptoms were ameliorated with supplementation.

**Effects of Dietary Milk Fat Globule Membrane on Brain Lipid Metabolism and Gene Expression.** A.L. Zhou, R. Ward, Utah State University, Logan, UT, USA

Effects of dietary MFGM on brain lipid metabolism and gene expression were compared to dietary anhydrous milk fat (AMF) and corn oil (CO) in Fischer-344 Rats. Tissue lipid profiles were similar among groups given the fact that CO diet had quite different fatty acids profile among diets. Although CO diet had much higher 18:2n-6 compared to other diets, CO group only had slightly higher 18:2n-6 and 22:4n-6 in PL, DAG and FFA fractions compared to other groups. AMF and MFGM groups had more DHA in PL. Since CO diet had more ALA while AMF and MFGM diets had more EPA and DPA, the brain may utilize EPA and DPA more efficiently than ALA in making DHA. Compared to AMF, MFGM upregulated cholesterol synthesis gene (LCAT) and neurotransmitter (GABA) synthesis gene (GAD1). MFGM suppressed genes with phospholipase and lipoprotein lipase activities (PLCB1, PLD3 and LPL), genes associated with phospholipid binding (ANXA11, ANXA4 and ANXA7), genes related to cholesterol synthesis (HMGCR) and fatty acid esterification (ACSL5). In general, the brain may maintain tight control over tissue lipid profile regardless of dietary lipids uptake, the brain may utilize the desaturation and elongation products of ALA more efficiently and dietary MFGM may regulate genes related to lipid transport and metabolism without affecting general lipid profile in the brain.

**Discussion.**

**LOQ 4 / H&N 4.1: Omega-3 Challenges: Stability, Processing, and Human Nutrition**

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Chair(s): S. Raatz, USDA, ARS, NPA, USA; S.-J. Yoo, Martek Biosciences Corp., USA; and S.-C. Liang, DuPont, USA

**Everything You Wanted to Know About DHA.** N. Salem, A. Ryan, Martek Biosciences Corp., Columbia, MD, USA

One frequently asked question concerning omega-3 fatty acids is "Which one do I need?" Alpha-linolenic acid (ALA) is predominant in the Western diet and the extent to which it is metabolized to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) will determine its efficacy in meeting requirements for the longer chain, more unsaturated n-3 fatty acids. Many compositional and stable isotope studies have indicated that ALA metabolism in higher mammals (cats, dogs and primates) occurs but is very limited and does not lead to increases in blood stream DHA, although EPA and DPAn-3 are generally increased. Thus preformed DHA is required in the diet to support higher organ DHA content and nervous system DHA, in particular. Increased organ and nervous system DHA does, in turn, yield many benefits for physiological function and health. Conversely, deficiency of n-3 fatty acids leads to many deficits in function with the nervous system the most studied organ in this respect. Some recent clinical study results in which DHA supplements were given will be summarized. Recent findings have indicated a benefit for cognitive function maintenance during normal aging leading to interest in possible uses of DHA for treatment of dementias or amelioration of their symptomology.

**Linoleic Acid-specific and Mixed Polyunsaturate Dietary Interventions have Different Effects on CHD Risk: A Meta-analysis of Randomised Controlled Trials.** C. Ramsden<sup>1</sup>, J. Hibbeln<sup>1</sup>, S. Majchrzak<sup>1</sup>, J. Davis<sup>2</sup>, <sup>1</sup>National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA, <sup>2</sup>University of Illinois at Chicago, Chicago, IL, USA

Randomized controlled trials (RCTs) of mixed PUFA diets, and meta-analyses of their CHD outcomes, are considered convincing evidence in specifically advising consumption of "at least 5-10% of energy as n-6 PUFAs". Here we: 1) extracted detailed dietary and outcome data enabling a critical examination of all relevant RCTs; 2) determined if diets increased n-6 PUFAs specifically, or increased both n-3 and n-6 PUFAs (i.e. mixed n-3/n-6 PUFA diets); and 3) compared mixed n-3/n-6 to n-6 specific PUFA diets on CHD outcomes. n-3 PUFAs were increased substantially in 4 of 8 datasets and n-6 linoleic acid was raised with specificity in 4 datasets. For non-fatal myocardial infarction(MI)+CHD death, pooled risk reduction for mixed n-3/n-6 PUFA diets was 22% (RR=0.78 95%CI 0.65-0.93), compared to an increased risk of 13% for n-6 specific PUFA diets (RR=1.13 95%CI 0.84-1.53). Risk of non-fatal MI+CHD death was significantly higher in n-6 specific PUFA compared to mixed n-3/n-6 diets (Q=5.4, p=0.02). RCTs that substituted n-6 PUFAs for TFA and SFA without increasing n-3 PUFAs produced an increase in risk of death (RR=1.16 95%CI 0.95-1.42). Advice to specifically increase n-6 PUFA intake, based on mixed n-3/n-6 RCT data, is unlikely to provide intended benefits.

**\*\*Canceled\*\* Current Omega-3 Oil Enrichment Technologies.** P. Lembke, Bioseutica Usa, Inc., USA

**Use of n-3 Oil Seed Meals in Livestock Rations as a Source of n-3 Enriched Foods.** Eric

Murphy, University of North Dakota, Grand Forks, North Dakota USA

Oil seed meals from flax and camelina are sources dietary of alpha-linolenic acid (ALA) for use in livestock rations. Camelina meal is an excellent source of protein, energy, and n-3 fatty acids for use in poultry rations. In laying hens, camelina meal provides ALA for conversion to longer chain n-3 fatty acids providing an n-3 enriched egg, while also providing a meal rich in protein and balanced with n-6 fatty acids. Similarly, it is a dietary n-3 fatty acid source for conversion in broilers to longer chain n-3 fatty acids found in muscle. Because camelina is an emerging oil seed crop used as a source of oil for conversion to biofuels, the meal is an important additional source of value and adds a valuable meal into the livestock market. Ground flax is another source of ALA and we have used flax extensively as a dietary source of ALA in cattle. In cattle, we demonstrate a marked increase in longer chain n-3 fatty acids as well as an increased expression of PPAR $\gamma$  and adipocyte fatty acid binding protein (A-FABP). In the end, these studies demonstrate that meals from camelina and flax can add value to livestock products due to the enrichment in n-3 fatty acids thereby providing a more healthy product for consumers.

**Linolenic Acid (ALA): Stability, Processing, and Human Nutrition.** K.C. Fitzpatrick, Flax Council of Canada, Canada

Linolenic acid (ALA) is a major dietary (n-3) fatty acid. ALA is converted to longer-chain (n-3) PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The efficiency with which this conversion occurs and the factors that may modify it could have important public health implications. Efficacy of the conversion is affected by both amount of ALA fed and the linoleic (n-6) to ALA ratio. ALA plays an important role in growth and development, reproduction and vision; in maintaining healthy skin and cell structure; in the metabolism of cholesterol and in gene regulation. ALA has been linked to the prevention and/or amelioration of several chronic conditions including cardiovascular disease, rheumatoid arthritis and autoimmune disorders. Because of limitations in increasing the public's consumption of fish, the use of the plant-based (n-3) PUFA, ALA, may be an important alternative for providing optimal EPA and DHA concentrations in the plasma and cell membranes. ALA is the most commonly consumed n-3 fatty acid in the typical Western diet with flaxseed being an important source. Flaxseed and oil can be incorporated into common dietary items such as breads, rolls, cereals, muffins, margarines, and salad dressings. This presentation will describe the challenges related to communicating the health benefits of ALA and use in various food applications.

**Correlation of PUFA Profiles and Cognitive Impairment in Participants in the Cache County Study on Memory Health and Aging.** R.E. Ward, H. Wengreen, L. Ward, D. Pearce, Utah State University, Logan, UT, USA

To investigate the relationship between red blood cell PUFA and mild cognitive impairment the fatty acid profile of RBCs was analyzed from samples collected in the third wave of the study (2002-2004). RBCs were subjected to direct transesterification with acetylchloride in methanol and analyzed via gas chromatography with flame ionization detection. In total, we analyzed the RBC profiles of 1,795 individuals from Wave 3 of the Cache County Study and compared the results to several data parameters associated with the subjects. There was no effect of age or sex, but was for BMI. The most interesting significant effects were the association of the Omega-3

index with fatty fish consumption and with Wave 3 3MS scores, a measure of cognition. According to the results, the RBC Omega-3 index is positively and significantly associated with improved cognition. While the effect was not significant for Dementia, the trend was for protection. The results for the n6 HUFA were very similar to the Omega-3 index. These measures are similar, yet the former potentially accounts for potential negative effects of high dietary n6 fatty acids. According to the data, the Omega-3 index (RBC EPA +DHA) can account for most of the protection against cognitive decline.

**Processing and Stability of Omega-3 Oil from Microbial Sources.** R.D. Orlandi, K.W. Hutchenson, M. Avogousti, J.M. Odom, S.-C. Liang, DuPont Applied BioSciences, Wilmington, DE, USA

Omega-3 fatty acids have been widely studied for their health benefits. There is abundant scientific evidence supporting the beneficial effects of these fatty acids, especially EPA and DHA. While fish currently remains the main source for EPA and DHA Omega-3 oil, there is growing interest and demand for microbial sources of Omega-3 oil, which is shown to have similar or better oxidative stability and is free of environmental contaminants. While there are many commonalities in oil processing between the microbial and marine sources, there are some unique aspects about extracting, refining, and stabilizing microbial oil in order to achieve the desired product quality. In this presentation, we will review various extraction methods and technologies pertaining to microbial oil. We will also cover some of the developments in oil extraction using supercritical CO<sub>2</sub> technology, and the stability of the extracted crude oil.

**Modeling the Kinetics of Fish Oil Oxidation.** J.C. Sullivan<sup>1</sup>, S.M. Budge<sup>1</sup>, M St-Onge<sup>2</sup>,  
<sup>1</sup>Dalhousie University, Halifax, NS, Canada, <sup>2</sup>Ascenta Health, Dartmouth, NS, Canada

The quality of commercial fish oil products can be difficult to maintain because of the rapid oxidation attributable to the high number of polyunsaturated fatty acids (PUFA), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). While it is known that oxidation in fish oil is generally the result of a direct interaction with oxygen and fatty acids, there are very few studies that investigate the oxidation kinetics of fish oil supplements. This study uses hydroperoxides, a primary oxidation product, to model the oxidation kinetics of two commercially available fish oil supplements with different EPA and DHA contents. Pseudo first order kinetics were assumed, and rate constants were determined for temperatures between 4 and 60 °C. This data was fit to the Arrhenius model, and activation energies (E<sub>a</sub>) were determined for each sample. Both E<sub>a</sub> agreed with values found in literature, with the lower PUFA sample having a lower E<sub>a</sub>. The oil with a lower PUFA content fit the first-order kinetics model at temperatures  $\geq 20$  °C and  $\leq 40$  °C, while the higher PUFA oil demonstrated first-order kinetics at temperatures  $\geq 4$  °C and  $\leq 40$  °C. When the temperature was raised to 60 °C, the model no longer applied. This indicates that accelerated testing of fish oil should be conducted at temperatures  $\leq 40$  °C.

**Emulsification Technique Affects Oxidative Stability of Fish Oil-in-Water Emulsions.** A.F. Horn<sup>1</sup>, L.H.S. Jensen<sup>2,3</sup>, N.S. Nielsen<sup>1</sup>, A. Horsewell<sup>2,3</sup>, C. Jacobsen<sup>1</sup>, <sup>1</sup>National Food Institute, Technical University of Denmark, Kgs Lyngby, Denmark, <sup>2</sup>Center for Electron Nanoscopy, Technical University of Denmark, Kgs Lyngby, Denmark, <sup>3</sup>Department of Mechanical

Engineering, Technical University of Denmark, Kgs Lyngby, Denmark

In oil-in-water emulsions, lipid oxidation is expected to be initiated at the oil-water interface. The properties of the emulsifier used, and the structure at the interface is therefore expected to be of great importance for lipid oxidation in emulsions. Previous studies have shown that e.g. homogenization pressure can affect how proteins locate themselves at the interface of an emulsion. The hypothesis is therefore that emulsions produced with different emulsification equipments differ in their oxidative stability due to differences in the behaviour of the proteins at the interface. The aim of this study was therefore to compare lipid oxidation in 10% fish oil-in-water emulsions prepared by two different kinds of high pressure homogenizers i.e. a microfluidizer and a two valve high pressure homogenizer. Emulsions were made with equal droplet sizes, and with either 1% sodium caseinate or 1% whey protein isolate. Emulsions were characterised and investigated by microscopy. Lipid oxidation was assessed by PV and the formation of secondary volatile oxidation products. Results showed that the different emulsification techniques had an influence on lipid oxidation and that the effect of the emulsification technique depended on the type of protein used as an emulsifier.

**Quality and Technical Challenges Facing the Omega-3 Industry as a Result of Sustained Rapid Market Growth.** A. Ismail, Global Organization for EPA and DHA Omega-3s, Salt Lake City, UT, USA

The market for omega-3 fatty acids has experienced rapid growth for more than two decades. While the growth has been driven by science, specific quality initiatives by the industry have prevented quality problems from derailing the growth trajectory of omega-3s. The industry growth has brought a number of new entrants, research into new sources of EPA and DHA, and increasing regulatory attention to the space. Various governments have instituted new rules for hygiene and contaminants related to omega-3s, while literature shows that there are still many products that can bring negative attention to the space by producing low quality products. Additionally, new technical methods are being developed that will allow for greater quality assurance and traceability, but they have not yet become generally accepted standards. It is imperative that the industry continue to innovate on quality and analytical issues in order to provide a solid foundation for growth.

**AFTERNOON**

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**H&N 5: General Nutrition II**

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Chair(s): A. Zhou, Utah State University, USA; and R. Ward, Utah State University, USA

**Novel Approach to Develop Functional Fermented Fish Meat Paste by Japanese *Koji* Fermentation Technique.** A. Giri<sup>1</sup>, T. Ohshima<sup>1</sup>, <sup>1</sup>College of Light Industry and Food Sciences, South China University of Technology, Guangzhou, China, <sup>2</sup>Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Shinagawa, Tokyo, Japan

A fermented fish meat paste product, fish miso, was developed from a variety of fish meat by

using *Aspergillus oryzae* inoculated rice malt, *koji*, in view of bioactive novel health aspects of traditional soy miso. In vitro assays for certain radicals and lipid oxidation models revealed that the aqueous extract of fish miso significantly suppressed the oxidation of linoleic acid by scavenging radical initiators. The peptides and amino acid profile in fish miso indicated the occurrence of certain peptides of low molecular weight in the early stages of fermentation suggesting the involvement in radical scavenging activity as well as reducing power ability of the miso. An online HPLC analysis of DPPH radical confirmed the involvement of the peptides in radical scavenging activity of the aqueous extract. Fractionation of aroma compounds in miso and subsequent volatile analysis revealed that 4-ethylguaiacol (4-EG) is a key compound contributing to radical scavenging in the dichloromethane extract. Analysis of phenolics extracted by ethyl acetate clearly suggested that 4-EG in the fish miso possibly the bioconversion product of ferulic acid produced by *koji*. Thus, the fish miso fermented using *koji* inoculated with *A. oryzae*, possessing high antioxidant activity, could be regarded as a functional food.

### **Millet Phenolics as Natural Antioxidants in Food and Biological Systems.** G.A.

Chandrasekara, F. Shahidi, Memorial University of Newfoundland, St John's, NL, Canada

Lipid oxidation is a major cause of deterioration in food systems and oxidized low density lipoprotein (LDL) cholesterol, a culprit in the development of atherosclerosis. Phenolic extracts from six millet varieties, namely kodo, finger, proso, foxtail, little and pearl were evaluated for their inhibitory effects on lipid peroxidation in several model systems, namely cooked comminuted pork, stripped corn oil, linoleic acid emulsion and in vitro copper induced human LDL cholesterol oxidation. Total phenolic content (TPC) and free radical scavenging activities were measured and major phenolic compounds identified and quantified using HPLC. The TPC ranged from 1156 to 146  $\mu\text{mol}$  ferulic acid eq/g extract. All six varieties exhibited effective inhibition of lipid oxidation in pork model, stripped corn oil and linoleic acid emulsion systems. At a final concentration of 0.05 mg/mL, millet extracts inhibited LDL cholesterol oxidation by 15-35 %. Both hydroxycinnamic and hydroxybenzoic acids were identified along with some flavonoids such as catechin, taxifolin and isovitexin. In food systems kodo millet exhibited superior inhibition of lipid peroxidation, similar to butylated hydroxyanisole. Millets may serve as a natural source of antioxidants in foods and as a nutraceutical and functional food ingredient in health promotion and disease risk reduction.

### **Prune (*Prunus domestica* L., *europaea*) Fruits as a Source of Polyphenolic Nutraceuticals.**

L. Banelli<sup>1</sup>, S. Miele<sup>2</sup>, E. Bargiacchi<sup>3</sup>, A. Romani<sup>1</sup>, P. Pinelli<sup>1</sup>, <sup>1</sup>Department of Pharmaceutical Sciences, University of Firenze, Firenze, Italy, <sup>2</sup>Department of Agronomy & Agroecosystem Management, Pisa, Italy, <sup>3</sup>Consortium I.N.S.T.M, Firenze, Italy

Phenolic compounds in foods have been associated with reduced incidences of heart disease by acting as antioxidants for low-density lipoprotein (LDL). Prunes may be a good source of natural antioxidants: neochlorogenic acid, and flavonoids (quercetin derivatives, in particular rutin). During the drying process the anthocyanin content is lost, nevertheless prunes are a source of nutraceuticals, due to their high polyphenolic content and the antioxidant activity. A pitted prune extract inhibits LDL oxidation by 24, 82 and 98% at 5, 10, and 20  $\mu\text{M}$  gallic acid equivalents. Fresh and dried epicarp and mesocarp extracts from prunes grown in selected areas of Central Italy were analyzed by HPLC/DAD/MS/MS quali-quantitative analyses. The classes of detected

polyphenols were: hydroxycinnamic acids, flavonols and anthocyanins (where presents). 100 g of fresh fruits contained 120 mg of total polyphenols, with an average concentration of 1.81 mg/g of dried fruit. Total phenol contents were in the range 0.28-0.33% in the dried epicarp, and 0.98-1.07% in the dried mesocarp. The high polyphenol content of prune extracts makes these fruits very suitable to obtain nutraceuticals, supplements, functional foods and food ingredients as natural antioxidants, to enhance food quality and provide safer products.

**A High Canola/Flax Oil Blend Diet Improves Muscle Phospholipid Fatty Acid Composition but Does Not Alter Insulin Resistance in Diet-Induced Obese (DIO) Rats.** D. Hanke<sup>1</sup>, S. Mohankumar<sup>1</sup>, L. Siemens<sup>1</sup>, P. Zahradka<sup>1,2</sup>, C. Taylor<sup>1</sup>, <sup>1</sup>University of Manitoba, Winnipeg, Manitoba, Canada, <sup>2</sup>CCARM, Winnipeg, Manitoba, Canada

Dietary lipid consumption has a profound impact on phospholipid (PL) fatty acid (FA) composition. Altered PL FA composition is expected to change membrane fluidity and alter cellular functions including insulin sensitivity. This study assessed whole body and molecular markers of insulin resistance (IR) and skeletal muscle PL FA composition in 6 wk old Obese Prone Sprague Dawley rats fed high fat (55% en) diets for 12 wks. The diets were based on high oleic canola oil, canola oil, canola/flax oil (C/F, 3:1), safflower oil, soybean oil, or lard. Results showed that the C/F group had the highest total omega-3, alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahecaenoic acid (DHA) and the lowest arachadonic acid (AA) concentrations in muscle PL. However, the changes in PL FA composition did not affect molecular markers of IR in muscle including phospho-IRS (S636/639), phospho-IRS (S307), phospho-JNK, phospho-Akt, and phospho-Erk1/2. Likewise, results from whole body insulin and glucose tolerance tests indicated no differences among groups. In conclusion, a C/F diet high in ALA elevated total omega-3, ALA, EPA, and DHA and decreased AA concentrations in muscle PL, but did not affect whole body or molecular markers of IR in DIO rats.

**In-vitro Anti-microbial and Brine-Shrimp Lethality Potential of the Seed Kernels' Oil of Nahar (*Mesua ferrea*) Plant.** I.A. Ahmed, M.S. Elwathig, S.M. Aremu, J.I. Daoud, M.A. Mikail, International Islamic University Malaysia, Kuala Lumpur, Selangor, Malaysia

The growing interest in human health and increasing bacterial resistance to antibiotics among other reasons have resulted into an increasing need for the exploration of both the essential oils and other plant extracts in the food and pharmaceutical industries. This work was aimed at evaluating the antimicrobial activity, minimum inhibitory concentration as well as the cytotoxicity of the Nahar seed kernel oil using agar disc diffusion, micro broth dilution and Brine shrimp lethality bioassay respectively. The oil which was found to be 77.0±1.0% of the kernel on dry weight basis showed a remarkable antibacterial property against all the selected microbes (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*) with the inhibition zones ranging from 16.0±0.5 to 27.0±0.5mm for all the tested bacteria, and *S. aureus* found to be the most susceptible. The oil was found to be both bacteriostatic and bactericidal. The MIC ranges of 0.24-0.98 mg/ml with MBC value of 2mg/ml were obtained for all the tested bacteria. The oil was found to be toxic to the Brine shrimps with LC<sub>50</sub> value of 1.95ppm. The antimicrobial and cytotoxic activities of the oil, found in this study, may explain some of the traditional medicinal uses of the plants and thus suggesting that the extracts may contain bioactive compounds of potential therapeutic and prophylactic significance. These could

also be of particular interest in relation to find out its unexplored efficacy and can also be a potential of chemically interesting and biologically important drug candidates.

**Mowrah Butter: Nature's Novel Fat.** M.F. Ramadan, Zagazig University, Faculty of Agriculture, Zagazig, Egypt

*Madhuca longifolia* Syn. *M. indica* (Sapotaceae) is an important economic tree growing throughout the subtropical region of the Indo-Pak subcontinent. In the present contribution lipid composition and characteristics of mahua butter were determined. n-hexane extract of finely ground buttercup fruit-seed meal was found to be about 58%. The amounts of neutral lipids in the crude mahua fat was the highest (ca. 95.4% of total lipids), followed by glycolipids and phospholipids, respectively. Oleic, stearic, palmitic and linoleic were the major fatty acids in mahua fat. Mahua butter being characterized by a relatively high amount of phytosterols, wherein the sterol markers were  $\Delta^5$ -avenasterol and  $\beta$ -sitosterol.  $\gamma$ -Tocopherol was the major tocopherol isomer while the rest being  $\beta$  and  $\alpha$  isomers. When mahua butter and extra virgin olive oil were compared upon their radical scavenging activity toward DPPH radical mahua butter exhibited stronger RSA. In consideration of potential utilization, detailed knowledge on the composition of mahua butter is of major importance. The diversity of applications to which mahua butter can be put gives this substance great industrial importance.

**\*\*Cancelled\*\* Canadian Human Milk is Now Low in trans Fatty Acids.** N. Ratnayake, Health Canada, Canada

## Health and Nutrition Posters

Chair(s): S. Bhale, Mead Johnson Nutrition, USA

**\*\*Cancelled\*\* Anti-inflammatory Effect of Newly Synthesized D7-Eicosatrienoic Acid (D7,11,14-20:3) on Murine RAW264.7 cells.**

L.-T. Chuang, Yuanpei University, Taiwan

### **Determination of Phytosterols in Parenteral Lipid Emulsions.**

Thomas Pavlina<sup>1</sup>, Zhidong Xu<sup>3</sup>, Kevin Harvey<sup>3</sup>, Guy Dutot<sup>2</sup>, Mary Hise<sup>1</sup>, Gary Zaloga<sup>1</sup>, Rafat Siddiqui<sup>3,4</sup>, <sup>1</sup>Baxter Healthcare Corporation, Deerfield, IL 60015, USA, <sup>2</sup>Baxter SAS, Maurepas-Cedex, France, <sup>3</sup>Methodist Research Institute, Indianapolis, IN 46202, USA, <sup>4</sup>Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA

Parenteral lipid emulsions (PLEs) are oil-in-water suspensions which are made from vegetable and/or fish oils. Phytosterols (PSts) occur naturally in plant oils whereas cholesterol is the major sterol in animal oils. The quantitative analysis of sterols in PLEs is currently not available. We

determined the types and amounts of sterols and squalene (a steroid precursor) in PLEs. The quantification of PSts in a freshly opened bag/bottle of PLEs was performed using a SACTM-5 capillary column (Supelco, USA) on a Shimadzu GC2010 system. Sterols and squalene were analyzed using both the internal and external standard curve, and quantified using standard linear regression equation parameters. Total sterol content (mg/L) was as follows: Liposyn<sup>®</sup> III=662, Intralipid<sup>®</sup>=713, Lipofundin<sup>®</sup> MCT=498, and ClinOleic<sup>®</sup>=384. The content of cholesterol (mg/L) was: Liposyn<sup>®</sup> III=64, Intralipid<sup>®</sup>=274, Lipofundin<sup>®</sup> MCT=219, and ClinOleic<sup>®</sup>=110. Squalene content (mg/L) of the lipid emulsions was: Liposyn<sup>®</sup> III=9, Intralipid<sup>®</sup>=7, Lipofundin<sup>®</sup> MCT=6, and ClinOleic<sup>®</sup>=387. ClinOleic<sup>®</sup>, had the lowest levels of sterols (including cholesterol) and the highest level of squalene amongst the lipid emulsions. Further studies are required to determine the types of phytosterols that may cause tissue toxicity following intravenous administration

### **The Effects of Whey Milk Fat Globule Membrane Lipids on Barrier Function of Caco-2 Cell Monolayer Against LPS Stress.**

A.L. Zhou, R. Ward, K. Hintze, Utah State University, Logan, UT, USA

The aim of this study was to test the effects of gangliosides and whey MFGM lipids on Caco-2 cell monolayer integrity against LPS stress. Caco-2 cells were grown on transwell inserts to obtain monolayer systems. The membrane integrity of Caco-2 cell monolayer was determined routinely by measuring transepithelial electrical resistance (TEER). The monolayer was incubated with either: control media, control media with whey MFGM lipids or control media with GD3. After treatment, the monolayer was stressed by the addition of media with LPS. Gangliosides GD3 protected Caco-2 cell monolayer against LPS stress. The data did not show any time or dose dependency of the protecting effects. GD3 also prevented the recovery of monolayer integrity after LPS stress. Whey MFGM lipids did not protect the integrity of Caco-2 monolayer against LPS stress comparing with GD3 and control. But whey MFGM lipids treatment facilitated recovery of Caco-2 cell monolayer integrity after LPS stress. Preliminary analysis indicated that whey MFGM lipids contain both gangliosides and phospholipids. In summary, ganglioside GD3 may protect Caco-2 cell monolayer integrity against LPS stress while whey MFGM phospholipids may facilitate the recovery of barrier function.

### **Macular Pigment Optical Density, Body Weight and Diet in College-aged Students.**

N. Lam, L. Thibodaux, H. Durham, C. Lammi-Keefe, Louisiana State University, Baton Rouge, LA, USA

Evidence suggests obesity promotes progression of advanced age-related macular degeneration (ARMD), the leading cause of blindness in the elderly. Carotenoids, lutein (L) and zeaxanthin (Z), concentrated in the macula, and the long-chain polyunsaturated fatty acid, docosahexaenoic acid (DHA, 22:6n-3), found throughout the retina, have protective effects within the eye. We compared macular pigment optical density (MPOD) and dietary intakes of L and Z and DHA among college-aged students (age=18-28 years old) categorized into the following groups using body mass index (BMI (kg/m<sup>2</sup>)): normal weight (n=31, BMI= 18.5-24.9), overweight (n=20, BMI= 25-29.9) and obese (n=9, BMI=30-34.9). MPOD was measured using a Macular Densitometer<sup>™</sup> and dietary information was collected using a food frequency questionnaire and 24-hour dietary recalls. Differences between groups were assessed using analysis of variance. The results showed there was a trend for a difference in MPOD means between BMI groups

(MPOD:  $0.31 \pm 0.03$ ,  $.40 \pm .04$ ,  $.27 \pm .06$ ) ( $p=0.1$ ). There was not a difference between groups for the following: energy, L and Z and DHA. These data suggest MPOD may be associated with BMI. Based on a power calculation a larger sample size is needed to further assess the relationships between BMI, MPOD and diet.

### **Effect of Phospholipids Containing n-3 Polyunsaturated Fatty Acids on Rat Lipid Metabolism.**

K. Fukunaga<sup>1,2</sup>, M. Fukao<sup>1,2</sup>, R. Hosomi<sup>1</sup>, H. Arai<sup>2</sup>, S. Kanda<sup>3</sup>, T. Nishiyama<sup>3</sup>, M. Yoshida<sup>1</sup>,  
<sup>1</sup>Kansai University, Suita, Osaka, Japan, <sup>2</sup>Kitami Institute of Technology, Kitami, Hokkaido, Japan, <sup>3</sup>Kansai Medical University, Moriguchi, Osaka, Japan

It has previously been demonstrated that the amount and type of dietary fat are risk factors of arteriosclerosis and coronary or cerebral artery disease. This study investigated the effects of phospholipids (PLs) containing n-3 polyunsaturated fatty acids (PUFAs) extracted from squid (*Todarodes pacificus*) mantle muscle on lipid metabolism in rats. We also examined the combination effect of triglyceride (TG) type of fish oil and soybean PLs. Groups of male Wistar rats were fed an AIN93G diet containing soybean oil (SO, 7%), fish oil (1.2%) + SO (5.8%), soybean PLs (1.8%) + SO (5.2%), PLs containing n-3PUFAs (1.8%) + SO (5.2%), or soybean PLs (1.8%) + fish oil (1.2%) + SO (4.0%). Compared to the SO diet, PLs containing n-3PUFAs and soybean PLs + fish TG diets decreased the serum and liver cholesterol contents by enhancing fecal cholesterol excretion and suppressing the liver HMG-CoA reductase expression level. However, there was no difference in the serum and liver cholesterol contents between the PLs containing n-3PUFAs and the soybean PLs + fish TG diet. Hence, the combination of soybean PLs and fish TG is very useful and inexpensive. We thus conclude that highly functional foods can be developed without the use of n-3PUFAs containing PLs, and that these would be available for health promotion worldwide.

### **Fish Oil Maintained Levels of Essential Fatty Acid in Rats Treated with Chemotherapy.**

A Pant<sup>1</sup>, M C Pawlowicz<sup>1</sup>, H Xue<sup>2</sup>, V Baracos<sup>3</sup>, VC Mazurak<sup>1</sup>, <sup>1</sup>University of Alberta, Agricultural, Food and Nutritional Science, Edmonton, Alberta, Canada, <sup>2</sup>Department of Anesthesiology, University of Colorado Health Sciences Center, Aurora, CO, USA, <sup>3</sup>Department of Oncology, University of Alberta, Edmonton, Alberta, Canada

Colon cancer remains a major cause of cancer deaths. Irinotecan used for treatment may interfere with fatty acid (FA) metabolism. We hypothesized that treatment with Irinotecan leads to deficits in EPA (C20:5n-3) and DHA (C22:6n-3) and dietary fish oil would maintain EPA+DHA in plasma phospholipid (PL) and triglyceride (TG). We measured amount and types of FAs in plasma PL and TG in female Fischer 344 rats bearing Ward colon tumor and treated with irinotecan. Rats were fed control (semi-purified diet; CO; n=4) or fish oil diet (3.2%EPA + 0.8%DHA; FO; n=3). Reference animals not receiving tumor or irinotecan treatment were fed the fish oil diet (NT; n=4). When the tumor size reached 2cm<sup>3</sup>, irinotecan (150mg/kg/day) was administered on 3 consecutive days. Plasma was collected 7days later. A modified Folch was used to isolate FA from plasma and thin layer chromatography was used to separate PL and TG. Gas chromatography was used to determine amounts and types of FA. In plasma PL, C20:5n-3 was higher (p

### **Influence of Fat Emulsified State on the Kinetics of Postprandial Lipemia in Healthy Normal Weight and Obese Subjects.**

Cécile Vors<sup>1,3</sup>, Jocelyne Drai<sup>2,5</sup>, Gaëlle Pineau<sup>6</sup>, Stéphanie Lambert-Porcheron<sup>2</sup>, Martine Laville<sup>2,4</sup>, Hubert Vidal<sup>6</sup>, Marie-Caroline Michalski<sup>1,3</sup>, <sup>1</sup>INRA USC1235, CARMEN, Villeurbanne, France, <sup>2</sup>CRNH Rhône-Alpes, Oullins, France, <sup>3</sup>INSA-Lyon, IMBL, Villeurbanne, France, <sup>4</sup>Université de Lyon, Villeurbanne, France, <sup>5</sup>Laboratoire de Biochimie, Hospices Civils de Lyon, Oullins, France, <sup>6</sup>INSERM U1060, CARMEN, Oullins, France

Obese people present a chronic imbalance of fat distribution. In this context, the kinetics of postprandial lipemia appears to be one of the major elements of the physiopathology of obesity. However, the impact on postprandial lipid metabolism of different fat structures in food is poorly described. Our hypothesis is that non-emulsified fat and emulsified fat would result in different kinetics of lipid absorption and chylomicronemia. To verify this hypothesis, 5 normal weight subjects and 5 obese subjects digested, during a test breakfast, 40 g of dairy fat varying by its emulsified state (fat-containing drink + bread) or not (fat spread on bread + drink without lipids). Plasma samples were collected during 8h of digestion to analyse triacylglycerols and chylomicrons after ultracentrifugation. Our results show that non-emulsified spread fat resulted in a later peak of chylomicrons during digestion compared with emulsified fat, both in lean and obese subjects. This effect was most pronounced in obese subjects, with a much later peak of chylomicrons and of lower intensity during the first 5 hours of digestion of non-emulsified spread fat. Optimizing dietary fat structure could thus constitute a strategy to control postprandial lipemia in obese subjects.

### **Intestinal and Metabolic Impacts of Different Dairy Creams in Mice Fed a High Fat Diet.**

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Dairy creams can vary in nutritional profile because depending on cow diets they can be enriched in unsaturated fatty acids such as omega 3 and omega 6 with a reduction of saturated fatty acids (SFA). The objective of our study was to examine the impact of cream quality on metabolic and intestinal adaptations in mice fed with a high fat diet (HFD). Four groups of mice were designed by feeding different diets for 12 weeks: control group (low fat diet) or HFD groups with 20% w/w lipids from lyophilized creams with different compositions: (i) high SFA amount and high n-6/n-3 ratio (HSH6); (ii) lower SFA amount and high n-6/n-3 ratio (LSH6); and (iii) lower SFA amount and low n-6/n-3 ratio (LSL6). Mice fed with LSL6 diet showed a decreased food intake, fat mass and plasma triglycerides content without failure in intestinal fatty acid uptake compared to the other HFD groups. LSL6 mice showed an increase in mucus cell number in duodenum and colon compared to the other groups (at least 1.4-fold) and an increase in Paneth cell number in duodenum (1.3-fold). Cream quality seems to induce a marked impact on lipids metabolism and we reveal that dietary lipid quality can influence intestinal barrier quality.

### **Comparative Inhibitory Activity of Black Seed Oil, Black Sesame Seed Oil, and Curcumin Against the Biomarkers of Breast Cancer Stem Cells.**

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The aim of this study was to compare the inhibitory activity of black seed oil, black sesame seed oil, or curcumin against breast cancer stem cell biomarkers in vitro. Hormone-independent MDA-MB-231 breast cancer cells were incubated with 0.29% (v/v) of well defined black seed oil or black sesame seed oil or 100  $\mu$ M of curcumin all stimulated with insulin-like growth factor-I (100 ng/ml of IGF-I) and incubated at 37 °C and 5% CO<sub>2</sub> in a humidified incubator for 72 h. Flow cytometry was performed to analyze cell cycle. Cell viability was measured by the crystal violet assay. The inhibitory effect of the dietary bioactive compounds on breast cancer stem cell biomarkers was measured by Western blot. Black seed oil was more efficient than curcumin in down-regulating the expression of breast cancer stem cell biomarkers including CD44, ESA, ABCG2, aldehyde dehydrogenase, p53, and survival factors including Notch1, survivin, cdk4, Her-2, and Akt. Black sesame seed oil was ineffective. Black seed oil is more bioavailable than curcumin. Black seed oil can inhibit breast cancer stem cells, mutated p53, is bioavailable, and is a good candidate for further in vitro and in vivo investigations on the potential health benefits of functional foods in the prevention and treatment of breast cancer.

#### **Physical and Chemical Properties of Structured Lipids for Topical Application in Wounds.**

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Recently essential fatty acids have been successfully used in the treatment of skin lesions of difficult healing. Vegetable oils are rich sources of these essential fatty acids, presenting low cost and easy access. The objective of this work is to develop structured lipids based on different mixtures of vegetable oils for topical application in wounds. For this purpose, blends of sunflower and canola oil (BL1) and sunflower and linseed oil (BL2) were prepared and then interesterified using TL IM Lipozyme, producing structured lipids SL1 and SL2, respectively. The success of enzymatic reaction was verified using GC for fatty acid and triacylglycerol compositions, DSC for melting and crystallization behavior, and NMR for stereospecific distribution of fatty acids. A macroscopic study of wounds in rats was made by measuring their areas in photographs. The rats were subjected to different treatments during 15 days. BL1 presented 54.1% of linoleic acid, believed to present pro inflammatory function. 22.4% of linolenic acid, presenting anti inflammatory properties, were found in BL2. TAG composition, thermal behavior and stereospecific distribution ensured the success of the enzymatic process. The wounds treated with vegetable oils were healed faster than the ones treated with a saline solution.

#### **Monoacylglycerol Gel Structure does not Influence Postprandial Lipid and Glucose Responses but Improves Lipid Profiles in High and Low Moisture Baked Products.**

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This study examined the impact of the physical structure of a monoacylglycerol (MAG) gel shortening alternative, that contains 40% less fat than traditional shortening and can be formulated with various saturated and *trans*-free oils, on postprandial lipid and glucose responses to baked products. Sugar-free cakes (high moisture) and cookies (low moisture) containing 48g and 98g total carbohydrate, respectively, and 40g total fat and were produced with either structured MAG gel or unstructured MAG gel ingredients. On 4 occasions, healthy males (n=17, 19-40 y, BMI≤27 kg/m<sup>2</sup>, glucose *trans*-free alternative to traditional shortenings.

### **Absorption of Lipid Peroxidation-derived Products by a Human Intestinal Epithelium Caco-2/TC7.**

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Polyunsaturated fatty acids (PUFA) are susceptible to lipid peroxidation. The end-products of lipid peroxidation such as 4-hydroxy-2-hexenal (HHE) and 4-hydroxy-2-nonenal (HNE), respectively derived from omega-3 and omega-6 PUFA, are recognized markers of oxidative stress and can exert genotoxicity and cytotoxicity in cells and tissues. Our objective was to test whether 4-HHE and 4-HNE are absorbed by human intestinal Caco-2/TC7 cell monolayer and modify their oxidative status. No significant effect on the growth of Caco-2 cells was observed for any concentrations of 4-HHE or 4-HNE incubated for 8 hours. We demonstrate an increasing concentration of 4-HHE or 4-HNE in the basolateral medium after incubating the Caco-2 cells with increasing concentrations of 4-HHE or 4-HNE in the apical medium for 8 hours, both in the absence and presence of mixed lipid micelles. The detected amount of free aldehydes in the basolateral medium was ~ 0,2-1% of the amount incubated, with higher detected amounts of 4-HHE compared with 4-HNE. Our study thus provides evidence that 4-HHE and 4-HNE can be absorbed by the Caco-2 epithelial cells. This is important because these alkenals may disrupt intestinal redox homeostasis and form covalent adducts with macromolecules in intestinal cells and in plasma.

### **Omega-3, CLA and *trans* Fatty Acids Content in Preterm and Term Breast Milk.**

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This study aim was to determine and compare the fatty acid composition of colostrums and mature milk produced by nursing mothers of preterm and at-term newborns. Based on gas chromatographic analysis, the average content of linolenic acid (C18:3 n-3) was found to be 0.92 and 0.70% in colostrum and 0.74 and 0.86 in mature milk for preterm and term milk, respectively. Eicosapentanoic acid (C20:5 n-3) was 0.02% for colostrum (preterm and term milk), and 0.01% for mature milk (preterm and term milk). Docosahexaenoic acid (C22:6 n-3) was 0.10% and 0.09% for colostrum in preterm and term groups, respectively, and 0.5% and 0.03% for mature milk in preterm and term, respectively. Elaidic acid levels were significantly lower from colostrum to mature milk in the preterm group (from 1.45% to 1.02%) with no significant difference in term milk (1.41% to 1.67%). The content of conjugated linoleic acid (C18:2n9c11t) was significantly higher in mature milk in preterm group (0.04% to 0.05%) and significantly higher in colostrum of the mature milk between the pre and at-term groups (from

0.05% to 0.06%). The results show the greatest differences observed were between colostrums and mature milks for both groups and not between pre and at-term mothers.