

# 2013 Annual Meeting Abstracts

## Health and Nutrition

MONDAY

AFTERNOON

### H&N 1: Global Perspectives on Fat Intakes and Dietary Recommendations

Chair(s): M.C. Michalski, INRA, USA; P. Huth, PJH Nutritional Science, USA

#### Saturated fat and heart disease: A review of the evidence

D. Mozaffarian<sup>(1)</sup>

<sup>(1)</sup>Harvard School of Public Health, United States of America

Dietary and policy recommendations focus on reducing saturated fatty acid (SFA) consumption for improving cardiometabolic health. These recommendations are largely based on ecologic, biomarker, and animal studies. Advances in nutritional science now allow more robust and critical assessment of effects of SFA. This talk will review evidence from randomized trials of lipid and non-lipid risk factors, prospective cohort studies of disease endpoints, and randomized trials of disease endpoints, including whether effects vary depending on specific SFA chain-length; the replacement nutrient; or disease outcomes evaluated. Compared with carbohydrate, the TC:HDL-C ratio is nonsignificantly affected by myristic or palmitic acid, is nonsignificantly decreased by stearic acid, and is decreased by lauric acid. Insufficient evidence exists for different chain-length-specific effects on other risk pathways or, more relevantly, disease endpoints. Based on consistent human evidence, replacing SFA with polyunsaturated fat lowers coronary heart disease risk: ~10% risk reduction for a 5% energy substitution. Conversely, replacing SFA with carbohydrate has no benefit, and replacing SFA with monounsaturated fat has uncertain effects. Evidence for effects of SFA on vascular function, insulin resistance, diabetes, and stroke is mixed, highlighting need for further investigation of these endpoints. Public health emphasis on reducing SFA consumption without considering the replacement nutrient or, more importantly, the many other food-based risk factors for cardiometabolic disease is unlikely to produce substantial intended benefits.

#### Update of French Nutritional Recommendations for Fatty Acids; New Approach for Saturated Fatty Acids

P. Legrand<sup>(1)</sup>

<sup>(1)</sup>Agro Campus West, France

The French Food Safety Agency (ANSES) recently published the adult French population reference intakes ?Apports Nutritionnels Conseillés? (ANC) for fatty acids. Some FA are essential, some are called ?conditionally essential? and other FA (polyunsaturates, monounsaturates, and saturates) are nutrients that can be synthesized de novo. The goal is to help build a credible diet, qualitatively and quantitatively, for maintaining good health of healthy individual. ANC is a reference value encompassing the physiological requirements for almost the entire population, similarly to ?adequate intake? (AI). ANC are innovative because recommendations for each FA studied has been established from minimum physiological requirements and by considering the physiopathological aspects. This led to: New proportion of lipids in

energy intake; Confirmed RI for linoleic acid (cardiovascular prevention and risk of excess), maintain of the linoleic acid/?-linolenic acid ratio under 5; Increased value for ?-linolenic acid upward (cardiovascular prevention; increase conversion to EPA and DHA); Increased value for DHA; RI for EPA and oleic acid? Concerning saturated fatty acids (SFA), distinction among SFA has been made. Dietary saturated fatty acids (SFA) are usually associated with deleterious consequences because of their impact on atherosclerosis biomarkers, when consumed in excess. However, each SFA exhibits metabolic and physiological specificities, some of them having important biochemical functions. The review will focus on: new aspects on the metabolism and biological functions of SFA and current investigations on their physiological roles. Myristic acid has new regulatory and signal functions through protein acylation. A large review of the SFA functions suggests that SFA

## **Perspectives on fat intake, health claims, and circulating fatty acids in Canadians.**

D. Ma<sup>(1)</sup>

<sup>(1)</sup>University of Guelph, Canada

This presentation will provide a broad overview of current perspectives on Canadian consumption of dietary fat, health claims centred on fats, and data on circulating plasma levels of fatty acids. Findings from the Canadian Community Health Survey (CCHS) provide important insights into fat intake which suggests that many Canadians are reducing their fat consumption. As part of the broader aim to educate consumers about nutrition, disease risk reduction health claims are permitted but only after rigorous evaluation. Claims related to saturated, trans, and unsaturated fats are approved for use by food manufacturers. A recently approved health claim for the replacement of saturated fat by unsaturated fat for reducing blood cholesterol will be presented. While dietary fats importantly influence health, little is known about the relationship between circulating plasma fatty acids and health. Recent data on circulating levels of plasma fatty acids in a cohort of young Canadians will be presented. Overall, dietary fat continues to remain an important area of health concern and study.

## **RBC Omega-3 Status of South Asian and Caucasian Canadians**

N. Ratnayake<sup>(1)</sup>, R. Nagasaka<sup>(2)</sup>, C. Gagnon<sup>(3)</sup>, E. Swist<sup>(4)</sup>, I. Rondeau<sup>(5)</sup>, P. Laffey<sup>(6)</sup>, I. Massarelli<sup>(7)</sup>, W. Cheung<sup>(8)</sup>

<sup>(1)</sup>Health Canada, Canada <sup>(2)</sup>Health Canada, Canada <sup>(3)</sup>Health Canada, Canada <sup>(4)</sup>Health Canada, Canada <sup>(5)</sup>Health Canada, Canada <sup>(6)</sup>Health Canada, Canada <sup>(7)</sup>Health Canada, Canada <sup>(8)</sup>Health Canada, Canada

Omega-3 index, which is defined as % EPA + DHA in total fatty acids of red blood cells (RBC) is a biomarker for omega-3 intake and coronary heart disease (CHD) risk. Canadian population has not yet been assessed for the omega-3 status. Canadians of South Asian descent makes up one of the largest non-European groups in Canada and they are at increased risk for CHD. This study determined the omega-3 index relative to cutoffs defined for CHD high risk (?4%), moderate risk (4 ? 8%) and low risk (?8%) for South Asians and Caucasians aged 20 to 79 years living in the National Capital Region of Canada in winter and summer 2012. Approximately 20% of the South Asians had omega-3 index ? 8% for both winter and summer, whereas only 9.4% and 11% of Caucasians in winter and summer, respectively, met this cutoff of for low risk CHD. For both ethnic groups, most of the population was in the moderate CHD risk range of 4-8%, for which the prevalence was 82-83% and 74-75% for Caucasians and South Asians, respectively, for both winter and summer. These data demonstrate that consumption of fish and other sources of EPA and DHA should be encouraged in Canada to meet the omega-3 index of 8% for cardioprotection.

## **Molecular and supramolecular structures of lipids in foods: impact on fatty acid bioavailability and lipid**

## metabolism

M. Michalski<sup>(1)</sup>, o. Steering Committee<sup>(2)</sup>

<sup>(1)</sup>INRA USC1362, CarMeN laboratory, France, Metropolitan <sup>(2)</sup>RMT LISTRAL, France

The nutritional properties of lipids may be studied beyond their energy content and fatty acid profile. Indeed, dietary fatty acids are building blocks of different lipid molecules such as triacylglycerols and phospholipids, themselves organised in various supramolecular structures. They can present different thermal behaviours and be embedded in complex food matrixes. Recent studies have revealed that the multiscale structures of lipids and their liquid or solid state at the body temperature influence both the digestibility and metabolism of dietary fatty acids. This presentation will review recent knowledge on the impact on fatty acid digestion, absorption and metabolism of the intramolecular structure of triacylglycerols, the phospholipids vs triacylglycerols carriers of fatty acids, and the supramolecular organization and physical state of lipids in different food products. Integrating such data in dietary recommendations should be considered in the future.

## Panel Discussion and Q&A

## TUESDAY

### MORNING

#### **H&N 2: Nutritional Needs of the Military**

Chair(s): J. Rood, Pennington Biomedical Research Center, USA; C. Lammi-Keefe, Louisiana State University, USA

#### **Omega-3s and Their Use for the Prevention, Treatment, and Rehabilitation of Traumatic Brain Injury (TBI) and Concussions**

M. Lewis<sup>(1)</sup>

<sup>(1)</sup>Brain Health Education and Research Institute, United States of America

Omega-3 fatty acids have numerous proven benefits including support of cardiovascular and psychiatric health. An emerging concept is the use of omega-3 fatty acids for the prevention, treatment, and rehabilitation of traumatic brain injuries and concussions. Omega-3s are the nutritional foundation of the brain and without an optimal supply, the brain is less likely to be able to heal following injury. Omega-3s provide benefits at the cellular level through the modulation of the neuroinflammatory cascade, promotion of neuronal survival, and neurogenesis and neurite development. Promising research and evolving clinical experience indicate that omega-3s are useful and effective for brain injuries. The basic science of omega-3s and brain injury will be discussed along with numerous clinical vignettes. Straight forward, common sense clinical protocols are proposed for the prevention of head trauma and treatment of sports concussions and more severe injuries. Given their safety profile, availability, and affordability, omega-3s should be considered as part of the care and protection regimen for those at risk or having experienced head trauma.

## **Poultry diets low in omega-6 and high in omega-3 fats on chicken eggs and meat: utility in U.S. Military Diets**

B. Marriott<sup>(1)</sup>, J. Hibbeln<sup>(2)</sup>, A. DiChiara<sup>(3)</sup>, F. DeMeester<sup>(4)</sup>, M. Hawes<sup>(5)</sup>, C. Champagne<sup>(6)</sup>

<sup>(1)</sup>Medical University of South Carolina, United States of America <sup>(2)</sup>NIAAA, NIH, United States of America <sup>(3)</sup>U.S. Army Natick Soldier Systems Center Laboratories, United States of America <sup>(4)</sup>DMF-U.S. Inc., United States of America <sup>(5)</sup>DMF-U.S. Inc., United States of America <sup>(6)</sup>Pennington Biomedical Research Center, United States of America

Eggs and chicken meat are the most widespread protein products in the U.S. diet. Poultry products also provide the highest level of protein at the best economic price point in the U.S. For the nearly 3 million active duty and reserve U.S. military personnel, poultry products can serve as a delivery mechanism for nutrients to improve military resilience and optimal performance. Poultry products also deliver substantial amounts of essential fatty acids. Diets low in omega-6 and high in omega-3 fatty acids are of interest not only for cardiovascular outcomes, but also for reducing mental ill health. It has long been established that lowering omega-6 linoleic acid (LA) in rodent diets increases endogenous conversion of alpha-linolenic acid (ALA) to eicosapentanoic (EPA) and docosahexanoic acid (DHA), which we applied here to broiler and layer hen diets. In a series of diet levels of LA, ALA, and EPA/DHA were altered to achieve maximal concentration of EPA/DHA and minimal concentrations of arachidonic acid (AA) in poultry eggs and meat. Products were evaluated for palatability and sensory properties. These products are currently being feed to volunteers in a randomized controlled trial to model possible changes in military garrison diets. Support: USAMRAA: W81XWH-06-2-0009, Military Operational Medicine Research Programs (MOMRP) to the Samueli Institute; NIAAA; the U.S. Army Natick Soldier Systems Center; Wenger feeds; DuPont/Pioneer.

## **Vitamin D status and military populations: implications for bone health**

J. McClung<sup>(1)</sup>

<sup>(1)</sup>US Army Research Institute of Environmental Medicine, United States of America

Vitamin D is an essential nutrient for maintaining bone health. Previous work from our laboratory indicates that vitamin D status may decline in Soldiers during US Army initial military training (IMT). Furthermore, a significant portion of Soldiers entering IMT may have serum vitamin D levels below cut-off values utilized to identify individuals with poor vitamin D status. Stress fractures may affect up to and 5% of male and 21% of female Soldiers during training, resulting in attrition in up to 60% of affected personnel. A recent report indicates that vitamin D and calcium supplementation may attenuate stress fracture risk by up to 20% in female US Navy recruits. However, biochemical measures of nutritional status and associated markers of bone health were not collected in that study. The objective of this presentation is to detail studies assessing vitamin D status in military populations, and to outline a randomized, double-blind, placebo-controlled trial that will aim to determine the effect of daily vitamin D and calcium supplementation (800 IU and 2000 mg/day, respectively) on biochemical indicators of nutritional status, to include serum vitamin D and markers of bone turnover during IMT.

## **Omega-3 fatty acids and acute stress in military personnel: interpreting fatty acid measures**

E. Farina<sup>(1)</sup>, J. McClung<sup>(2)</sup>, H. Lieberman<sup>(3)</sup>

<sup>(1)</sup>US Army Research Institute of Environmental Medicine, United States of America <sup>(2)</sup>US Army Research Institute of Environmental Medicine, United States of America <sup>(3)</sup>US Army Research Institute of Environmental Medicine, United States of America

Observational data indicate positive relationships between n-3 fatty acids (FA) and mental health. FA metabolism may be disrupted in depression, but relationships between n-3 FAs and acute stress have not been examined. Thus, we

utilized multiple regression modeling to determine associations between fasted baseline serum n-3 FA expressed as % of total FA [ $\alpha$ -linolenic acid (ALA, 18:3), eicosapentaenoic acid (EPA, 20:5), and docosahexaenoic acid (DHA, 22:6)]; midday baseline saliva hormone concentrations; and mood among US Army Soldiers ( $n=59$ ,  $26.9\pm 2.9$  y) during Survive, Evade, Resist, Escape (SERE) school. Mood, assessed by the Profile of Mood States, was evaluated following an acutely stressful simulated interrogation conducted during ~12 days of intermittent sleep and food deprivation. ALA was associated with increased cortisol ( $P<0.01$ ), and EPA was inversely associated with testosterone ( $P=0.04$ ) and dehydroepiandrosterone-sulfate ( $P=0.02$ ), after adjustment for covariates. EPA was associated with increased total mood disturbance ( $P=0.01$ ), tension ( $P=0.02$ ), depression ( $P=0.03$ ), anger ( $P=0.05$ ), fatigue ( $P=0.02$ ), and confusion ( $P=0.01$ ), after adjustment for hormones and covariates. These data suggest higher levels of EPA prior to stress exposure may be negatively associated with acute stress resilience. (Support: Office of Naval Research/US Army Medical Research and Materiel Command)

## Panel Discussion and Q&A

### AFTERNOON

#### EAT 3/H&N 3: Algal Oil - Food Applications and Nutritional Aspects

Chair(s): W. Rakitsky, Solazyme, Inc., USA; E. Bailey-Hall, DSM Nutritional Products, USA

#### Microalgae Triglyceride Oils: a new Source of oil for Food Industry Applications

W. Rakitsky<sup>(1)</sup>, R. Bond<sup>(2)</sup>

<sup>(1)</sup>Solazyme, Inc., United States of America <sup>(2)</sup>Solazyme, United States of America

Solazyme, Inc. is a renewable oil and bioproducts company that transforms a range of low-cost plant-based sugars into high-value tailored oils. Headquartered in South San Francisco, Solazyme's renewable products can replace or enhance the properties of triglyceride oils derived from the world's three dominant sources: petroleum, plants and animals. Harnessing the oil-producing capabilities of microalgae, Solazyme's biotechnology platform utilizes industrial standard fermentation equipment to efficiently scale and accelerate microalgae's natural oil production time from weeks to merely a few days. By feeding simple plant sugars to proprietary microalgae strains in industrial fermentation vessels, the company is in effect taking advantage of "indirect photosynthesis", in contrast to the traditional open-pond approaches most often associated with microalgae. Through Solazyme's unique tailoring technology ability, the company has been able to optimize specific oil profiles ideal for various food applications. These profiles have superior performance characteristics when compared to currently available food oils and provide unprecedented opportunities for optimizing the balance of health and wellness with flavor and texture delivery. Throughout this presentation, Dr. Walt Rakitsky will discuss the innovative benefits that Solazyme's algal oils provide the food industry, including providing alternatives to partially hydrogenated vegetable oils, animal fats, oils and palm oil fractions, and beyond the possibilities of traditional plant and seed oils. He will also explore the impact of Solazyme's tailoring on important oil characteristics such as viscosity, oxidative stability, solid fat content profiles and pour points, among many others.

#### Functionality Matching of two Algal Oils With Vastly Different Molecular Compositions

A. Marangoni<sup>(1)</sup>, E. Co<sup>(2)</sup>, W. Rakitsky<sup>(3)</sup>

<sup>(1)</sup>University of Guelph, Canada <sup>(2)</sup>University of Guelph, Canada <sup>(3)</sup>Solazyme Inc., United States of America

The nutritional and functional properties of edible fats have been traditionally associated with specific chemical compositions and crystallization conditions. Switching from one oil source to another is usually a difficult task since both the melting behavior and structure of the fat changes dramatically, leading to adverse changes in functionality. In recent history, we can recall the painful period when partially hydrogenated fats were replaced with palm oil and palm oil fractions. In this work we demonstrate how the yield stress, elastic modulus, polymorphism, microstructure and melting profile of two fats with vastly different chemical compositions can be matched. Solazyme oil A contains greater than 62% (w/w) medium chain fatty acids, or MCT (C8:0-C14:0), 23% (C16:0+C18:0) and 9% C18:1, while Solazyme oil B contains less than 2% C8:0-C14:0, 54% (C16:0+C18:0) and 29% C18:1. Oil A is a medium chain triglyceride rich fat, while Oil B resembles palm oil. Both oils had a solid fat content of ~45% at 20°C, and very similar SFC versus temperature profiles. DSC melting profiles showed two major peaks centered around ~12-13°C and ~28-35°C. Both fats were in the beta-prime polymorphic form and displayed asymmetric, elongated crystallite morphology with characteristic features. The yield stresses and storage moduli (G') of Solazyme A and B oils were 520-550 Pa, and 7x10<sup>6</sup>Pa-1.8x10<sup>7</sup>Pa, respectively. A yield stress and G' in this range suggests a satisfactory functionality as a roll-in shortening. Using algal biotechnology, it was possible to alter the chemical composition of a food while retaining its lamination functionality.

### **Evaluation of the performance of a range of algal oils in food frying applications**

M. Matlock<sup>(1)</sup>, T. Tiffany<sup>(2)</sup>, M. Pietz<sup>(3)</sup>, R. Bond<sup>(4)</sup>

<sup>(1)</sup>ADM, United States of America <sup>(2)</sup>ADM, United States of America <sup>(3)</sup>ADM, United States of America <sup>(4)</sup>Solazyme, United States of America

Results generated in ADM's R&D laboratory will be presented from 10-day frying tests exploring the properties of Solazyme's tailored algal oils versus commercially available high-stability vegetable frying oils. Quantitative measurements of quality and fry life such as development of total polar materials, free fatty acid levels, para-anisidine values, and oxidative stability index (OSI) over time will be discussed. Other parameters measured include color stability and GC/MS headspace analysis of volatile products, as well as sensory properties by a trained internal panel. Variables explored include the impact of natural and synthetic antioxidants and frying with and without defoamers. The three different algal triglyceride oils tested had fatty acid compositions ranging from a mid-oleic (60%) to an oil composed of >85% oleic with near-zero polyunsaturates. Results indicated that all three algal oils evaluated in this frying procedure exhibited similar or improved oxidative, hydrolytic stability and color stability to that of a commercial premium vegetable frying oil. One algal oil showed a higher OSI at the end of ten days frying ? without antioxidant additives ? than the control oil, a high oleic/low linolenic canola oil, had at the beginning of testing. Remarkably, this same algal oil, without dimethylpolysiloxane antifoamer additive, did not show foaming even after ten days of frying. Throughout this presentation, a thorough analysis of frying characteristics will be used to demonstrate how the compositions of algal oils derived from Solazyme's technology platform affected performance under different experimental conditions, as seen through ADM's experienced laboratory frying protocols.

### **Challenges of PUFA Fortifications in Food and Beverages**

W. Wang-Nolan<sup>(1)</sup>, X. Tang<sup>(2)</sup>, M. Stefanski<sup>(3)</sup>, O. Pena<sup>(4)</sup>, G. Su<sup>(5)</sup>

<sup>(1)</sup>DSM Nutritional Products, United States of America <sup>(2)</sup>DSM Nutritional Products, United States of America <sup>(3)</sup>DSM Nutritional Products, United States of America <sup>(4)</sup>DSM Nutritional Products, United States of America <sup>(5)</sup>DSM Nutritional Products, United States of America

Fortifications of algal oil in food, even at 32 mg DHA/serving of food, can be meaningful based on daily intake of long-chain omega-3 polyunsaturated fatty acids from a typical American diet. To make the food and beverage products successful in maintaining product shelf life, both algal oil stability as well as its stability after incorporation into foods are important. The types of measures to maintain the product stability largely depend on the oxidation mechanisms; thus they are functions of food matrices, flavors, storage conditions, packaging and shelf life requirements.

## **Algal DHA , Nutrition and Cognitive Activity**

E. Nelson<sup>(1)</sup>

<sup>(1)</sup>DSM Nutritional Lipids, United States of America

The reason for studying algal DHA as a bioactive for improving cognitive ability is based on several lines of evidence, including 1. Elevated prenatal DHA levels are associated with improved infant IQ, 2. Breast feeding data suggesting benefits and 3. Consumption of DHA (and other LCPUFA) from fish is linked to a lower incidence of Alzheimer's Disease (AD). We conducted, sponsored or were associated with several studies which demonstrated the bioactivity of algal DHA in benefiting cognitive behavior in some subject populations. These studies included nursing infants, preschool and school age children, and older adults with or without AD. They were carried out in the US and UK. All were double blind placebo controlled trials. The overall conclusion from these studies is that a cognitive benefit of DHA can be shown in certain subject populations, when the correct subject population is selected, the correct measurements are used, a proper amount of DHA is selected and a reasonable time frame of use is allowed. I will review these trials and place them in context with literature associated with this field.

## **Long-Chain Polyunsaturated Fatty Acid (LCPUFA) Nutrition for Athletes**

E. Barrett<sup>(1)</sup>

<sup>(1)</sup>DSM Nutritional Products, United States of America

DHA and EPA are long-chain polyunsaturated fatty acids (LCPUFAs) that are important for serious athletes as well as weekend warriors. LCPUFAs support a healthy heart which is essential for athletic performance. LCPUFAs reduce inflammation, muscle damage, and muscle soreness induced by exercise. LCPUFAs are also being investigated as a therapy for concussions. Although concussions are recognized as a major public health concern, an effective therapy for concussions is not available. DHA is the primary structural LCPUFA in the brain and a nutrient critical for brain development and cognitive functions throughout life. As such, DHA is being evaluated as a therapy for concussions. In animal studies, DHA supplementation before or after injury protects the brain by reducing axonal damage, reducing inflammation and cell death, and limiting injury-induced brain dysfunction. Exercise places increased demands on the cardiovascular system, making heart health essential for athletic performance. Many components of the diet affect heart health, and LCPUFAs in particular are recognized as essential for normal heart function. In clinical trials LCPUFA supplementation reduces heart rate at rest and during exercise. LCPUFAs also increase stroke volume, cardiac output, and blood flow and oxygen delivery to exercising muscles. Inflammation is a normal response to prolonged and intense exercise. Unresolved inflammation, however, results in prolonged muscle soreness and reduced physical performance. In clinical studies, LCPUFAs counteract exercise-induced inflammation, reducing blood markers for inflammation and muscle damage. They also reduce levels of perceived pain, improve range of motion, and reduce arm/thigh circumference (swelling).

## **The effect of phytosterols and omega 3 fatty acids on fatty streak in LDLr-knockout mice**

I. Castro<sup>(1)</sup>, P. Botelho<sup>(2)</sup>, K. Mariano<sup>(3)</sup>, J. Guimaraes<sup>(4)</sup>

<sup>(1)</sup>Faculty of Pharmaceutical Sciences of University of São Paulo, Brazil <sup>(2)</sup>Faculty of Pharmaceutical Sciences of University of São Paulo, Brazil

<sup>(3)</sup>Faculty of Pharmaceutical Sciences of University of São Paulo, Brazil <sup>(4)</sup>Faculty of Pharmaceutical Sciences of University of São Paulo, Brazil

Atherosclerotic process begins early in life and is progressive. As drug therapy is not recommended for healthy children, supplementation with bioactive compounds may be an alternative approach by which to prevent atherosclerosis. To evaluate this hypothesis, LDLr knockout mice were supplemented by gavage with omega 3 fatty acids (n-3 FA) (stearidonic acid - SDA from Echium oil and docosahexaenoic acid - DHA from algae oil) with or without phytosterols during the first 2 months of life. Subsequently, dyslipidemia and oxidative stress were induced by a high-fat diet intake for 2 months. Instead of being reduced, an increase was observed in fatty streaks lesion area in the aorta artery following isolated phytosterol supplementation. This effect was fully reversed by co-supplementation with both forms of n-3 FA. The mechanisms by which n-3 FA reversed the phytosterol-induced increase in fatty streaks area involved the activation of fatty acid oxidation by Peroxisome Proliferator Activated Receptor ? (PPAR-?) and a reduction in fatty acid synthesis by Liver X Receptor ? LXR-?). Moreover, supplementation with Echium oil exhibited a clear trend (p=0.048) to reduce polyunsaturated fatty acid oxidation in the liver, by a mechanism that did not involve the modulation of antioxidant enzyme activity or expression. We conclude that to reduce the risk of atherosclerosis, the most desirable food supplementation for children was conferred by n-3 FAs, especially SDA from Echium oil. More studies are necessary to clarify the phytosterol effect as a promoter of fatty streak lesion when associated with a high-fat diet.

## WEDNESDAY

### MORNING

#### **ANA 4.1/H&N 4: Advances in Analytical Aspects of Lipid Nutrition**

Chair(s): S. Bhandari, Silliker Inc., USA; B. Ward, Utah State University, USA

#### **Assessing Stability of Fingertip Prick and Venous Whole Blood During Long-term Storage and Associated Mechanisms of Degradation**

A. Methere<sup>(1)</sup>, K. Stark<sup>(2)</sup>

<sup>(1)</sup>University of Waterloo, Canada <sup>(2)</sup>University of Waterloo, Canada

High-throughput omega-3 profiling can be enabled by rapid blood collection techniques such as fingertip prick (FTP) blood sampling. However, improved ease of collection increases the number of samples collected and places pressure on storage capacities. In the past, fatty acid oxidation of highly unsaturated fatty acids (HUFA) in erythrocytes has been observed at storage temperatures of -20°C. The tendency for oxidation of HUFA in FTP and venous whole blood has not been well characterized. Presently, FTP and venous whole blood samples were stored for up to 6 months under multiple temperature (room temperature (RT), 4°C, -20°C, -75°C), antioxidant (butylated hydroxytoluene(BHT), no BHT) and omega-3 content (low vs. high) conditions. Significant reductions in HUFA levels were observed starting after 1 day at -20°C, 30 days at 4°C and 60 days at RT. No degradation was observed at -75°C. Mechanisms involved in the expedited -20°C degradation were further assessed by storing samples with glycerol, a cryoprotection agent, and/or deferoxamine, an iron chelator. Cryoprotection of FTP whole blood with glycerol prevents haemolysis and HUFA degradation as compared with controls. In addition, iron chelation significantly reduced HUFA degradation to 10% after 7 days of storage at -20°C compared to 72% in untreated controls. In conclusion, BHT treatment protects against FTP and venous whole blood HUFA degradation and high omega-3 blood is more resistant to degradation, and



degradation during storage at -20°C appears to be due to an iron-mediated peroxidation mechanism as a result of haemolysis during the freeze-thaw process.

## **Improved Lipid Profile in Hyperbilirubinemic Subjects Contributes to Cardiovascular Protection**

K. Wagner<sup>(1)</sup>

<sup>(1)</sup>University of Vienna, Austria

Gilbert's syndrome (GS) is characterized by a benign, mildly elevated bilirubin concentration in the blood and affects up to 10% of the population. Recent reports show clear protection from cardiovascular disease in this population. Protection of lipids, proteins and other macromolecules from oxidation by bilirubin represents the most commonly accepted mechanism contributing to protection in this group. However, a recent meta-analysis estimated that bilirubin only accounts for ~34% of the cardioprotective effects within analysed studies. To reveal the additional contributing variables we have explored circulating cholesterol and triglyceride concentrations, which appear to be decreased in hyperbilirubinemic individuals and animals, and are accompanied by lower body mass index in highly powered studies. These results suggest that bilirubin could be responsible for the development of a lean and hypolipidemic state in GS. Within the presentation it will also be discussed the possible contributing mechanisms that might reduce circulating cholesterol and triglyceride concentrations, proinflammatory cytokines or LDL subfractions in individuals with syndromes affecting bilirubin metabolism/excretion. Further the antioxidative potential of bilirubin will be addressed.

## **Plasma, Erythrocytes and Whole Blood Fatty Acids: Translating Compositional Data**

K. Stark<sup>(1)</sup>, J. Aristizabal Henao<sup>(2)</sup>, A. Metherel<sup>(3)</sup>, L. Pilote<sup>(4)</sup>, f. GENESIS PRAXY investigators<sup>(5)</sup>

<sup>(1)</sup>University of Waterloo, Canada <sup>(2)</sup>University of Waterloo, Canada <sup>(3)</sup>University of Waterloo, Canada <sup>(4)</sup>McGill University Health Centre, Canada <sup>(5)</sup>McGill University Health Centre, Canada

Fatty acid composition can be determined from a variety of blood fractions with erythrocytes recommended for determining eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) status. Plasma and whole blood samples are often used and can be difficult to compare with studies using erythrocytes. The fatty acid composition of total lipids of plasma (PTLE), erythrocytes (RBC) and whole blood (WB) from participants of the GENESIS PRAXY study (n = 789) were determined by gas chromatography. The fatty acid composition of plasma phospholipids (PPL), plasma triacylglycerols (PTAG), plasma cholesteryl esters (PCE) and plasma free fatty acids (PFFA) were also examined for a subset of 40 participants. The relative percentage of eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) was  $3.82 \pm 1.20\%$  in RBC,  $2.91 \pm 0.97\%$  in WB, and  $2.67 \pm 0.97\%$  in PTLE (n= 789). RBC EPA+DHA was significantly correlated to PTLE and WB EPA+DHA ( $r > 0.70$ ,  $P < 0.001$ , for both, n = 789) with  $RBC\ EPA+DHA = 0.93(PTLE\ EPA+DHA) + 1.34$  and  $RBC\ EPA+DHA = 1.11(WB\ EPA+DHA) + 0.6$ . PPL, PCE and PTAG EPA+DHA were very strongly correlated to RBC EPA+DHA ( $r > 0.80$ ,  $P < 0.001$ , for all) with  $RBC\ EPA+DHA = 0.93(PPL\ EPA+DHA) + 0.55$ ,  $RBC\ EPA+DHA = 1.59(PCE\ EPA+DHA) + 2.05$ , and  $RBC\ EPA+DHA = 2.71(PTAG\ EPA+DHA) + 2.56$ . PFFA EPA+DHA was also correlated to RBC EPA+DHA ( $r = 0.67$ ,  $P < 0.001$ ). In conclusion, EPA+DHA determined from various blood fractions including whole blood can be used to estimate the EPA+DHA in erythrocytes.

## **Relative Role of Dietary fat Amount and Structure in the Meal on the Secretion of Chylomicrons and Associated Endotoxin Transport in Lean and Obese Humans**

C. Vors<sup>(1)</sup>, F. Laugerette<sup>(2)</sup>, G. Pineau<sup>(3)</sup>, H. Vidal<sup>(4)</sup>, M. Laville<sup>(5)</sup>, M. Michalski<sup>(6)</sup>

<sup>(1)</sup>INRA USC1362, CarMeN Laboratory, France <sup>(2)</sup>INRA USC1362, CarMeN laboratory, France <sup>(3)</sup>INRA USC1362, CarMeN laboratory, France <sup>(4)</sup>INSERM U1060, CarMeN laboratory, France <sup>(5)</sup>CENS, France <sup>(6)</sup>INRA USC1362, CarMeN laboratory, France

Postprandial hyperlipemia and low-grade inflammation are major metabolic features in obesity that increase the risk of cardiovascular diseases. Moreover, we recently reported the role of gut endotoxin absorption during the digestion of lipids, partly due to endotoxin transport by chylomicrons during their secretion. Our new objective was to test the impact of different lipid amounts and structures in the meal on both chylomicronemia and associated endotoxin transport in humans. Therefore, 8 lean and 8 obese volunteers digested 10g or 40g of unemulsified dairy fat (spread on bread + drink) or 40 g emulsified in a drink (+ bread). Plasma and chylomicrons were collected during 8h of digestion. Chylomicron triglycerides and endotoxemia were analyzed. Increasing fat amount from 10g to 40g resulted in increased postprandial chylomicronemia both in lean and obese subjects, as expected. However, in obese subjects, this was associated with an increased postprandial accumulation of endotoxins in plasma, which was not observed in lean subjects ( $P < 0.05$ ). When 40g fat was emulsified, chylomicron triglycerides increased sooner and sharper than with 40g of unemulsified fat, in both groups. In lean subjects, this did not affect endotoxin transport by chylomicron, while in obese subjects, this enhanced early endotoxemia of the chylomicron fraction 60 min after meal ( $P < 0.05$ ). Altogether, our results show that (i) the amount and structure of fat in the meal modify the kinetics of chylomicronemia and (ii) this has an impact on the properties of postprandial endotoxemia in obese subjects. Long-term consequences on low-grade inflammation thus deserve to be elucidated.

### **LCMS Analysis of Vitamins D2, D3, 25(OH)D2 and 25(OH)D3 in Food Samples**

S. Bhandari<sup>(1)</sup>, H. Wu<sup>(2)</sup>, T. Gallegos-Peretz<sup>(3)</sup>

<sup>(1)</sup>Silliker Laboratories, United States of America <sup>(2)</sup>Silliker Laboratories, United States of America <sup>(3)</sup>Silliker Laboratories, United States of America

Studies were performed to validate a LC-MS/MS method to quantify vitamins D2, D3, 25(OH)D2 and 25(OH)D3 in various food matrices. The method gave a linear response in relation to analyte concentration in tested range for all the four analytes. The method was found to be accurate for vitamin D3 analysis by testing the certified and in house reference materials and by comparing results with that of a reference method. The accuracy of the method for all the analytes was also demonstrated by a satisfactory spike recovery of the respective analyte in a variety of matrices. The replicate analysis of analytes in different matrices, spiked and unspiked demonstrated a satisfactory precision of the method. Identity of the analytes in the tested samples was established by a satisfactory ratio of the quantifier to qualifier transitions for the analyte in the tested matrices. The study also determined the LOD and LOQ of the method for all the analytes. The method differs in extraction from the conventional methods in the use of a solid- supported liquid-liquid extraction (SLE) instead of liquid-liquid extraction (LLE). The SLE method is about 4 times more efficient than the one reported earlier using LLE.

### **Effect of dairy product consumption on cognitive performance among elderly participants of the Cache County Study on Memory, Health and Aging.**

R. Ward<sup>(1)</sup>

<sup>(1)</sup>Utah State University, United States of America

The objective of this study was to investigate any role dairy product consumption may have in protection against cognitive decline among elderly participants in the Cache County Study on Memory, Health and Aging. This entailed analysis of the fatty acid methyl esters (FAMES) obtained from approximately 2,000 red blood samples with a focus

on those associated with dairy fat, C15:0, C16:1n7t and C17:0. Data from the Modified-Mini Mental State Exam (3MS) was available from three waves of the study. Among the 3,364 subjects evaluated at baseline, eleven categories of dairy products were estimated: skim milk, 2% milk and higher fat, chocolate milk, cream, sour cream, ice cream, yogurt, cottage cheese, cream cheese, other cheese, and butter. Intakes were estimated from the Food Frequency Questionnaires. Mean baseline intake was 2.7 cups per day. Approximately 9% of the respondents abstained from dairy. Plasma phospholipid C16:1n7t content was below the detection limit in RBC samples while 15:0 and 17:0 were 0.2% and 0.4% of RBC FAMES, respectively. Baseline 3MS scores were compared between groups separated by dairy intake and found to be highly significant. 2.5 units of dairy was associated with the highest 3MS scores at baseline. The association remained at wave 3 when there was a FFQ administered and it is from this time point that the RBCs were collected. In sum, our data indicate that intake of 2.5 units of dairy per day was associated with the highest 3MS scores.

## **Generating Meaningful Results with Lipidomics**

S. Watkins<sup>(1)</sup>

<sup>(1)</sup>Metabolon, United States of America

Metabolomics has progressed over the last ten years to become a routine analytical technology for discovery applications, monitoring bioprocessing reactions and for clinical and diagnostic discovery and development. The quality of most metabolomic approaches can be assessed using the conventional measures of quantitation, throughput, breadth and cost. Data sets are often simply lists of metabolite identities and quantities within a given biological matrix. These data sets are largely sufficient to map the results to biological pathways and to begin to make interpretations of study results. Lipids, however, are special cases among metabolites and require unique handling. This is because the pathways that regulate lipids operate at multiple and distinct levels including, at least, the levels of: intermediary (fatty acids, sterols), complex (triacylglycerol, phospholipids) and aggregate (membranes, lipoproteins) lipid metabolism. Thus, there are more dimensions of relationships among lipid metabolites than are described in a standard biochemical pathway map, and changes in lipid metabolism occurring at one level of metabolism have an impact on the entire lipid profile. The techniques for measuring and reporting lipids in an interpretable way must therefore be capable of identifying not only the changes but the causes of the change. As an example, one should probably not interpret a global drop in plasma triglyceride levels as an independent decrease in the concentration of hundreds of individual triglyceride species. This talk will identify some of the major challenges facing building a lipidomics platform that yields interpretable results, and provide both real world examples and potential solutions to these problems.

## **Targeted Lipidomics Of Signaling Sphingolipids In Health And Disease**

E. Berdyshev<sup>(1)</sup>

<sup>(1)</sup>University of Illinois at Chicago, United States of America

Our knowledge about distribution, qualitative and quantitative composition of lipid molecules in natural systems is experiencing a tremendous rise during last fifteen years. This would not be possible without recent progress in analytics of non-volatile lipid molecules. This progress was achieved through the invention of ion sources capable of handling solvent flows of ordinary HPLC systems with a resulting expansion in LC/MS-based methods for direct analysis of complex lipid mixtures without their preliminary chemical processing. In case of signaling sphingolipids, the progress in their analytics was supported by relative stability of analytes and by great attention given to these molecules playing critical role in such fundamental processes as cell survival, proliferation, and death. Great examples of such bioactive sphingolipids are sphingosine-1-phosphate and ceramides often playing opposite role in physiological responses. We are still learning about their physiological functions and deciphering the complexity of

their interaction with other signaling systems. Our better understanding of signaling pathways regulated by these sphingolipids now offers novel avenues for the treatment of multiple diseases and pathological conditions including cardiac arrest, pulmonary inflammation and fibrosis, and cancer.

## **Lipidomic Analysis of Essential Fatty Acids and their Metabolites in the Fat-1 Transgenic Mouse**

J. McKenzie<sup>(1)</sup>, G. Astarita<sup>(2)</sup>, J. Kang<sup>(3)</sup>

<sup>(1)</sup>Massachusetts General Hospital and Harvard Medical School, United States of America <sup>(2)</sup>Georgetown University, United States of America

<sup>(3)</sup>Discovery and Life Science, Waters Corporation, United States of America

Essential fats, such as omega-3 and omega-6 fatty acids, must be obtained through the diet and cannot be synthesized de novo in mammals. In the modern Western diet, n-6 consumption is dramatically higher than n-3 consumption, and many research studies demonstrate that this imbalance may impact many chronic diseases through differential modulation of inflammation. The fat-1 transgenic mouse model can endogenously convert omega-6 to omega-3 fatty acids to achieve a balanced omega-6/omega-3 tissue ratio. Studies have revealed that the fat-1 mouse is protected against a wide variety of chronic diseases including inflammatory diseases, atherosclerosis, diabetes, and cancer. However, a comprehensive characterization of lipid profiles in the fat-1 mouse using sensitive analytical techniques remains to be explored. In this study, we employed state-of-the-art, high-throughput assays (such as LC-MS/MS) to analyze bioactive lipid species in plasma and liver samples from fat-1 and wild-type mice, and revealed significant differences in lipid metabolites resulting from alteration of the omega-6/omega-3 tissue ratio. Our study has yielded valuable new clues about the underlying pathways and mechanisms involved in the health benefits associated with a balanced omega-6/omega-3 tissue ratio.

## **Dietary Fat Metabolism in Humans Using Deuterated Fatty Acids: Perceptions, Realities, Questions**

E. Emken<sup>(1)</sup>

<sup>(1)</sup>Midwest Research Consultants, United States of America

Perceptions, realities and questions related to the metabolism and health effects of nearly all the fatty acids in US diets were addressed by a multiple-labeled stable isotope experimental protocol that has significant advantages over single-isotope studies. For example, a series of human studies with <sup>2</sup>H-18:1 positional isomers dispelled allegations that *trans* isomers were poorly absorbed, accumulate in phospholipids, and adversely competed with oleic acid. The reality is that negative physiological properties reported for *trans* 18:1 isomers are inconsistent with their metabolic properties. The perception that humans were unable to convert 18:3n-3 to 20:5n-3 and 22:6n-3 but can readily converted 18:2n-6 to 20:4n-6 was investigated by feeding subjects a mixture of <sup>2</sup>H-18:3n-3 and <sup>2</sup>H-18:2n-6. The reality was that 18:3n-3 is converted to 20:5n-3 and 22:6n-3 and conversion of 18:2n-6 to 20:4n-6 is not greater than 18:3n-3 to 22:6n-3 conversion. The unresolved question: why is there a large variability between results from various studies and individuals for conversion of 18:3n-3 to 22:6n-3 and 18:2n-6 to 20:4n-6? The belief that interesterified fats may have negative health effects was addressed by dosing subjects with triglycerides labeled with <sup>2</sup>H-16:0 and 2H-18:2n-6 at specific *sn*-1(3) and *sn*-2 positions. The reality is that chylomicron TAG structures are rearranged after absorption and the *sn*-1(3) and *sn*-2 positions of dietary TAG does not influence fatty acid accretion but conversion of 18:2n-6 to 20:4n-6 was influenced. The questions: How is the rearrangement of chylomicron TAG structures accomplished? Why does the acyl TAG position influence conversion but not accretion?



**H&N 5: General Health and Nutrition**

Chair(s): H. Durham, Louisiana State University, USA; A. Metherel, University of Waterloo, Canada

**Food Matrix Effects on in Vitro Digestion of Microencapsulated tuna oil Powder**

Z. Shen<sup>(1)</sup>, C. Apriani<sup>(2)</sup>, R. Weerakkody<sup>(3)</sup>, L. Sanguansri<sup>(4)</sup>, M. Augustin<sup>(5)</sup>

<sup>(1)</sup>CSIRO Animal, Food and Health Sciences, Australia, Australia <sup>(2)</sup>CSIRO Animal, Food and Health Sciences, Australia <sup>(3)</sup>CSIRO Animal, Food and Health Sciences, Australia <sup>(4)</sup>CSIRO Animal, Food and Health Sciences, Australia <sup>(5)</sup>CSIRO Animal, Food and Health Sciences, Australia

Effects of food matrix on lypolysis of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs) in food fortified with microencapsulated (ME) tuna oil (TO) powder during in vitro treatment, were investigated. Orange juice, yogurt and cereal bar, used as food matrices, fortified with ME TO powder and neat ME TO powder, as a control sample, were exposed to simulated gastric fluid (SGF), or sequentially in SGF and simulated intestinal fluid (SIF). The changes in particle size of oil droplets during in vitro treatment were influenced by the type of food matrix used to deliver the ME TO powder. The released free LC n-3 PUFAs were analysed by GC with subtraction of fatty acid methylester (FAME) by alkali methylation from FAME by acid methylation. There was negligible or very low lipolysis of LC n-3 PUFAs after SGF exposure (4.4?6.1% EPA and ? 1.5% DHA). After sequential exposure to SGF and SIF, significantly higher levels ( $p < 0.005$ ) of lipolysis of LC n-3 PUFAs occurred to all samples. The released free EPA and DHA were 73.2?78.6% for the neat powder, fortified orange juice and yogurt and 60.3?64.0% for the fortified cereal bar. This research demonstrated that the nature of the food matrix may modify the availability of LC-n3 PUFAs in microencapsulated tuna oil incorporated into it, to lipolysis.

**The Relation of Alpha-linolenic Acid Consumption via Flaxseed oil Supplementation to Insulin Resistance in Type 2 Diabetics- the Delivery Throws a Curve Ball and so now What?**

D. Barre<sup>(1)</sup>

<sup>(1)</sup>Cape Breton University, Canada

Dietary alpha-linolenic acid (ALA, 18:3 n-3) consumption correlates negatively and significantly with homeostatic model assessment-insulin resistance (HOMA-IR) in type 2 diabetics (T2D) but this relationship has, to now, been mechanistically unexplored. It was hypothesised that elevated plasma mol % free ALA (FALA) would correlate significantly ( $p < 0.05$ ) and negatively with HOMA-IR due to plasma FALA-induced manipulation of fasting plasma glucose (FPG) and/or fasting plasma insulin (FPI) concentrations in T2D consuming flaxseed oil (FXO) (average 5.5 g/day of ALA) for three months. The purpose was to test this hypothesis. Patients were assigned to FXO (n=18) or placebo (safflower oil (SFO) (n=14)) capsules in this parallel, double blind, randomized, controlled (including smoking, diet, exercise, and medications) trial. Equal FXO or SFO oil mass per kg body weight was consumed per day. At baseline, no statistically significant correlation between HPLC-analysed plasma mol % FALA and HOMA-IR existed. Only FXO consumption yielded a significant Pearson correlation between plasma mol % FALA and HOMA-IR ( $r = - 0.552(p=0.022)$ ). FPG, FPI and HOMA-IR levels were unchanged (treatment versus placebo) when comparison, by unpaired T-test, of differences yielded by subtraction of post-treatment values from pre-treatment values for FPG, FPI and HOMA-IR levels for FXO versus SO consumption was done. It is concluded that FXO yielded plasma FALA's negative correlation with HOMA-IR but any contributory potential of elevated plasma FALA to HOMA-IR reduction was overcome by ALA's FXO delivery (dose and form). Otherwise formatted ALA delivery may decrease HOMA-IR in T2D via increased plasma mol % FALA.

## Hepatic $\Delta$ 6-desaturase and Docosahexaenoic Acid are Increased by Supplementation of Ovariectomized Rats With $17\beta$ -estradiol, but not Progesterone

Higher docosahexaenoic acid (DHA) in women compared with men and in pregnant compared to post-partum women suggests that ovarian hormones increase DHA synthesis. However, the individual effects of  $17\beta$ -estradiol and progesterone and the underlying mechanisms are not clear. To investigate, rats were ovariectomized or sham-operated at 8 weeks of age, and ovariectomized rats were implanted with a pellet providing  $17\beta$ -estradiol, progesterone, both, or neither and were sacrificed after 14 days. Enzyme expression was measured in liver by immunoblot, and transcriptomes of ovariectomized and sham-operated rats were compared by high-density microarray. Fatty acid analysis was performed on liver and plasma. Ovariectomy increased hepatic  $\Delta$ 6-desaturase expression by both microarray and immunoblot, but no difference in phospholipid DHA was observed. Supplementation of ovariectomized rats with  $17\beta$ -estradiol or  $17\beta$ -estradiol+progesterone increased hepatic  $\Delta$ 6-desaturase (increase of 39% and 42%, respectively), corresponding to higher DHA in hepatic phospholipids (increase of 34% and 40%, respectively) and plasma phospholipids (increase of 70% and 74%, respectively). Supplementation with progesterone alone did not affect  $\Delta$ 6-desaturase or DHA. These results indicate that  $17\beta$ -estradiol, but not progesterone, increases circulating DHA through increased hepatic  $\Delta$ 6-desaturase, possibly explaining differences in DHA content between sexes and during pregnancy.

## Functional Lipids and Risk of Metabolic Syndrome

T. Yanagita<sup>(1)</sup>, K. Nagao<sup>(2)</sup>

<sup>(1)</sup>Nishikyushu University, Japan <sup>(2)</sup>Saga University, Japan

The metabolic syndrome is a cluster of metabolic disorders, such as abdominal obesity, dyslipidemia, hypertension and impaired fasting glucose that contribute to increased cardiovascular morbidity and mortality. Although the pathogenesis of metabolic syndrome is complicated and the precise mechanisms have not been elucidated, dietary lipids have been recognized as contributory factors in the development and the prevention of cardiovascular risk clustering. We review the physiological functions and molecular actions of functional lipids, especially n-3 polyunsaturated fatty acids (EPA, DPA, and DHA), conjugated fatty acids, and phospholipids (PC, omega3-PC, PI and PIPS), in the development of metabolic syndrome. Dietary functional lipids suppress the accumulation of abdominal adipose tissue and lipids in the liver and serum, and alleviate hypertension and type 2 diabetes through the transcriptional regulation of lipid and glucose metabolism. Peroxisome proliferator-activated receptors (PPARs), sterol regulatory element binding proteins, and nuclear factor  $\kappa$ B contribute to these nuclear actions of functional lipids. Additionally, our studies have demonstrated the striking ability of functional lipids to regulate the production of adipocytokines, such as adiponectin and MCP-1. In particular, the property of functional lipids as dietary adiponectin inducers deserves attention with respect to alleviation of metabolic syndrome by dietary manipulation. Reference: T. Yanagita and K. Nagao. *Asia Pac. J. Clin. Nutr.*, 17, 189-191 (2008). K. Nagao and T. Yanagita. *Prog. Lipid Res.*, 47, 127-146 (2008). B. Shirouchi, et al. *J. Oleo Sci.* 58, 111-115 (2009). N. Gotoh, et al. *J. Agric. Food Chem.*, 57, 11047-11054 (2009). B. Shirouchi, et al. *Biosci. Biotechnol. Biochem.*, 74, 2333-2335 (2010).

## Trans Fatty Acid Content of Selected Foods in Chinese Market

Y. Jiang<sup>(1)</sup>, S. Xia<sup>(2)</sup>, Y. Zhang<sup>(3)</sup>, J. Yu<sup>(4)</sup>, P. Hu<sup>(5)</sup>, X. Xu<sup>(6)</sup>

<sup>(1)</sup>Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd, China <sup>(2)</sup>Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd, China <sup>(3)</sup>Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd, China <sup>(4)</sup>Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd, China <sup>(5)</sup>Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd, China <sup>(6)</sup>Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd, Denmark

This study aims to provide reliable and comparable data on trans-fatty acid (TFA) content of foods in China via a survey. 581 bottled cooking oil samples (no more than 5L, covered 15 oil varieties and 133 brands), 74 catering cooking oil samples (18-22L, 5 categories) and 257 popular oil-bearing processed food samples (76 brands) were collected from market and analyzed. Except rice bran oil, the average TFA content of other oil varieties was less than 2%, similar to the data in 2010. And non-refined oil contained lower TFA. Besides, the average TFA content of 5 categories collected catering oil ranged from 0.45% to 1.68%, rarely appeared in published papers. Furthermore, a significant reduction was shown in oil-bearing processed foods compared with the data in 2010. The percentage of foods with more than 2% TFA was reduced by 50%. Only the average TFA content in cake, bread & pie and cookies exceed 1%. Overall, TFA content of selected foods was controlled well and declined stability because of the efforts made by government and oil industry. For example, industry employed mild refining condition to avoid high temperature, added specific oils and applied interesterification/fractionation technology to reduce/replace partially hydrogenated oil.

## Health and Nutrition Poster Session

Chair(s): K. Stark, University of Waterloo, Canada

### **Hypolipemiant and Antioxidant Effect of CLnA Rich Nanocapsules Tested on rat Model**

A. Sengupta<sup>(1)</sup>, M. Ghosh<sup>(2)</sup>

<sup>(1)</sup>Bengal Engineering and Science University, Shibpur, India <sup>(2)</sup>University of Calcutta, India

Conjugated linolenic acid (CLnA), group of octa-deca-trienoic acid isomers that have three double bonds in conjugation, is highly prone to oxidative damage due to its typical structural configuration. To utilize the full potential of CLnA as an anti-oxidant, encapsulation technique can be adopted to protect it from degradation. The present study was designed to clarify the ameliorative effects of two types of CLnA rich oil nanocapsules :CLnA rich oil nanocapsules with natural antioxidant, lipoic acid and CLnA rich oil nanocapsules with synthetic antioxidant, tert-butylhydroquinone [TBHQ], in comparison to CLnA rich oil on hypercholesterolemia induced by feeding a high-fat diet to male albino rats. CLnA nanocapsules were prepared by ultrasonication followed by freeze-drying technique. The feeding experiment was carried out for a period of 30 days. Results showed that both CLnA rich oil and their nanocapsules reduced blood and tissue lipids which were increased by the introduction of cholesterol in the diet but the reduction was more in case of nanocapsules. Adding cholesterol to the diet decreased the activities of antioxidant enzymes and increased tissue and plasma MDA concentrations. Inclusion of CLnA both in the form of oil and nanocapsules in the diet of hypercholesterolemic rats partly prevented the effects of the increased cholesterol and prevention is more in case of nanocapsules. Capsules with lipoic acid as antioxidant showed overall better protective effect than capsules with TBHQ as anti-oxidant.

### **Studies on Comparative Efficacy of alpha-linolenic Acid and alpha- Eleostearic Acid on Prevention of Methyl Mercury -induced Hyperlipidimia in Kidney and Liver of Rat**

M. Pal<sup>(1)</sup>, M. Ghosh<sup>(2)</sup>

<sup>(1)</sup>University of Calcutta, India <sup>(2)</sup>University of Calcutta, India

Methylmercury (MeHg) is the principal form of organic mercury historically associated with organ toxicity. After Minamata incident in 1950, MeHg has been recognized as one of the most hazardous environmental pollutant. In the aquatic environment, MeHg is accumulated in fish, which represent a major source to human exposure. Literature suggests that MeHg toxicity is mainly associated with oxidative stress which in turn generates lipid peroxidation and hyperlipidemia. The present study will demonstrate the hypolipidemic effect of conjugated and non-conjugated isomers of linolenic acid,  $\gamma$ -eleostearic acid (CLA) and  $\gamma$ -linolenic acid (ALA) against MeHg induced hyperlipidimia, especially in liver and kidney of rat. Male albino rats were treated with Methylmercury (II) chloride (MeHgCl) (5 mg/Kg BW/day) to induce oxidative stress. The healing effect that occurred due to ingestion of CLA and ALA was

monitored by total body and organs weight, total lipid content, total triglyceride, cholesterol and phospholipid content and fatty acid profile of lipid component. The ameliorative activity of CLA and ALA was established through the study and the best result was obtained when the stressed rats were treated with 1% (w/w of total lipid given) CLA and ALA. There was no significant difference in ameliorative activity with both the isomers.

### **Phytochemical Protection Against Carbofuran Toxicity**

S. Datta<sup>(1)</sup>, M. Sinha<sup>(2)</sup>, D. Das<sup>(3)</sup>, S. Ghosh<sup>(4)</sup>, S. Ghosh<sup>(5)</sup>, P. Dhar<sup>(6)</sup>, M. Ghosh<sup>(7)</sup>

<sup>(1)</sup>University of Calcutta, India <sup>(2)</sup>University of Calcutta, India <sup>(3)</sup>University of Calcutta, India <sup>(4)</sup>University of Calcutta, India <sup>(5)</sup>University of Calcutta, India <sup>(6)</sup>University of Calcutta, India <sup>(7)</sup>University of Calcutta, India

PENDING REVISION -- The study evaluated plausible interactions between food contaminants and natural constituents in vivo and focused on protective effect of polyphenols present in Ipomoea aquatica (IA) against carbofuran toxicity in Wistar rats. Chromatographic study identified flavonoids like rutin, apigenin and quercetin as major components present in IA. Determinations based on antioxidant enzymes viz GSH, GPx, SOD and catalase activities in tissues (liver and brain) and plasma of pesticide treated group showed significant alterations while the polyphenolic extracts from IA attenuated their activities given along with carbofuran. IA extract decreased enhanced lipid peroxidation levels in plasma and erythrocyte membrane and cholesterol levels in brain and plasma. IA extract also minimized histopathological degenerative changes produced by carbofuran. While single cell gel electrophoresis showed that secondary metabolites in leafy vegetables produced a combinatorial effect with pesticide in cellular level, DNA fragmentation levels in bone marrow cells showed a decline in the IA extract treated rats. Adverse affects of various toxic contaminants present in food can be combated by plant polyphenols and secondary plant constituents that can be found together in bolus. Therefore, the present study gives an insight into this protective role of naturally found polyphenols against pesticide toxicity.

### **Dietary Sunflower Fiber is Effective in Decreasing Body Weight**

N. Watanabe<sup>(1)</sup>, A. Okano<sup>(2)</sup>, Y. Takamura<sup>(3)</sup>, M. Takumi<sup>(4)</sup>, K. Fujimoto<sup>(5)</sup>

<sup>(1)</sup>Showa Women's University, Japan <sup>(2)</sup>Showa Women's University, Japan <sup>(3)</sup>Mottainai Biomass Co. Ltd., Japan <sup>(4)</sup>Koyo Sangyo Co. Ltd., Japan <sup>(5)</sup>Koriyama Women's University, Japan

In this study, we showed that compared to dietary crystallized cellulose, dietary sunflower fiber obtained from sunflower stem decreased body weight and weight of white adipose tissue in C57BL/6J mice fed a high-fat diet (containing 20% oil: 10% corn oil + 10% lard). Furthermore, the mice showed decreased plasma levels of triacylglycerol and total cholesterol on intake of sunflower fiber. Triacylglycerol and cholesterol contents in the feces of mice fed sunflower fiber were higher than the corresponding contents in the feces of mice fed crystallized cellulose. Furthermore, the feces of mice fed sunflower fiber were smaller and blacker. Analysis of sunflower fiber by using a scanning electron microscope showed structural features resembling thin sawdust and an extensive surface area. In vitro assay findings helped validate the low absorption of triacylglycerol (in salad oil) by sunflower fiber, which may be attributed to the structural characteristics of the fiber. On the other hand, the bile salt-binding ability of sunflower fiber remains to be elucidated. These results suggest that dietary sunflower fiber may effectively prevent obesity and metabolic syndromes.

### **Dietary Effects of Trans, Trans Rich Conjugated Linoleic Acid soy oil on egg Yolk Lipids and egg Quality**

S. Shinn<sup>(1)</sup>, A. Proctor<sup>(2)</sup>

<sup>(1)</sup>University of Arkansas Dept of Food Science, United States of America <sup>(2)</sup>University of Arkansas, United States of America

The objective of the study was to determine the effect of chicken feed containing conjugated linoleic acid (CLA) rich soy oil on CLA accumulation in egg yolk and egg quality. Studies using commercial CLA sources reported adverse effects on the albumen color and yolk size of eggs, but CLA rich soy oil high in trans, trans isomers has not been used in poultry studies. A CLA-rich soy oil was produced consisting of 15.2% CLA, of which 73% of the CLA was trans, trans isomers. Twenty-four, 44-week old broiler-breeder hens were fed one of three chicken feed diets containing either 0% soy oil, 7.4% conventional soy oil, and 7.4% CLA-rich soy oil, for 26 days. Albumen color, yolk index and yolk size were measured. Yolks contained a maximum of 1.94% CLA (110 mg of CLA per egg) after 11 days, as



measured by GC-FID FAME analysis. JMP 9.0.2 ANOVA was used to compare percent fatty acid means and quality markers. Dietary CLA significantly decreased oleic and palmitoleic acid levels but significantly increased saturated fatty acid concentrations, relative to the controls. Arachidonic acid concentrations were not significantly affected. Yolk quality was also not affected by the CLA dietary treatment.

### **Comparison of the Effect of Consuming Olive oil and Sunflower oil on the Lipid Profile in Menopause Women With Type 2 Diabetes Mellitus**

S. Armin<sup>(1)</sup>, F. Taleban<sup>(2)</sup>, F. Tahbaz<sup>(3)</sup>, Y. Mehrabi<sup>(4)</sup>, Z. Kamali<sup>(5)</sup>

<sup>(1)</sup>Guelph General Hospital, Canada <sup>(2)</sup>Shahid Beheshti University of Medical Sciences, Iran <sup>(3)</sup>Shahid Beheshti University of Medical Sciences, Iran <sup>(4)</sup>Shahid Beheshti University of Medical Sciences, Iran <sup>(5)</sup>National Nutrition and Food Technology Research Institute, Iran

In type 2 diabetes mellitus, high postprandial blood triglyceride and low HDL-C levels are common, which might be managed by dietary interventions; substituting the SFA by MUFA or PUFA. The aim of study was to compare the effects of olive and sunflower oils, as the substitutes for a portion of daily fat intake, on postprandial lipid profile in type 2 diabetes mellitus. In a randomized crossover clinical trial, 15 menopause women with diabetes ( $51.7 \pm 1.2$  years old), taking Oral Hypoglycemic Agents and consuming hydrogenated margarine/oil within 3 weeks prior to the study, were selected. The participants were randomly assigned to one of the groups of olive oil or sunflower oil, (replaced for their hydrogenated margarine/vegetable oil,  $16.1 \pm 2.3$  g/day) for 3 weeks. Then, following a washout period of 3 weeks, the subjects switched to the other oil for the next 3 weeks. Anthropometric measurements, physical activity level, and biochemical tests were done at the beginning of the study and at the end of weeks 3, 6, and 9. Compared to sunflower oil, olive oil substitution resulted in a decrease in fasting blood glucose ( $-46.2 \pm 2.1$ ) and triglyceride ( $-68.2 \pm 19.9$ ), ( $p < 0.01$ ), postprandial blood triglyceride ( $-60.1 \pm 20.6$ ), and TG/HDL-C ( $-1.9 \pm 0.8$ ), ( $p < 0.05$ ). No significant difference were seen between blood cholesterol, LDL-c and HDL-c levels, anthropometric measurements, physical activity, energy ( $1494.3 \pm 72.2$ ) and fat ( $24.1 \pm 1.3\%$ ) intake of the participants. Replacing dietary sources of SFA ( $< 7\%$  of total energy) by olive oil may help reduce CVD factors.

### **Fatty Acid Composition of Plasma Phospholipid Fractions of Women During Pregnancy and Postpartum: a Pilot Study**

A. Chalil<sup>(1)</sup>, K. Stark<sup>(2)</sup>

<sup>(1)</sup>University of Waterloo, Canada <sup>(2)</sup>University of Waterloo, Canada

Docosahexaenoic acid (DHA), an omega-3 fatty acid, plays an important role in the fetal brain development. Evidence suggests that there are maternal physiological adaptations in order to supply DHA to the fetus. Estrogen, which is elevated during pregnancy, appears to increase DHA biosynthesis. Estrogen has also been implicated in the synthesis of phospholipids, specifically the regulation of phosphatidyl ethanolamine methyltransferase (PEMT) that methylates phosphatidyl ethanolamine (PE) to form phosphatidyl choline (PC). In the present study we examine the fatty acid composition of the lipid classes of the plasma from women ( $n=3$ ) during third trimester of pregnancy, at 4 months and 1 year post partum. A non-pregnant control group ( $n=10$ ) was also examined. DHA was found to be elevated in PC during pregnancy as compared with post partum and control groups, but did not reach statistical significance. Further study is warranted, but PEMT may play a role in mobilizing DHA from PE to PC in lipoproteins of maternal plasma in order to increase its availability to the developing fetus.

### **Dietary Hemoglobin Reduce Serum and Liver Cholesterol Contents and Increase Fecal Fatty Acids, Cholesterol, and Bile Acids in Rats.**

K. Fukunaga<sup>(1)</sup>, R. Hosomi<sup>(2)</sup>, H. Arai<sup>(3)</sup>, S. Kanda<sup>(4)</sup>, T. Nishiyama<sup>(5)</sup>, M. Yoshida<sup>(6)</sup>

<sup>(1)</sup>Kansai University, Japan <sup>(2)</sup>Tottori College, Japan <sup>(3)</sup>Kitami Institute of Technology, Japan <sup>(4)</sup>Kansai Medical University, Japan <sup>(5)</sup>Kansai Medical University, Japan <sup>(6)</sup>Kansai University, Japan

The aim of this study was to elucidate the effects of porcine hemoglobin on serum and liver lipid contents in rats, and the ability of hemoglobin hydrolysates to disrupt the lipid absorption. After the rats had been on casein and hemoglobin containing diets for 4 weeks, their serum and liver lipid contents and fecal cholesterol, bile acids, and nitrogen excretion contents were measured. To elucidate the mechanism of lipid absorption by dietary hemoglobin,

lipase activity, micellar solubility of cholesterol, and bile acid binding activity in the presence of hemoglobin hydrolysates were also examined. Hemoglobin consumption decreased serum and liver triglyceride and cholesterol contents and increased fecal fatty acids, cholesterol, and bile acids excretions. In addition, hemoglobin hydrolysates prepared by in vitro digestion had the inhibition of lipase activity compared with casein hydrolysates. These results suggest that the hypolipidemic effect of hemoglobin is mediated by increased fecal lipid excretions and that lowering lipase activity of hemoglobin is at least partially responsible for the effect. These effects were documented by 8 g/kg hemoglobin diet, which is content lower than other studies therefore hemoglobin may play beneficial roles in the prevention of lifestyle-related diseases.

### **Immunomodulatory and Cytotoxic Properties of Oils and Fatty Acids from Leaves, Flowers, and Stems of *Clerodendrum volubile***

O. Erukainure<sup>(1)</sup>, O. Ebuehi<sup>(2)</sup>, M. Choudhary<sup>(3)</sup>, A. Mesaik<sup>(4)</sup>, A. Shukralla<sup>(5)</sup>, M. Aliyu<sup>(6)</sup>, G. Elemo<sup>(7)</sup>

<sup>(1)</sup>Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria, Nigeria <sup>(2)</sup>University of Lagos, Nigeria <sup>(3)</sup>University of Karachi, Pakistan <sup>(4)</sup>University of Karachi, Pakistan <sup>(5)</sup>University of Karachi, Pakistan <sup>(6)</sup>University of Karachi, Nigeria <sup>(7)</sup>Federal Institute of Industrial Research, Nigeria

Suppression and cytotoxic activity affecting the function of the immune system has been reported for many synthetic and natural therapeutic agents. Some medicinal plants have been shown to possess immunomodulatory and cytotoxic properties against cancerous cells. *Clerodendrum volubile* locally known as obenetete is among other medicinal plants in south-south Nigeria, with dearth of medicinal data base. Oil was extracted with hexane from the leaves, flower and stem of *C. volubile* using soxhlet apparatus. They were transesterified with methanolic KOH. The oils and resulting Fatty acid methyl esters (FAME) were subjected to GC-MS analysis. The oils and Fatty acids were studied in detail to explore their immuno-modulatory potentials on whole blood phagocytes ROS (reactive oxygen species) production using Luminol-amplified chemiluminescence technique after activation with serum opsonized zymosan standard methods and cytotoxic effect on PC-3 (PC3) human prostate cancer cell lines. GC-MS analysis revealed a high concentration of unsaturated fatty acids in all the oils. The oils exhibited no effects. Fatty acid from the stem showed inhibitory activity on ROS production following opsonized zymosan, indicating an immunomodulatory potency (IC<sub>50</sub>= 18.52 + 0.5), while the others showed little or no inhibition. Fatty acid from the leaves were able to inhibit the growth of PC-3 cell lines (IC<sub>50</sub>= 9.68 + 0.04), indicating a cytotoxic potential against human prostate cancer cells. The others showed little or no inhibition. These results show the medicinal potentials of fatty acids from *C. volubile* in the management of immune suppressing ailments and human prostate cancer.

### **Tissue-specific differences in fatty acid desaturation and fat accumulation by pioglitazone-administrated rats**

M. Ochiai<sup>(1)</sup>, T. Matsuo<sup>(2)</sup>

<sup>(1)</sup>Faculty of Agriculture, Kagawa University, Japan, Japan <sup>(2)</sup>Faculty of Agriculture, Kagawa University, Japan

Fatty acid (FA) metabolism in skeletal muscle with insulin resistance (IR) is not fully clarified. In this experiment, we investigated the effects of pioglitazone (PGZ) on FA desaturation and fat accumulation in rat tissues. Wistar rats aged 4 weeks were divided into control (C) or PGZ (P) group, and then all rats were fed a diet high in beef tallow and sucrose for 8 weeks. PGZ (3 mg/kg) or vehicle was orally administered daily to rats in the P group or C group, respectively. The administration of PGZ significantly increased serum adiponectin level, and suppressed serum glucose level. Triacylglycerol (TG) content in the liver was significantly decreased, but in contrast, TG contents in the heart and skeletal muscles were significantly increased by PGZ administration. In parallel with the increase of tissue TG level, FA desaturation indexes, ratios of palmitoleate / palmitate and oleate/stearate, of the heart, adipose tissue and skeletal muscles were significantly increased. The FA desaturation index of the liver was conversely decreased by PGZ administration. In conclusion, the administration of PGZ influenced the fat accumulation and FA profiles tissue-specifically. The FA desaturation indexes in the liver and skeletal muscles could be important indicators of IR in rats.

### **Digestibility and Nutritional Quality of Enzyme-treated Pulse Flours**

A. Aryee<sup>(1)</sup>, J. Boye<sup>(2)</sup>

<sup>(1)</sup>Agriculture & Agri-Food Canada, Canada <sup>(2)</sup>Agriculture & Agri-Food Canada, Canada

In vitro protein digestibility (IVPD) studies provide simple and useful measures of in vivo protein digestibility in

evaluating the nutritional value, quality, and bioavailability of amino acids (AAs) in food ingredients. The ability to improve the digestibility of lentil flours and isolate, and bioavailability of AAs by treatment with free and encapsulated enzymes were studied. Proximate analysis of the raw lentil flour (RLF), cooked lentil flour (CLF), and lentil protein isolate (LPI) showed varying protein, moisture, lipid, and ash contents with values ranging between 27.29 and 90.15%, 0.49 and 6.68%, 0.14 and 0.68%, and 2.37 and 3.39%, respectively. Other biochemical characteristics such as total phenolic, phytic acid, and trypsin inhibitor content, and protein solubility also varied with flour type. The IVPD of RLF, CLF, and LPI using a one-step-multi-enzyme (trypsin-?-chymotrypsin-peptidase), one-step-single-enzyme (papain, bromelain, acid protease or actinidin) or a two-step-multi-enzyme (pepsin-pancreatin or pepsin-trypsin-?-chymotrypsin) system was assessed. IVPD values ranged between 77.11 and 85.37%. Food-sourced enzymes such as papain, actinidin, and bromelain were also encapsulated to model a controlled release system of these enzymes during digestion and serve as aid for individuals with reduced enzyme production or suffer from a reduced function of the pancreas. The effectiveness of the support in immobilization and the in vitro enzyme release of the encapsulated enzymes were evaluated.

### **The novel iFat1 transgenic mouse is capable of inducible endogenous tissue enrichment of n-3 PUFA**

S. Clarke<sup>(1)</sup>, J. Kang<sup>(2)</sup>, D. Ma<sup>(3)</sup>

<sup>(1)</sup>University of Guelph, Canada <sup>(2)</sup>Massachusetts General Hospital/Harvard Medical School, United States of America <sup>(3)</sup>University of Guelph, Canada

The fat-1 transgenic mouse, which endogenously synthesizes n-3 polyunsaturated (PUFA) from n-6 PUFA, is a powerful tool in nutritional research which has facilitated enhanced insight into protective health effects of lifelong tissue n-3 PUFA enrichment. However, the relative impact of timing of n-3 PUFA enrichment on health related outcomes remains poorly defined. To this end, the inducible fat-1 (iFat1) mouse model, which carries a Cre-recombinase dependent version of the *C. elegans* fat-1 gene, has been developed. The objective of this study was to characterize the utility of the iFat1 transgenic mouse as a model of conditional endogenous n-3 PUFA enrichment. iFat1 transgene function was verified in-vitro. For in-vivo analysis, iFat1 females were crossed with R26-Cre-ER(T2) males, a tamoxifen inducible Cre expression model. At 6 weeks of age R26-Cre-ER(T2)/iFat1 double hybrid males (n=3/group) were transiently treated with either tamoxifen or vehicle control. Tissues were collected at 9 weeks of age and phospholipid fatty acid composition determined using gas chromatography. Relative to their vehicle treated controls, tamoxifen treated double hybrids experienced an approximate 2-fold, or more, reduction (p<0.05) in the n-6/n-3 PUFA ratio within major phospholipid fractions, phosphatidylethanolamine (PE) and phosphatidylcholine (PC), of each of the liver, kidney and muscle. Total saturated, monounsaturated and PUFA did not generally differ between groups. These results suggest that the iFat1 transgenic mouse has potential application as tool in addressing the temporal effects of n-3 PUFA enrichment in disease prevention and treatment.

### **Relationship between ruminant trans fatty acids intake levels and blood lipids in healthy subjects: results from a systematic review and meta-regression of randomized clinical trials**

C. Gayet-Boyer<sup>(1)</sup>, F. Tenenhaus-Aziza<sup>(2)</sup>, J. Chardigny<sup>(3)</sup>, B. Lamarche<sup>(4)</sup>, C. Marmonier<sup>(5)</sup>

<sup>(1)</sup>CNIEL/ French Dairy Council, France <sup>(2)</sup>Cniel/ French Dairy Board, France <sup>(3)</sup>INRA and Clermont University, France <sup>(4)</sup>Institute of Nutraceuticals and Functional Foods, Laval University, Canada <sup>(5)</sup>CNIEL, Nutrition and Health Department, France

Background: Trans fatty acids (TFA) have been linked to harmful effects as they induce unfavorable changes in many cardiovascular risk factors including lipid and lipoprotein levels. A growing body of evidence suggest that impact of TFA on plasma lipids may vary respectively to their origin (from ruminants; R-TFA vs. industrially produced; IP-TFA). However, the topic is still under debate. To help in drawing conclusion, we conducted a systematic review and meta-regression analysis to assess the impact of R-TFA intake levels on changes in lipid ratios. Methods: A literature search was conducted using PubMed and Scopus data bases. Of 1313 studies, thirteen clinical trials were selected as appropriate for inclusion in the analysis. Based on extracted data, post vs. pre diet changes in both ratios of total to HDL cholesterol (TC/HDL-C) and of LDL-C/HDL-C were calculated. A univariate meta-regression approach was used to quantify the relationship between R-TFA doses and changes in TC/HDL-C. To consider potential confounders, we also performed multivariate regression analysis. Results: Altogether, the 13 trials yielded a total of 23 independent experimental groups of subjects. R-TFA doses ranged from 0.12 to 4.19 % of daily energy intake. The meta-regression analysis showed no significant association between doses of R-TFA and changes in the TC/HDL-C and LDL-C/HDL-

C ratios. Conclusion: While adverse cardiometabolic effects of IP-TFA are well established, data from this meta-regression analysis indicate that R-TFA intake levels have no impact on cholesterol ratios in healthy individuals. Discrimination between TFA from the two sources may be considered for further dietary guidelines.

### **Oxygenated fatty acids fermented by *Lactobacillus plantarum* decrease LXR $\alpha$ -induced cellular triacylglycerol in HepG2 cells**

Hyperlipidemia is a main risk factor of atherosclerosis. Since liver cells function in both lipid synthesis and  $\alpha$ -oxidation, dysfunction of liver leads to cardiovascular disease (CVD). Recently, new oxygenated fatty acids were discovered by fermentation of *Lactobacillus plantarum* from unsaturated fatty acids like linoleic acid (LA), however, their effect on lipid metabolism is still obscure. Hence, we aimed to clarify the ameliorating effect of these fatty acids on lipogenesis in liver cells by concentrating on liver X receptor  $\alpha$  (LXR $\alpha$ ) and sterol regulatory element-binding protein-1c (SREBP-1c) pathway which plays the crucial role in lipid metabolism in the liver, comparing with eicosapentaenoic acid (EPA). We first screened the effect of the oxygenated fatty acids on SREBP-1c expression and cell viability in synthetic LXR $\alpha$  agonist (T0901317)-treated HepG2 cells and found that 10-hydroxy-12Z-18:1 (HYA), 6Z-10-hydroxy-12Z-18:2 ( $\alpha$ HYA), 10-keto-12Z-18:1 (KetoA), and 6Z-10-keto-12Z-18:2 ( $\alpha$ KetoA) significantly decreased the expression of SREBP-1c induced by T0901317. The luciferase assay revealed that these four oxygenated fatty acids diminished activation of LXR $\alpha$  in the same manner with EPA. Nevertheless, they significantly inhibited maturation of SREBP-1 more than EPA. Interestingly, HYA and KetoA also significantly suppressed the expression of ACC2, which is a major player in fatty acid  $\alpha$ -oxidation and lipid accumulation, more than EPA. Corresponding with aforementioned results, HYA, KetoA, and  $\alpha$ KetoA significantly reduced TG synthesis of HepG2 cells more potently than LA and EPA. Our findings suggest that these four oxygenated fatty acids might be applied to utilize in atherosclerosis therapy due to their effective hypolipidemic effect.

### **Effect of carnosic acid on tumor necrosis factor- $\alpha$ -mediated inflammation and insulin resistance in 3T3-L1 adipocytes**

Obesity and insulin resistance have been linked to a low-grade chronic inflammatory response. Carnosic acid (CA), found in rosemary, has been reported to have antioxidant, antiinflammation and antiadipogenic properties. Therefore, we examined the effects of CA on inflammation and insulin resistance in 3T3-L1 adipocytes treated with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). In this study, we found that CA attenuated TNF- $\alpha$ -induced mRNA expression of inflammatory genes including interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1). CA attenuated TNF- $\alpha$ -mediated activation of extracellular signal-related kinase (ERK), c-Jun NH 2-terminal kinase (JNK), and nuclear translocation of p65 as well as DNA binding activity of nuclear factor-kappa B (NF- $\kappa$ B). CA or PP242 (mTOR inhibitor) suppresses TNF- $\alpha$  induced protein expression of mTOR, P70S6K, eIF4E and IL-6. Moreover, CA attenuated TNF- $\alpha$  mediated the suppression of peroxisome proliferator activated receptor  $\alpha$  (PPAR $\alpha$ ), adiponectin and adipocyte protein 2 (aP2). CA reversed TNF- $\alpha$  mediated the suppression of insulin-stimulated glucose uptake, phosphorylation of Tyr632 insulin receptor substrate-1 (IRS-1), Akt and FoxO1, but decreased TNF- $\alpha$  induced phosphorylation of Ser307 IRS-1 as well as total FoxO1. In conclusion, CA attenuates TNF- $\alpha$ -mediated inflammation and insulin resistance in 3T3-L1 adipocytes.

### **Fucoxanthin suppresses macrophage infiltration into white adipose tissue through regulation of chemokine production in obese/diabetic mice**

M. Hosokawa<sup>(1)</sup>, A. Koide<sup>(2)</sup>, M. Yim<sup>(3)</sup>, M. Oyama<sup>(4)</sup>, K. Miyashita<sup>(5)</sup>

<sup>(1)</sup>Hokkaido University, Japan <sup>(2)</sup>Hokkaido University, Japan <sup>(3)</sup>Hokkaido University, Japan <sup>(4)</sup>Hokkaido University, Japan <sup>(5)</sup>Hokkaido University, Japan

Fucoxanthin is a characteristic carotenoid present in edible brown seaweeds, such as *Undaria pinnatifida* and *Laminaria japonica*. Its chemical structure including an allenic bond, epoxide, and acetyl residue, is different from that of other carotenoids such as beta-carotene and lutein. Dietary fucoxanthin (0.2% in diet) attenuated body and white adipose tissue (WAT) weights gain in diabetic/obese KK-Ay mice after 4 weeks feeding. In obese WAT, immune cells

such as macrophages and T cells infiltrate and lead to inflammation by interaction with adipocytes. The chronic inflammation induced in WAT has a critical role in the pathogenesis of insulin resistance and obesity-related type-2 diabetes. Dietary fucoxanthin suppressed infiltration of macrophages and CD8+ T cells into WAT in obese/diabetic KK-Ay mice. Furthermore, fucoxanthin down-regulated mRNA expression of chemoattractive chemokines such as SDF-1 (CXCL12) and RANTES (CCL5) as well as MCP-1 (CCL2), which can recruit immune cells. These results show that anti-inflammatory effect of fucoxanthin is an important mechanism for its anti-obesity and anti-diabetic effects.

### **Protein digestibility of yogurt, pasta and muffin formulated with raw and pre-treated pea and lentil ingredients**

F. Zare<sup>(1)</sup>, J. Boye<sup>(2)</sup>

<sup>(1)</sup>Agriculture and Agri-Food Canada, Canada <sup>(2)</sup>Agriculture and Agri-Food Canada, Canada

Pulses are high in protein, fiber and antioxidants and are reported to have health-promoting benefits. Nevertheless, pulse consumption in North America remains low. Formulating novel food products with pulse ingredients could increase consumption. Pulse supplementation of foods could affect the physico-chemical, rheological and sensory properties of the finished food product. Additionally, nutritional properties such as protein digestibility could be altered. In this study, formulation of food products including yogurt, pasta and muffin supplemented with pulse ingredients was considered. Yogurt samples were prepared by supplementing 2% of skim milk with 16 different pulse ingredients followed by fermentation with *S. thermophilus*, *L. bulgaricus* and *S. acidophilus*. 10 supplemented pasta and 16 supplemented muffins were prepared by substituting 30% and 50% of durum semolina and white flour, respectively, with pulse ingredients. Pulse ingredients including yellow pea flour and/or fiber and red lentil flour were either raw or treated using processes such as dehulling, splitting, germination, fermentation, cooking and roasting. The effect of the formulation and processing on protein digestibility of the food products were studied by *in vitro* protein digestibility and SDS-gel electrophoresis which showed differences depending on the type of pulse ingredient, processing or food matrix.

### **Anti-inflammatory activity of black raspberry seed oil**

H. Lee<sup>(1)</sup>, H. Jung<sup>(2)</sup>, K. Hwang<sup>(3)</sup>

<sup>(1)</sup>Seoul National University, Korea, Republic of <sup>(2)</sup>Seoul National University, Korea, Republic of <sup>(3)</sup>Seoul National University, Korea, Republic of

This study was to determine anti-inflammatory effect of black raspberry seed (BRS) oil containing abundant  $\omega$ -linolenic acid. Dried black raspberry seeds were ground and oil was extracted with hexane. Corn, olive, canola, soy bean, grape seed and sunflower seed oils were used for comparison. RAW264.7 cell viability was measured by MTT assay. Anti-inflammatory effects were determined by measuring LPS-induced NO, PGE2 and COX-2 productions in RAW264.7 cells treated with the BRS oil and the other vegetable oils. COX-2 expression was determined by western blotting. The oil samples tested in this study had no noticeable cell cytotoxicity. Inhibitory effects of 0.5 mM BRS oil on NO production in the LPS-treated cells significantly increased ( $p < 0.05$ ). The BRS oil at the concentrations of 0.1 and 0.3 mM showed significantly higher inhibitory effects on PGE2 production than other oils except olive oil ( $p < 0.05$ ). COX-2 expression was significantly reduced compared with LPS-treated control at 5  $\mu$ M oil ( $p < 0.05$ ). However, there were no significant differences in COX-2 expression between oils. The results suggest that BRS oil have anti-inflammatory activity.

### **Effects of stage of maturity and post-harvest storage condition on the ergothioneine and phenolic contents, and antioxidative properties of select mushroom varieties**

H. Nguyen<sup>(1)</sup>, R. Nagasaka<sup>(2)</sup>, T. Koyama<sup>(3)</sup>, T. Ohshima<sup>(4)</sup>

<sup>(1)</sup>Tokyo University of Marine Science and Technology, Japan <sup>(2)</sup>Tokyo University of Marine Science and Technology, Japan <sup>(3)</sup>Tokyo University of Marine Science, Japan <sup>(4)</sup>Tokyo University of Marine Science and Technology, Japan

Ergothioneine is a promising, safe, and effective antioxidant. Mushrooms are the best source of ergothioneine. Evaluating bioactive properties at different stages of fruiting body maturity is important to archive the ideal stage for consumption concerning nutritional value. In this study, we examined the influence of maturity stage and storage conditions on the contents of ergothioneine and phenolics, and antioxidant capacities of two edible mushrooms, *Grifola*

frondosa and *Lentinula edodes*. Mushroom samples were harvested at four different developmental stages (S-I, S-II, S-III, S-IV). A HPLC post-column method was used for the quantitative determination of ergothioneine; total phenolic content and antioxidant activity were determined by the Folin-Ciocalteu method and DPPH assay, respectively. For *G. frondosa*, the highest contents of ergothioneine and total phenolics as well as antioxidant activity were observed at the S-I; the ergothioneine and total phenolic contents were 4 and 1.5 times, higher than those of S-IV, respectively. For *L. edodes*, the highest contents of antioxidant compounds and antioxidant activities were observed at S-IV. The online HPLC-DPPH method demonstrated that ergothioneine is the most abundant antioxidant in the mushroom extracts. Ergothioneine was quite stable at refrigerated storage conditions up to 9 days. The present study revealed that the changes in antioxidant compounds and antioxidant potentials vary between mushroom species. The results must be useful to find the proper stage to achieve better functional and nutritional properties.

### **Effect of 4-hydroxy-2-nonenal on chemical mediators release from mast cells.**

M. Takasugi<sup>(1)</sup>, T. Hirata<sup>(2)</sup>, S. Matsumoto<sup>(3)</sup>, S. Sekimoto<sup>(4)</sup>, R. Hosomi<sup>(5)</sup>, K. Fukunaga<sup>(6)</sup>, H. Arai<sup>(7)</sup>

<sup>(1)</sup>Kyushu Sangyo University, Japan <sup>(2)</sup>Kitami Institute of Technology, Japan <sup>(3)</sup>Kitami Institute of Technology, Japan <sup>(4)</sup>Kitami Institute of Technology, Japan <sup>(5)</sup>Tottori College, Japan <sup>(6)</sup>Kansai University, Japan <sup>(7)</sup>Kitami Institute of Technology, Japan

It has been suggested that lipid peroxidation mediated by reactive oxygen species plays an important role in inflammation. However, it is not clear whether lipid peroxidation affects on allergy reaction. In type I allergy including hay fever and food allergy, chemical mediators such as histamine and leukotrienes (LT) released from mast cells are responsible for the symptoms. In this study, we investigated effects of 4-hydroxy-2-nonenal (HNE), a secondary product generated by the decomposition of lipid hydroperoxides, on histamine and LTB<sub>4</sub> release from mast cells in vitro. Rat basophilic leukemia cell line (RBL-2H3) and mouse mast cell line (PB-3c) was used for histamine and LTB<sub>4</sub> release assay, respectively. RBL-2H3 and PB-3c were treated with HNE in Tyrode buffer for 10 min and then stimulated with calcium ionophore or cross linking of FcεRI with specific IgE and allergen. The secreted histamine and LTB<sub>4</sub> were determined by HPLC. HNE enhanced histamine release from RBL-2H3 stimulated with calcium ionophore or cross linking of FcεRI, and the effect was in a dose-dependent manner. However, HNE did not affect LTB<sub>4</sub> release from PB-3c. As a molecular basis for the increase of histamine release by HNE, enhancement of ERK1/2 phosphorylation was observed in the presence of HNE.

### **Inhibitory effect of tellimagrandin I in *Rosa rugosa* petals on allergic reaction**

H. Arai<sup>(1)</sup>, Y. Takaya<sup>(2)</sup>, S. Sasayama<sup>(3)</sup>, K. Toda<sup>(4)</sup>, R. Takahashi<sup>(5)</sup>, T. Yamagishi<sup>(6)</sup>, A. Utsunomiya<sup>(7)</sup>, R. Hosomi<sup>(8)</sup>, K. Fukunaga<sup>(9)</sup>, M. Takasugi<sup>(10)</sup>

<sup>(1)</sup>Kitami Institute of Technology, Japan <sup>(2)</sup>Kitami Institute of Technology, Japan <sup>(3)</sup>Kitami Institute of Technology, Japan <sup>(4)</sup>Kitami Institute of Technology, Japan <sup>(5)</sup>Kitami Institute of Technology, Japan <sup>(6)</sup>Kitami Institute of Technology, Japan <sup>(7)</sup>Kyushu Sangyo University, Japan <sup>(8)</sup>Tottori College, Japan <sup>(9)</sup>Kansai University, Japan <sup>(10)</sup>Kyushu Sangyo University, Japan

*Rugosa rose* is a species of rose native to eastern Asia. We have reported that *Rugosa rose* petals contains tellimagrandin I as a major hydrolyzable tannins, however its physiological functions are unknown. In this study, we investigated effect of tellimagrandin I on the release of chemical mediators such as histamine and leukotriene B<sub>4</sub> (LTB<sub>4</sub>) from mast cells in vitro, which is responsible for type I allergy reaction. Rat basophilic leukemia cell line (RBL-2H3) and mouse mast cell line (PB-3c) were used for histamine and LTB<sub>4</sub> release assay, respectively. RBL-2H3 and PB-3c were pre-incubated with tellimagrandin I in Tyrode buffer. The stimulation of mast cells was induced by calcium ionophore (A23187) or cross linking of FcεRI with specific IgE and allergen. Then the secreted histamine and LTB<sub>4</sub> were determined by HPLC. The phosphorylation of intracellular signaling molecules was analyzed by western blotting. Histamine and LTB<sub>4</sub> releases from mast cell were suppressed by tellimagrandin I in dose-dependent manner. The phosphorylation of protein kinases, known to be critical for the chemical mediators release, was inhibited in the presence of tellimagrandin I. The results suggest that tellimagrandin I may have the anti-allergic function through the inhibition of signal transduction followed by chemical mediators release.

### **Soybean $\beta$ -conglycinin improves carbohydrate metabolism in type 2 diabetes mellitus models, GK rats.**

The effects of dietary soybean  $\beta$ -conglycinin on carbohydrate and energy metabolism were studied in male GK rats of type 2 diabetes mellitus models. Male GK rats (5 weeks old) were fed an AIN-93G diet containing casein or  $\beta$ -conglycinin for 5 weeks. After 3 weeks of the feeding period, though there was no significant difference in OGTT, serum glucose and insulin levels were significantly lower in the  $\beta$ -conglycinin group as compared with the casein group. After experimental feeding, final body weight was significantly lower in the  $\beta$ -conglycinin-fed rats than in the casein-fed rats. As the result of energy metabolism measurement, carbohydrate consumption was higher and fat consumption was lower in the  $\beta$ -conglycinin-fed rats than in the casein-fed rats. Total energy consumption was the same between the two groups. Although serum leptin was no difference between rats fed both diets, serum adiponectin was significantly higher in the  $\beta$ -conglycinin-fed rats than in the casein-fed rats. Serum triacylglycerol, total-cholesterol and phospholipids levels in the  $\beta$ -conglycinin-fed rats were significantly lower than that in the casein-fed rats. These results suggest that  $\beta$ -conglycinin prevents the development of type 2 diabetes mellitus through the improvement of carbohydrate metabolism and insulin sensitivity in GK rats.

### **Physiological functions of dietary ozonated-olive oil in mice**

K. Nagao<sup>(1)</sup>, M. Sakoh<sup>(2)</sup>, S. Kai<sup>(3)</sup>, K. Kojima<sup>(4)</sup>, T. Nagai<sup>(5)</sup>, T. Yanagita<sup>(6)</sup>

<sup>(1)</sup>Saga University, Japan <sup>(2)</sup>Saga University, Japan <sup>(3)</sup>Saga University, Japan <sup>(4)</sup>Tsukishima Foods Industry Co., Japan <sup>(5)</sup>Tsukishima Foods Industry Co., Japan <sup>(6)</sup>Nishikyushu University, Japan

PENDING REVISION -- Lifestyle-related diseases, such as hyperlipidemia, diabetes mellitus, hypertension, and arteriosclerosis, are widespread and increasingly prevalent diseases in industrialized countries. Although several favorable therapeutic effects of ozone and ozonated vegetable oils have been reported, there is not enough data concerning physiological function of dietary ozonated-vegetable oil in vivo. In the present study, we investigated the effect of dietary ozonated-olive oil on lipid metabolism in C57BL/6J and db/db mice. Mice were fed semisynthetic diets that contained either 6.5% corn oil + 0.5% olive oil, or 6.5% corn oil + 0.5% ozonated-olive oil for 4 weeks. Feeding of ozonated-olive oil did not show any side effects in C57BL/6J mice. Feeding of ozonated-olive oil markedly alleviated hepatomegaly and hepatic lipid accumulation in obese, diabetic db/db mice. Enzyme activities of hepatic injury markers in serum were also significantly lowered in ozonated-olive oil-fed db/db mice. These results indicate that dietary ozonated-olive oil would be useful to prevent or alleviate metabolic syndrome in obese, diabetic db/db mice.

### **Antioxidative and Antihypertensive Hemp seed (Cannabis sativa L.) Protein-Derived Peptides**

A. Girgih<sup>(1)</sup>, M. Offendengen<sup>(2)</sup>, J. Wu<sup>(3)</sup>, R. Aluko<sup>(4)</sup>

<sup>(1)</sup>University of Manitoba, Canada <sup>(2)</sup>University of Alberta, Canada <sup>(3)</sup>University of Alberta, Canada <sup>(4)</sup>University of Manitoba, Canada

Abstract The purpose of the study was to characterize antioxidant and antihypertensive peptides present in a hemp seed protein hydrolysate (HPH). The HPH was fractionated two consecutive times using reversed-phase high-performance liquid chromatography (RP-HPLC). Twenty four peptide sequences were characterized using LC-MS/MS analysis, and their antioxidant and antihypertensive potentials were determined. Results showed that the tetra- and pentapeptides exhibited stronger antioxidant and antihypertensive activities than their tripeptide counterparts. Interestingly, dipeptides were not identified in this study which goes to show that peptides with amino acid sequences greater than three could have been responsible for the observed multifunctional properties of the HPH. The identified peptides could be utilized in functional foods and nutraceuticals formulation that can be used as therapeutic agents for the prevention and treatment of chronic diseases.

### **Regiospecific distribution of fatty acids in intraesterified high-CLA milk fat**

D. ZOPE<sup>(1)</sup>, P. ANGERS<sup>(2)</sup>, Y. CHOUINARD<sup>(3)</sup>, J. ARUL<sup>(4)</sup>

<sup>(1)</sup>LAVAL UNIVERSITY, Canada <sup>(2)</sup>LAVAL UNIVERSITY, Canada <sup>(3)</sup>LAVAL UNIVERSITY, Canada <sup>(4)</sup>LAVAL UNIVERSITY, Canada

Interesterification is emerging as the major fat modification process to improve the functionality of fats and oils with the decline of hydrogenation. Intraesterification of a specific oil or fat suffers from slow rate of reaction and limited modification of the triacylglycerols (TG), and hence, has received little attention compared with interesterification of fats and oils blends. We studied effect of intraesterification on the regiospecific distribution of fatty acids in high-CLA milk fat and its fraction. The major changes in regiospecific distribution of fatty acids occurred with respect to medium

chain fatty acids (C12-C15) and long chain fatty acids (C16-C18) in both milk fats. The medium chain fatty acids and the long chain saturated fatty acids tended to shift from the sn-2 position to the external (sn-1, 3) positions, whereas oleic acid shifted from the external positions to sn-2 position. There was only negligible change in the position of CLAs as a result of intraesterification, and about two-thirds of CLA was located at the external positions in both milk fats. However, trans-vaccenic acid shifted almost completely to sn-1 position from the external positions. The short chain fatty acids (C6-C10) were preferentially located at the external positions in both the high-CLA milk fat and its fraction. The changes in the fatty acid distribution in triglycerides by intraesterification resulted in the modification of the physical properties of the milk fats fat.

### **Premature infants may oxidize additional fatty acids from supplements.**

Z. Hossain<sup>(1)</sup>, J. Friel<sup>(2)</sup>, B. Diehl-Jones<sup>(3)</sup>, A. Chiu<sup>(4)</sup>, D. MacKay<sup>(5)</sup>

<sup>(1)</sup>Manitoba University, Canada <sup>(2)</sup>Manitoba University, Canada <sup>(3)</sup>Manitoba University, Canada <sup>(4)</sup>Manitoba University, Canada <sup>(5)</sup>Manitoba University, Canada

Human milk fortifiers (HMF) are routinely added to mother's milk (HM) for the premature infant. We reported data on 65 premature infants (birth weight < 1500 g) who were either fed HM with varying levels of HMF or formula (F) while they were in hospital receiving oral feeds. A significant increase ( $P < 0.05$ ) in urine\_F2-isoprostanes was found as HMF supplementation increased. Isoprostanes are prostaglandin-like compounds formed in vivo from the free radical-catalyzed peroxidation of essential fatty acids primarily arachidonic acid (AA C20:4n6). Linoleic acid (LA 18:2n6) is a polyunsaturated fatty acid used in the biosynthesis of AA. LA in HM ranges from 7 to 24 % of total fatty acids. LA in HMF is 140 mg per packet. We hypothesized that premature infants who had higher (n=7, providing total samples 20) F2 isoprostanes in urine would have more LA in their plasma from BM + HMF than those who were lower (n=10, providing total samples 31). In vitro study revealed that HMF increased the lipids peroxidation. Results (%w/w) were: High; LA 21.3 + 1.6 vs low 23.8 + 0.8,  $P < 0.05$ ) and AA: High; 6.5 + 0.3 vs Low 6.8 + 0.4 NS). The hypothesis is supported and suggests that increased oxidation of LA may account for elevated isoprostanes in premature infants fed HMF. [Supported by CIHR and MICH]

### **[Program](#)**