A number of non-traditional biomarkers for cardiovascular disease (CVD) risk have made their way into clinical practice in recent years. Recently, the National Lipid Association (NLA) convened an expert panel of lipid specialists with the aim of reviewing the available evidence in order to develop consensus recommendations regarding the appropriate use of selected non-traditional biomarkers [C-reactive protein, lipoprotein-associated phospholipase A2, apolipoprotein B, LDL particle concentration, lipoprotein(a), LDL and HDL subfractions] for CVD risk stratification and evaluation of response to treatment in clinical practice. This presentation will summarize the recommendations of the NLA expert panel and review areas of controversy and unanswered questions relating to clinical application of advanced lipoprotein testing and inflammatory biomarkers.

A Biomarker Discussion - Should "Bad" and "Good" Cholesterol Remain the Focus of Clinical Attention? J. Otvos, LipoScience, Inc., Raleigh, NC, USA

It is well established that low density lipoprotein (LDL) particles are atherogenic and high density lipoprotein (HDL) particles are anti-atherogenic. The "bad" LDL cholesterol (LDL-C) and "good" HDL cholesterol (HDL-C) biomarkers used universally to assess coronary heart disease (CHD) risk and a patient’s need for LDL-lowering or HDL-raising treatment were not selected on the basis of superior analytical or clinical performance, but instead became the standard of care by historical default. Because the per-particle cholesterol content of LDL and HDL particles varies widely between individuals, LDL-C and HDL-C levels and LDL and HDL particle concentrations (LDL-P and HDL-P) do not agree in many patients. Recent data from the Framingham Heart Study and Multi-Ethnic Study of Atherosclerosis (MESA) demonstrate that in patients with discordance between cholesterol and particle measures of LDL and HDL, CHD risk tracks with the particle measures. These findings have potentially important implications for CHD risk management because many lifestyle and drug interventions alter the cholesterol and particle measures of LDL and HDL differentially.

LDL Subfractions and Risk of Cardiovascular Disease. Ronald M. Krauss, Children’s Hospital Oakland Research Institute and University of California-San Francisco, Oakland, CA, USA

Subfractions of LDL defined by differences in particle size and density have been associated to varying degrees with risk of cardiovascular disease (CVD). Assessment of these relationships has been clouded by lack of standardization among the various analytic methodologies as well as the strong correlations of the subfractions with each other and with standard lipid and lipoprotein risk markers. While several recent studies have shown independent relationships of levels of LDL size subclasses to CVD risk, the two largest studies, employing nuclear magnetic resonance and ion mobility, respectively, did not find that these measurements improved risk assessment compared with standard lipid and lipoprotein assays. In the latter study, principal component analysis was used to group multiple subfraction measurements into three distinct and statistically independent clusters that were related both to CVD and to specific genotypes that may reflect underlying metabolic determinants. Thus, although there is as yet inconclusive evidence as to the extent to which LDL subfraction measurements improve clinical assessment of CVD risk beyond conventional...
lipid risk markers, recent findings suggest that more refined analyses of lipoprotein subspecies may lead to further improvements in CVD risk evaluation and particularly in identification of appropriate targets for therapeutic intervention in individual patients.

**Biomarkers for Management of Dietary Carbohydrate.** Jeff S. Volek, University of Connecticut, Storrs, CT, USA

People vary widely in their ability to metabolize dietary carbohydrate. Since the inability to properly metabolize dietary carbohydrates is the direct result when insulin action is impaired, insulin resistance manifests functionally as carbohydrate intolerance. In insulin resistance (carbohydrate intolerance), there is a propensity to divert dietary carbohydrate away from skeletal muscle and towards the liver where it is converted to fat. Over time this can lead to fatty liver, metabolic syndrome and type-2 diabetes. A number of markers may provide early clues to this mismanagement of dietary carbohydrate. These include many of the markers of metabolic syndrome, especially the plasma concentration and fatty acid composition of triglycerides. Specifically, levels of palmitoleic acid (16:1) may be a specific biomarker of newly made fat to quantitate the degree of ongoing lipogenesis. The advantage of measuring these biomarkers is that it provides individualized feedback that can be used to adjust and optimize a person’s carbohydrate intake.

**Surrogate Endpoints Used by FDA for the Premarket Review of Health Claims.** Paula R. Trumbo, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD, USA

Health Claims describe the relationship between a substance (food or food component) and a disease or health-related condition. FDA conducts pre-market science and regulatory reviews of health claims. As part of the science review, FDA relies on clinical and surrogate endpoints for evaluating the association between a substance and disease risk. This presentation will discuss how surrogate endpoints are used in the scientific review of health claims.

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**TUESDAY**

**MORNING**

**H&N 2: Omega-3 Fatty Acids and Brain Health**

Chair(s): S. Bhale, Hill's Pet Nutrition, USA; and B. Berg, Mead Johnson Nutrition, USA

**Maintaining Brain PUFA Concentrations: Uptake and Rapid Metabolism.** Richard Bazinet, University of Toronto, Canada

The brain is particularly enriched in glycerophospholipids with either arachidonic or docosahexaenoic acid esterified in the stereospecifically numbered-2 position. In this talk, I will review how polyunsaturated fatty acids (PUFA) enter the brain, and the mechanisms that regulate their concentrations within brain phospholipids. Whereas little evidence exists to support the incorporation of PUFA from lipoproteins into the brain, the incorporation rates of arachidonic and docosahexaenoic acid from the plasma unesterified pool into brain phospholipids closely approximate independent measures of their consumption rates by the brain. Thus, with the use of radiolabelled fatty acids, it is possible to image and quantify their entry and uptake into the brain in rodents and, with positron emission tomography, in humans. Upon entry into the brain, certain PUFA are highly conserved with extensive recycling within phospholipids, whereas others, such as eicosapentaenoic acid, are rapidly and extensively removed from the brain, in part, due to b-oxidation. Altered PUFA metabolism has been implicated in several neurological disorders, including bipolar disorder and Alzheimer’s disease. Identifying the mechanisms by which PUFA enter and are handled within the brain could lead to a better
understanding of nutritional requirements for the brain as well as new therapeutic targets and novel imaging methods.

**Consumption of Docosahexaenoic Acid (DHA, 22:6n–3) in Pregnancy Decreases Risk for Postpartum Depression (PPD).** C.J. Lammi-Keefe¹, M.P. Judge², C.T. Beck², H. Durham¹, M.M. McKelvey², ¹Louisiana State University, Baton Rouge, LA, USA, ²University of Connecticut, Storrs, CT, USA

DHA is important to nervous system functioning. Low dietary DHA and preferential placental transfer deplete DHA. Our objective: to investigate efficacy of DHA supplementation in pregnancy for decreasing PPD symptoms. In a randomized, double-blind, placebo controlled trial pregnant women (n=26/group) consumed i) 300 mg DHA or ii) placebo, 24 wks to birth. PPD symptoms were assessed (2 & 6 wks; 3 & 6 mos) with the Postpartum Depression Screening Scale (PDSS). With Proc Mixed, DHA intervention was associated with a lower mean depression score (p=0.006). For RBC DHA (wt %) there was a trend for a group × time effect (p=0.10) and a significant group effect; DHA was higher in the DHA group at 36-38 wks (p≤0.0001; 3.64% vs 2.70%). Our findings confirm efficacy of DHA for improving PPD symptomatology. Given teratogenic effects of some medicines to treat depression, the need for alternatives is underscored. Our findings provide a foundation for efforts to increase awareness of the value of DHA/fish consumption for maternal mental health. The importance of preventing and treating PPD is underlined by findings that PPD can have adverse effects on children's cognitive and emotional development. (Funded in part by Donaghue Medical Research Foundation; Loders Croklaan; National Fisheries Institute)

**Sex-Specific and Dietary Regulation of Intrinsic Protective Lipid Circuits.** K. Gronert, University of California, Berkeley, CA, USA

The dynamic and temporally defined interplay of distinct populations of leukocytes is critical but a poorly defined feature of inflammatory events. By design inflammation is a frequent and self-resolving response that is essential for protection and integrity of all tissues. Successful execution of healthy inflammation depends on tightly regulated activation of leukocytes and their active resolution to return tissues to homeostasis. Dysregulation of this highly regulated process and stalled inflammation leads to PMN mediated tissue injury, activation of the inflammatory macrophage phenotype, transition to chronic inflammation and may promote/initiate adaptive immune responses. Hence, pathways that promote the resolution of PMN are of primary interest. Their function and regulation in the eye is just beginning to unfold and of considerable interest as the delicate visual axis has evolved a highly sophisticated intrinsic and protective lipid circuit. Our studies have identified a resident lipid circuit in the cornea that counter-regulates pro-inflammatory signals, promotes wound healing, controls pathological angiogenesis and regulates dynamic in vivo leukocyte behavior. The presentation is an update on current state of the field and new developments, which demonstrate that this intrinsic protective lipid circuit is regulated by dietary PUFA and exhibits sex-specific differences. Unraveling regulation of these intrinsic protective lipid circuits may provide insights into the etiology or pathogenesis of ocular inflammatory diseases.

**The DHA Metabolome Gives Rise to Potent Mediators of Neuroinflammation and Cell Survival.** Nicolas Bazan, LSU Health Sciences Center, New Orleans, LA, USA

**Role of Docosahexaenoic Acid in Brain Development and Function: Emerging Evidence from Magnetic Resonance Imaging.** Robert K. McNamara, Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati College of Medicine, Cincinnati, OH, USA

A body of evidence suggests that docosahexaenoic acid (DHA), the principal omega-3 fatty acid in brain gray matter, is positively is positively associated with cognitive development. To elucidate underlying brain mechanisms, the present study determined the effects of DHA supplementation on functional cortical activity during sustained attention in healthy male children (aged 8-10 years). Subjects were randomized to receive placebo or one of two doses of DHA (400 or 1,200 mg/d) for 8 weeks. Relative changes in cortical activation patterns during sustained attention at baseline and endpoint were determined by functional magnetic resonance imaging. At 8 weeks, erythrocyte membrane DHA composition increased significantly from baseline in subjects receiving low- and high-dose DHA, but not placebo.
During sustained attention, both DHA dose groups exhibited significantly greater change from baseline than placebo in the activation of dorsolateral prefrontal cortex (DLPFC), and the high-dose DHA group exhibited greater decreases in cerebellar cortex. Erythrocyte DHA composition was positively correlated with DLPFC activation, and inversely correlated with reaction time, at baseline and endpoint. These data demonstrate that dietary DHA intake and associated elevations in erythrocyte DHA composition are associated with alterations in functional activity in cortical attention networks during sustained attention in healthy male children.

**DHA Function and Metabolism in the Nervous System.** Norman Salem, Jr., Nutritional Lipids, DSM Nutritional Products LLC, USA

Docosahexaenoic acid (DHA) is an essential component of the nervous system, including both brain and retina. This essentiality is evidenced from various perspectives. When the brain levels of DHA fall, this leads to losses in function as measured behaviorally and a variety of such studies have been performed. Reversal of deficiency leads to reversal of the behavioral deficits that correlate quantitatively with the extent of the recovery of brain DHA. There are several neurological diseases that are associated with a loss of brain DHA. A variety of biological functions for DHA are known including regulation of GPCR?s, apoptosis, synapse formation, gene expression and bioactive compound generation (neuroprostanes, anandamide analogues, acylglycerols, etc). Another aspect of essentiality for a nutrient entails it being the essential species and that it cannot be made in an adequate quantity from its precursors in a particular organism. Rodents are known to have fairly active alpha-linolenic acid (ALA) metabolism, however, studies from several laboratories have shown that maximal brain and retinal levels of DHA can only be obtained when preformed DHA is given to various rodents. Such studies also demonstrate that DHA has a much greater potency in supporting tissue levels of DHA than does ALA and this applies also the intermediate EPA. In human, both compositional and stable isotope metabolic studies show that ALA and EPA are indeed metabolized to DHA but that this occurs only at trace levels. As a consequence, even very high levels of ALA supplementation to humans produce no change in blood stream DHA content. The conclusion is that preformed dietary DHA must be supplied in order to support increased levels of organ DHA.

**Therapeutic Effects of Omega-3 Fatty Acids on Brain Trauma.** Fernando Gomez-Pinilla, Dept. Integrative Biology and Physiology, and Dept. Neurosurgery, UCLA, USA

Traumatic brain injury (TBI) is characterized by a diffuse pathology resulting in long-term deficits in higher order cognitive and intellectual functions. In particular, posttraumatic stress disorder, which is characterized by emotional and cognitive disability, is a common sequel of TBI. The broad mode of action of dietary factors makes of them a unique tool to counteract main broad aspects involved in the TBI pathology. Our research indicates that the efficacy of nutritional factors is displayed at the level of processes involved in re-establishing energy homeostasis and providing structural substrates that can foster neuronal signalling. For example, dietary DHA has important actions on the mechanisms that maintain neuronal communication, providing support to cognitive and emotional abilities, and have a great potential to circumvent TBI pathobiology. The actions of dietary factors appear complementary, such as the curry spice curcumin, can complement the action of omega-3 fatty acids in the brain. In addition emerging studies indicate that exercise is capable of boosting the healthy effects of certain diets. Therapy based on DHA, curcumin, and exercise can benefit TBI, and have long-term consequences on molecular systems responsible for maintaining synaptic function, underlying higher order operations such as learning and memory, and emotions. The overall evidence indicates that the non-invasive broad neuroprotection provided by diet and exercise could be implemented to counteract the diffuse nature of TBI and other neurological disorders. (Studies supported by NIH awards R01 NS056413, and NS04665).ReferencesGómez-Pinilla F. Brain foods: the effects of nutrients on brain function. Nature Rev Neurosci. 9(7) (2008) 568-78.Chytrova G, Ying Z, Gomez-Pinilla F. Exercise contributes to the effects of DHA dietary supplementation by acting on membrane-related synaptic systems. Brain Res. (2010) 1341: 32-40.Gomez-Pinilla, F., The Collaborative Effects of Exercise and Foods in the Wage Against Neurological and Cognitive Disorders, Prev. Med., 2011 Jun 1; 52 Suppl 1:S75-80.Wu A, Ying Z, Gomez-Pinilla F. , The salutary effects of DHA dietary supplementation on cognition, neuroplasticity, and membrane homeostasis after brain trauma, J. Neurotrauma, 2011; 28(10):2113-22.Bhattia, H, Agrawal R, Sharma S, Yi-Xin Hu, Gomez-Pinilla F, Omega-3-Fatty Acid deficiency during brain development and maturation reduces neuronal and behavioral plasticity in adulthood, PLoS One. 2011; 6(12):e28451.
Impact of Omega-3 Fatty Acids on Alzheimer's Disease. G.M. Cole, Mary S. Easton Center for Alzheimer's Disease Research at UCLA, Los Angeles, CA, USA

Increased intake of omega-3 EPA and DHA from fatty fish is associated with reductions in cognitive decline, brain shrinkage and Alzheimer disease (AD). Some studies report protection from fish is only relevant in subjects lacking Apolipoprotein E4, the most common and potent genetic risk factor for AD. EPA has anti-inflammatory effects, primarily in non-neuronal cells. Preferentially neuron-enriched, DHA has neuroprotective activity in culture and animal models suggesting increased intake could protect from AD. However, DHA relevance and mechanisms for prevention or treatment of AD remain unclear. DHA may benefit pre-dementia but failed to slow progression in mild to moderate AD but may have benefited non-ApoE4 carriers. DHA reduces production and accumulation of the Aβ42 peptide that may initiate AD. Aβ accumulation effects are most relevant to prevention. Ongoing animal diet studies show robust ApoE4 x dietary fatty acid Aβ effects. DHA's pleiotropic neuroprotective effects derive from both esterification into membrane phospholipids and transformation into neuroprotective metabolites (protectins). The relative high safety and low cost of omega 3 fatty acids and the huge economic costs of an aging population at risk for dementia provide a compelling argument for seriously pursuing omega-3 potential for AD prevention.

Dietary Linoleic Acid and n-3 HUFAs Determine Endocannabinoids, Satiety and Obesity. Joseph R. Hibbeln, Anita R. Alvheim, LMBB, NIAAA, NIH, USA

Suppressing hyperactive endocannabinoid tone is a critical target for reducing obesity. The backbone of both endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA) is the omega-6 fatty acid arachidonic acid (AA). Here we posited that excessive dietary intake of linoleic acid (LA), the precursor of AA, would induce endocannabinoid hyperactivity and promote obesity. Linoleic acid was isolated as an independent variable to reflect the dietary increase in LA from 1 percent of energy (en%) to 8 en% occurring in the US during the 20th century. Mice were fed diets containing 1 en% LA, 8 en% LA and 8 en% LA + 1 en% eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) in medium fat diets (35 en% fat) and high fat diets (60 en%) for 14 weeks from weaning. Increasing LA from 1 en% to 8 en% elevated AA -phospholipids in liver and erythrocytes, tripled 2-AG+1-AG and AEA associated with increased food intake, feed efficiency and adiposity in mice. Reducing AA - phospholipids by adding 1en% long-chain omega-3 fats to 8 en% LA diets resulted in metabolic patterns resembling 1 en% LA diets. Selectively reducing LA to 1 en% reversed the obesogenic properties of a 60 en% fat diet. These animal diets modeled 20th century increases of human LA consumption, changes that closely correlate with increasing prevalence rates of obesity. In summary, dietary LA increased tissue AA, and subsequently elevated 2-AG+1-AG and AEA resulting in the development of diet-induced obesity. The adipogenic effect of LA can be prevented by consuming sufficient EPA and DHA to reduce the AA ?phospholipid pool and normalize endocannabinoid tone.

AFTERNOON

BIO 3.1/H&N 3: Food Form and Functionality of Lipids

Chair(s): M.-C. Michalski, INRA, France; and D. Hildebrand, University of Kentucky, USA

Understanding Lipid Structures in Foods in Relation to Lipid Digestibility. Harjinder Singh, Riddet Institute, Massey University

The importance of lipids in the human diet has led to major advances in understanding the mechanisms of lipid digestion and absorption. With these advances has come new recognition that the matrix in which lipids are presented (i.e. food structure) in the diet could influence the rate of lipid digestion and hence the bioavailability of fatty acids. Lipids in natural foods occur generally as in the form of complex structure in which triglycerides particles are
coated with a stabilizing layer or multi-layer of membrane phospholipids and proteins. Breaking down the surrounding structures and releasing the lipid droplets from the cells, seed bodies or whatever locating matrix, will have a profound influence on our ability to digest the lipid. In processed foods, lipids may also be incorporated within the food matrix in the form of emulsions. Here phospholipids can be used as emulsifiers, but monoacylglycerols and proteins also often feature as emulsifiers and stabilizers. This paper will review the current knowledge on the state of lipids in different foods and how these systems are modified as they traverse through the gastrointestinal tract. Particular emphasis will be placed on colloidal aspects of lipid droplets and lipid digestion.

Role of Lipid Structure and Food Matrix on Lipid Digestion and Absorption. A.J. Wright, University of Guelph, Guelph, ON, Canada

Lipids play various biological roles and their consumption has critical implications in health and disease. They also contribute to the desirable and characteristic attributes of many foods. Indeed, there is intense interest in modifying lipid composition and formulating foods so as to retain these functional properties, while optimizing potential health benefits and minimizing possible deleterious consequences associated with lipid consumption. One particularly active area of study is the impact of digestive processing on food structure, lipid digestion and absorption. Progress is being made through the applications of tools to model the gastrointestinal environment, as well as interdisciplinary and multilevel research approaches. The impact of lipid and food matrix composition and structure on digestion, nutrition and health outcomes will be highlighted using examples from our team, including structured lipids and results from simulated gastrointestinal digestion experiments of lipid–based systems in relation to bioavailability. Ongoing investigations underscore the importance of food microstructure and ingredient interactions on lipid-related nutritional outcomes. A better understanding of the relationships involved will enable the rationale design of foods to optimize health and support health authorities and professionals in making recommendations.

Enhanced Absorption of n–3 Fatty Acids from Emulsified Compared with Encapsulated Fish Oil. S. Raatz1,2, D. Bibus3,2, 1USDA, Human Nutrition Research Center, Grand Forks, ND, USA, 2University of Minnesota, Minneapolis, MN, USA, 3Lipid Technologies, LLC, Austin, MN, USA

The omega–3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have important nutrition and disease management properties. Presently fish oil (FO) supplementation relies on capsular triglyceride. Flavored emulsified lipid preparations may provide an improved approach to FO delivery. Oil in water emulsions are hypothesized to possess advantages in the digestion and absorption of their fatty acids thus increasing the bioavailability of the active components. A randomized, crossover designed study compared absorption kinetics of EPA and DHA in 10 subjects consuming 4g of FO of emulsified FO or the parent FO in capsular form. Blood was obtained at 0, 2, 4, 8, 24 and 48 hours. During this 48–hour period, there was enhanced absorption of total n–3 and EPA (0.67±0.16%, 0.45±0.06%; p<0.01; 0.34±0.05%, 0.23±0.04%; p=0.05; emulsified FO and capsular FO, respectively) observed for the emulsified FO treatment. Our findings indicate that a single dose of emulsified FO resulted in enhanced absorption of total n–3 and EPA as evidenced by changes in phospholipid fatty acid composition compared with the capsular FO during the 48–hour observation period. Oil in water emulsions are a highly bioavailable, highly tolerable method of delivery for long chain omega–3 fatty acids from FO.

Emulsified Fat Enhances Postprandial Lipemia and Exogenous Lipid Oxidation Compared with Spread Fat in Lean and Obese Humans. M.C. Michalski1,3, C. Vors1,2, G. Pineau1,2, L. Gabert3, M. Laville3,2, H. Vidal2,3, 1INRA USC1235, Lyon University, Univ Lyon-1, INSA-Lyon, CarMeN Laboratory, Villeurbanne, France, 2INSERM U1060, Lyon University, Univ Lyon-1, CarMeN Laboratory, Oullins, France, 3CRNH-RA, CENS, Oullins, France

Obese people present an imbalance of fat distribution linked to postprandial lipemia kinetics and resulting exogenous lipid beta–oxidation. We showed in rodents that the latter could be modified using different emulsions or bulk oil. Our objective was to show in humans that non–emulsified fat and emulsified fat can modulate lipid absorption kinetics and ultimately lipid oxidation. Therefore, 10 lean and 10 obese volunteers digested 40 g of dairy fat either emulsified in a drink (+ bread) or not (spread on bread + drink). Plasma and chylomicrons were collected during 8h of digestion. Fat labelling with 13C–triaclyglycerols allowed to characterize the kinetics of exogenous lipid oxidation using 13CO2
breath test and indirect calorimetry. Spread fat resulted in a later peak of chylomicrons compared with emulsified fat. This was enhanced in obese subjects, with a much later and flatter chylomicron peak during the first 5 hours of spread fat digestion. Moreover, breath $^{13}$C appeared earlier and more sharply using emulsified fat. In obese subjects, emulsion even increased cumulated exogenous lipid oxidation. Dietary fat structure in food could thus be specifically adapted using the new concept of “slow” and “fast” lipids in order to control postprandial lipemia and lipid beta-oxidation in obese subjects.

Effects of Supplementation of Rodent Diets with Milk Fat Globule Membrane on Lipid Metabolism and Gut Microflora in Fisher 344 Rats. Robert Ward, Albert Zhou, Korry Hintze, Rafael Jimenez, Nutrition, Dietetics and Food Sciences, Utah State University, Logan, UT, USA, 2Dairy Science Department, California Polytechnic University, San Luis Obispo, CA, USA

Production, Characterization, and Functional Properties of Structured Triacylglycerols. C.C. Akoh, University of Georgia, Athens, GA, USA

Enzymatic synthesis of structured triacylglycerols (TAGs) is now an accepted process for designing functional for the food industry and healthful lipids for enhanced nutrition. Lipases are used in the design or synthesis of alternative lipids or analogs of existing lipids such as human milk fat analogs (HMF) for infant formula, healthful lipids containing physiologically beneficial fatty acids such as eicosapentaenoic (EPA), docosahexaenoic (DHA), and gamma linolenic acids (GLA) or for the production of functional lipids such as cocoa butter equivalents, trans-free fats for spreads, shortenings, and margarines. Fat analogs must possess desirable physical and chemical properties for possible food and clinical applications. These characteristics must be analyzed and confirmed before the application of structured TAGs in foods and nutrition. Examples of desirable properties include product purity, identity, oxidative stability, antioxidant content, melting behavior, crystal forms and morphology, FFA, n-6/n-3 ratios, TAG molecular species, positional distribution of FAs within the TAG, and sensory properties. Structured TAGs hold great promise in formulating many food products and the consumer quest for healthy good for you fats and oils.

Omega-3 Fatty Acids: Health Benefits and Sources for such Acids. I.A. Guschina, C Bascoul-Colombo, J.L. Harwood, Cardiff School of Biosciences, Cardiff, Wales, UK

There is increasing awareness of the health benefits of dietary omega-3 polyunsaturated fatty acids. Because of the inefficient conversion of alpha-linolenic acid into the long chain metabolites, eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), most attention has been paid to human epidemiological surveys as well as animal experiments using EPA and DHA. There is robust evidence that the latter are of benefit for prevalent diseases such as arthritis, dementia and cardio-vascular complaints. In addition, there is experimental evidence for possible benefit in a host of other diseases and/or psychological complaints. As a result of the above there is an escalating need for increased supplies of EPA, DHA and other omega-3 fatty acids. Traditionally, fish oils have been used but this source is not sustainable and other sources are needed. New materials and genetically manipulated crops or algae will be discussed as well as attempts to improve extraction/processing of currently-used commodities.

Progress in Producing DHA in Oilseeds Using Algal PUFA Synthases. T. Walsh, J. Metz, 1Dow AgroSciences LLC, Indianapolis, IN, USA, 2DSM, Boulder, CO, USA

The omega-3 long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA) has well-established benefits for heart, brain and eye health. However typical western diets are deficient in this important omega-3 fatty acid, leading to an increased need for sustainable and convenient sources of DHA-containing oils. Vegetable oils that are low in saturated fatty acids and high in monounsaturated fatty acids are also considered heart-healthy. We have combined these healthy oil attributes by engineering high-oleic Omega-9 canola to produce DHA by seed expression of PUFA synthase genes from marine algae. PUFA synthases use malonyl-CoA as the sole substrate for direct de novo synthesis of DHA, rather than requiring elaboration of native fatty acids such as linoleic and linolenic acids. Thus DHA production is enabled in a vegetable oil background with low saturated and high monounsaturated fat. Progress toward
engineering both canola and soybean oils to contain DHA using algal PUFA synthase genes will be described.

**Tailored Triglyceride Oils for Food Industry Applications.** Walt Rakitsky, Solazyme, Inc., South San Francisco, CA, USA

Solazyme, Inc. is a renewable oil and bioproducts company that transforms a range of low–cost plant–based sugars into high–value oils. Utilizing an industrial biotechnology platform that harnesses the oil–producing capabilities of microalgae, Solazyme's technology is capable of using industrial fermentation equipment to efficiently accelerate microalgae's natural oil production time to a few days. By feeding their microalgae plant sugars in dark fermentation tanks, the company is in effect utilizing ?indirect photosynthesis,? in contrast to open–pond approaches. Solazyme’s technology allows them to optimize oil profiles– or ‘tailor’ them– with different carbon chain lengths, saturation levels and functional groups to modify important oil characteristics, allowing them to address specific customer requirements, while offering superior performance characteristics to convention oils. Solazyme’s tailored nutritional oils offer the food industry opportunities to: reduce saturated fat content in food products, replace partially hydrogenated vegetable oils (PHVO) while maximizing overall nutritional benefits, increase the life of cooking/frying oils, increase shelf life in packaged food products, extend the supplies of cocoa and shea butters and provides more sustainable alternatives to palm derived products.

**Stearidonic Acid (SDA) Effects on EPA Levels in Red Blood Cells.** E. Krul2, R. Mukherjea2, S. Lemke1, D. Goldstein1, R. Wilkes1, 1Monsanto Company, St. Louis, MO, USA, 2Solae, LLC, St. Louis, MO, USA

Stearidonic acid (SDA) is a product of delta–6 desaturase, the rate limiting enzyme in the conversion of alpha–linolenic acid (ALA) to eicosapentaenoic acid (EPA). Through biotechnology, soybeans can produce oil enriched with SDA omega–3 fatty acids. Previous studies demonstrated an increase in percent EPA in red blood cells in people consuming SDA enriched soybean oil or SDA ethyl esters. In one recent study, the effects of different doses and durations of treatment with encapsulated ethyl esters of SDA and EPA on levels of EPA in RBC was assessed. Repeated Measures Analysis showed that SDA and EPA both significantly increased % EPA in RBC and the omega–3 index compared to control. The relationship between time and EPA enrichment in RBCs appears to follow a first order kinetic model. In a second study, the efficiency of SDA when incorporated as a food ingredient on EPA enrichment of RBC membranes in healthy men and women was assessed. Food sources provided 7.5 g/day of oil in two baked bars and one beverage. Mean %EPA in RBC of 0.5% at end of treatment was significantly greater compared to control. SDA was found to increase RBC%EPA with 22% efficiency of EPA alone, similar to past studies. These studies confirmed that significant increases in RBC EPA can be achieved through the consumption of foods easily formulated to contain SDA enriched soybean oil.

**Optimization of Nanoliposome Formulation Encapsulating Natural Dipeptide Antioxidant by Mixture Design.** Behnoush Maherani, Elmira Arab–tehrany, Michel Linder, Institut national polytechnique de Lorraine, vandoeuvre lès nancy, Lorraine, France

Encapsulation of antioxidants by nanoliposomes could represent an ameliorative approach to overcome the problems related to a range of chemical changes in food systems, including enzymatic and chemical modification, as well as extreme pH and temperature. Mixture Design of Experiments is a technique that used to determine the optimum combination of chemical constituents that deliver a desired response. The model mixture design was created to characterize ten liposome formulations having different percentage and types of lipid(DOPC, POPC, DPPC) in their formulation. Nanoliposomes prepared by thin film hydration method, were assessed by considering their physicochemical properties. The dipeptide carnosine (fl–alanyl–L–histidine) was chosen because of its wide range of antioxidant functionality in nutritional and pharmaceutical aspects. The model proposed an optimal liposome formulation by considering the appropriate size, maximum entrapment efficiency, considered fluidity for bioactive releasing in controlled condition with desired stability. The optimal point estimated by model is: 46% DOPC, 12% POPC and 42% DPPC. This model could be an impressive approach which enables theoretical understanding of optimal liposome formulation including construction of liposomes with improved stability, favorable size, expected encapsulation efficiency and controlled interaction properties.
WEDNESDAY

MORNING

H&N 4: Lipids and Immune Function

Chair(s): R. Ward, Utah State University, USA; and H. Durham, Louisiana State University, USA

Role of Endogenous Endotoxin Absorption and Endotoxin Receptors in Inflammation Associated with High Fat Diets. M.C. Michalski1,3, F. Laugerette1, M. Alligier2,3, C. Vors1,2, G. Pineau1,2, C.O. Soulage1, A. Geloen1, M. Laville3,2, H. Vidal2,3, 1INRA USC1235, Lyon University, Univ Lyon-1, INSA-Lyon, CarMeN Laboratory, Villeurbanne, France, 2INSERM U1060, Lyon University, Univ Lyon-1, CarMeN Laboratory, Oullins, France, 3CRNH-RA, CENS, Oullins, France

Low-grade inflammation is now recognized as a major metabolic feature in obesity. Among possible causes, the role of gut endotoxin absorption during the digestion of lipids has recently been revealed. However, the impact of high fat diet and of lipid composition on innate immunity-related mechanisms involving endotoxin handling are poorly understood. Healthy volunteers were subjected to a dietary lipid overfeeding intervention. Moreover, mice were fed diets enriched with 20 wt% rapeseed oil, sunflower oil, palm oil or milkfat. We measured endotoxemia, endotoxin receptors LBP and scCD14, IL-6 and MCP-1. In adipose tissue, gene expression of TLR4, CD14 and cytokines was analysed by PCR. In humans, markers of endotoxemia were altered by dietary lipid overfeeding in part of the cohort, resulting in increased inflammation. In mice, palm oil diet provoked the greatest inflammatory outcomes, high plasma LBP, low scCD14 and increased expression of TLR4 and CD14 in adipose tissue. In contrast, rapeseed oil diet resulted in an endotoxin metabolism driven towards less inflammatory pathways. Mechanisms involving immunity appear to contribute to the development of metabolic inflammation. Moreover, lipid composition can contribute to modify the onset of inflammation through the quality of endotoxin receptors.

Inhibitory Effects of Carotenoids on Antigen-induced Activation of Immune Cells. Y. Manabe, S. Sakai, T. Sugawara, T. Hirata, Kyoto University, Kyoto, Japan

We have previously reported that some carotenoids, such as fucoxanthin, astaxanthin, zeaxanthin, and β-carotene, inhibit the mast cell degranulation via suppression of antigen-induced translocation of high affinity IgE receptor (FccRI) to lipid rafts. In this study, the effects of other carotenoids on the translocation of immunoreceptors to lipid rafts were evaluated. Rat basophilic leukemia (RBL-2H3) cells were used as a model of mast cells, and antigen-induced translocation of FccRI to lipid rafts was evaluated by immunofluorescence staining. Twelve out of twenty-two carotenoids inhibited the translocation but the others had no effects. The carotenoids suppressing the translocation of FccRI inhibited the degranulation of mast cells. We also investigated the effects of the carotenoids on activation of B cells via B cell receptor (BCR) using human Burkitt lymphoma (Ramos) cells as a model. Nine carotenoids, especially lycopene, markedly suppressed anti-BCR antibody-induced translocation of BCR to lipid rafts. These results indicate that the carotenoids have the structure-dependent inhibitory effect on the translocation of the immunoreceptors to lipid rafts. Further studies are needed to clarify the precise molecular mechanism by which the carotenoids affect the translocation of the immunoreceptors to lipid rafts.

Dietary Milk Polar Lipids Benefit Lipid Metabolism and Gut Barrier in Obese Mice Fed Moderate High-fat Diet. A.L. Zhou, R. Ward, Utah State University, Logan, UT, USA

Dynamic interactions among dietary fat, gut barrier integrity and lipid metabolism remain unclear during development.
of diet-induced obesity (DIO). To explore those interactions, 3 groups of C57BL mice for were fed for 15 weeks: (1) modified AIN-93G diet (CO); (2) CO with milk gangliosides (GG); (3) CO with milk phospholipids (PL). GG or PL did not affect food intake, weight gain or fasting plasma levels of glucose, cholesterol and triglycerides. Expressions of 13 genes on liver lipid metabolism were assessed by RT-qPCR. PL suppressed fatty acid synthesis gene ACC (vs GG) and up regulated cholesterol excretion gene SR-BI (vs CO & GG). PL increased body fat accumulation (vs CO & GG). Differential sugar tests revealed significant time effect and diet*time interactions on gut permeability. Intestinal barrier damage was proximal and then universal. Colon barrier integrity decreased gradually and recovered later. Early on, PL and especially GG protected intestinal barrier integrity. Upon the end, PL and especially GG facilitated recovery of colon barrier integrity. In general, this study revealed important dynamic changes in gut permeability and interesting interactions between dietary lipids and gut permeability during development of DIO. Milk polar lipids may have beneficial effects on lipid metabolism and gut permeability in DIO.

Differential Inhibition of PGE2 Synthesis by Sciadonic Acid (SCA) and Δ7-eicosatrienoic Acid (Δ7-ETrA) in Murine RAW264.7 Macrophage. Lu-Te Chuang1, Po-Jung Tsai2, Wen-Cheng Huang2, 1Department of Biotechnology, Yuanpei University, Hsinchu, Taiwan, 2Department of Human Development and Family Studies, National Taiwan Normal University, Taipei, Taiwan

Sciadonic acid (SCA; Δ5,11,14-20:3) and Δ7-eicosatrienoic acid (Δ7-ETrA; Δ7,11,14-20:3) are two rare non-methylene-interrupted eicosatrienoic acids (NMI-ETrA) present in gymnosperm seeds. Previously, we showed that SCA and Δ7-ETrA could replace arachidonic acid (AA) in phospholipids, and subsequently reduce LPS-stimulated PGE2 production. The purpose of this study was to determine if the same mechanism underlie the inhibition of PGE2 production by SCA or Δ7-ETrA in RAW264.7 cells. Results showed that SCA or Δ7-ETrA were both incorporated dose-dependently into cellular phospholipids, and that concomitantly each of these NMI-ETrA decreased the proportion of AA in phospholipids. Supplementation of cells with SCA or Δ7-ETrA (100 μM) significantly reduced PGE2 production by 29% and 74%, respectively, while down-regulating COX-2 expression by 60% and 13%, respectively. The fact that the extent of decrease in COX-2 expression caused by SCA or Δ7-ETrA did not result in proportional decrements in PGE2 production is consistent with the hypothesis that Δ7-ETrA competed much more effectively than SCA for COX-2. In conclusion, the observation that Δ7-ETrA appears to be a better substrate than SCA for cellular incorporation and COX-2 accounts for their differential effects on PGE2 production.

The Endocannabinoid Metabolome: Inflammatory Response Dyad in the Last Half of Pregnancy. H.A. Durham1, J.T. Wood2, J. Geaghan1, A. Makriyannis2, C.J. Lammi-Keefe 1, 1AgCenter, Louisiana State University, Baton Rouge, LA, USA, 2Center for Drug Discovery, Northeastern University, Boston, MA, USA

Endocannabinoids (ENDO) are lipid messengers and fatty acid analogs which play a fundamental role in inflammatory response. Emerging evidence indicates that the endocannabinoid-cytokine connection is responsible for many regulatory events during pregnancy. The objective of this study was to evaluate the relationship between inflammatory markers (IM) and plasma ENDOs in pregnant women during the second and third trimester (n=61). We quantified plasma ENDOs (anandamide, AEA; palmitoylethanolamine, PEA; oleoylethanolamine, OEA; docosaheaxaenoyl ethanolamine, DHEA; eicosanoyl ethanolamine; 2-arachidonoylglycerol; 2-palmitoylglycerol; & 2-oleoylglycerol) at 20-22, 23-26, 32 and 38-40 weeks using liquid chromatography-mass spectrometry. The IMs, IL-6, leptin, and adiponectin, were measured using Enzyme-Linked ImmunoSorbent Assay. Canonical correlations were used to evaluate relationships between IMs and ENDOs with adjustment for weeks. Preliminary findings suggest that during the second and third trimesters of pregnancy leptin and adiponectin are negatively related to AEA, PEA and DHEA, all ethanolamines (p=0.045). To our knowledge, this is the first report evaluating the relationship between the endocannabinoid metabolome and specific IMs during pregnancy. These data point to a relationship between the inflammatory response and the regulation of ENDOs in maintaining a successful pregnancy outcome.

The Macrophage and Plasma Lipidomes in Health, Nutrition and Disease. Edward A. Dennis, University of California, San Diego, La Jolla, CA, USA

The omics evolution began at the end of the 20th century with the cloning of the human genome. The 21st century has already seen the development of comprehensive proteomics analyses, but the emerging evolution is to metabolomics,
the definition of which is the identification and quantitation of all of the molecular constituents of the cell including its nucleic acids, amino acids, sugars, and fats. But by far, the largest number of distinct molecular species in cellular metabolism lies in the fats (or lipids) where tens of thousands of distinct molecular species exist in cells and tissues [Dennis, Proc.Natl.Acad.Sci.U.S.A.,106, 2089-2090 (2009)]. We have now applied novel liquid chromatographic-mass spectrometric based lipidomics techniques termed ?CLASS? [Harkewicz & Dennis, Annual Reviews of Biochemistry, 80, 301-25 (2011)] generally in the context of an overall omics analysis of immunologically-activated macrophages integrating transcriptomics, proteomics, and metabolomics of lipid metabolites [Dennis et al, J Biol Chem, 285, 39976-85 (2010)]. As part of the LIPID MAPS Consortium [see, LIPID MAPS/Nature Lipidomics Gateway www.lipidmaps.org], our laboratory has developed a robust and comprehensive approach to the lipidomic analysis of hundreds of fatty acids, acylethanolamines and inflammatory eicosanoids, including their numerous metabolites arising from an array of cyclooxygenases, lipoxygenases, cytochrome P450s and non-enzymatic oxidation producing isoprostanes, as well as combinations thereof [J. Lipid Res. 50, 1015-1038 (2009)]. We will discuss the application of lipidomic analysis to characterize cellular lipid signaling of Toll-like (TLR) and purinergic receptors and their ? synergy? in endotoxin stimulated macrophages as models for inflammation and infection [J. Biol. Chem., 282, 22834-22847 (2007)]. New results comparing various primary macrophages and analysis of the fluxes of metabolites as well as ?directed proteomics? of the system will be presented. Also lipidomic analysis of cells supplemented with small amounts of the omega-3 fatty acids eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) provides information on the overall effects of EPA and DHA on the inflammatory eicosadome. Human plasma has also been profiled to quantify almost six hundred distinct lipid molecular species present across all mammalian lipid categories [J Lipid Res., 51, 3299-3305 (2010)] and the implications for the future of clinical medicine and the understanding of the mechanisms of disease will be discussed [Quhenberger & Dennis, New England Journal of Medicine, 365, 1812 (2011)]. [Supported by LIPID MAPS Glue Grant U54 GM069338, R01 GM020501, and R01 GM064611]

**Anti-atherogenic properties of apolipoprotein E mimetic peptides.** Gaurav Nayar, Shaila P Handattu, David W. Garber, Vinod K. Mishra, Mayakonda N. Palgunachari, Geeta Datta, G.M. Anantharamaiah, UAB School of Medicine

Apolipoprotein E (apoE) is a protein component of chylomicrons, remnants, very low density lipoprotein (VLDL), and high density lipoproteins (HDL) and is required for the receptor mediated hepatic uptake of apoB-containing remnant lipoproteins. ApoE has been shown to play a central role in protecting the artery wall. We designed a novel peptide in which the cationic putative receptor binding domain from apoE (141-150 of apoE, LRKLRKRLLR) was covalently linked to a well-characterized apoA-I mimetic peptide 18A to yield a dual-domain peptide Ac-hE-18A-NH2. Administration of this peptide dramatically decreases plasma cholesterol in dyslipidemic animal models and inhibits atherosclerosis in apoE null mice. In addition we have also shown that oral administration of a single domain Arg rich peptide mR18L reduces atherosclerosis. Peptide Ac-hE18A-NH2 has also been shown to increase paraoxonase activity and improve HDL quality. Such studies are being performed with mR18L. Several intriguing observations regarding apoE secretion when hepatocytes and macrophages are exposed to oxidative stress and their relevance to inhibit atherosclerosis in dyslipidemic mouse models will be discussed.

**Omega-3 Fatty Acid Receptor, GPR120 Mediates Potent Anti-Inflammatory and Insulin Sensitizing Effects.** D.Y. Oh, J.M. Olefsky, University of California San Diego, La Jolla, CA, USA

Omega-3 fatty acids (ω-3 FAs), DHA and EPA, exert anti-inflammatory effects, but the mechanisms are poorly understood. Here we show that the G protein-coupled receptor 120 (GPR120) functions as an ω-3 FA receptor/sensor. Stimulation of GPR120 with ω-3 FAs or a chemical agonist causes broad anti-inflammatory effects in monocyteic RAW 264.7 cells and in primary intraperitoneal macrophages. All of these effects are abrogated by GPR120 knockdown. Since chronic macrophage-mediated tissue inflammation is a key mechanism for insulin resistance in obesity, we fed obese WT and GPR120 knockout mice a high fat diet with or without ω-3 FA supplementation. The ω-3 FA treatment inhibited inflammation and enhanced systemic insulin sensitivity in WT mice, but was without effect in GPR120 knockout mice. In conclusion, GPR120 is a functional ω-3 FA receptor/sensor and mediates potent insulin sensitizing and anti-diabetic effects in vivo by repressing macrophage-induced tissue inflammation.

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N-3 polyunsaturated fatty acids (PUFA) are beneficial for health but are highly susceptible to oxidation. However, little is known about the digestive fate of oxidation end-products from dietary PUFA and their metabolic impact. Therefore, we studied (i) the intestinal absorption of 4-hydroxy-2-hexenal (HHE) and 4-hydroxy-2-nonenal (HNE), end-products of n-3 and n-6 PUFA peroxidation, respectively and (ii) their impact on inflammation and oxidative stress. C57/B16 mice were fed for 8 weeks with high fat diets containing n-3 PUFA oxidized (OX) vs non-oxidized (Nox). In vitro, Caco-2/TC7 cells were incubated with 4-HHE or 4-HNE (1 to 100 µM). In mice, OX groups had increased plasma concentration of 4-HHE (4-5 fold) compared with Nox groups (P<0.05). Moreover, OX groups presented a greater NF-kB activation in the jejunum (P<0.05) and higher plasma levels of the proinflammatory chemokine MCP-1. In vitro, 4-HHE and 4-HNE (i) increased in the basolateral medium with the dose incubated on Caco-2/TC7 cells, indicating partial absorption and (ii) induced increased protein carbonylation and NF-kB activation. Altogether, results suggest that oxidized n-3 PUFA in the diet induce oxidative stress and inflammation, both in the intestine and in plasma, which can be due to the absorption and reactivity of 4-hydroxy-alkenals.

Effects of Oxidized EPA on Apolipoprotein A-I Expression in HepG2 Cells and Caco-2 Cells.

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Apolipoprotein (apo)A-I, the major protein component of high density lipoprotein, contributes to the process of reverse cholesterol transport. Increase in apoA-I levels in plasma can reduce cardiovascular disease risk. Eicosapentaenoic Acid (EPA) is well-known to decrease plasma triacylglycerol levels. However, the effects of EPA on apoA-I expression remain controversial. EPA can be easily oxidized, and diverse oxidation products are generated. In this study we investigated the effects of oxidized EPA on apoA-I expression in HepG2 cells and Caco-2 cells. EPA was incubated in a water bath at 40°C for 0-72 hours, and the oxidized EPA was analyzed by LC-PDA-MS. HepG2 cells and differentiated Caco-2 cells were treated for 24 hours with oxidized EPAs varying in oxidation times. Protein secretion and mRNA expression of apoA-I were evaluated by ELISA and real time RT-PCR, respectively. Oxidized EPAs upregulated apoA-I secretions from both HepG2 cells and Caco-2 cells in an oxidation time dependent manner. Several hydroxy and hydroperoxy EPAs were identified by LC-PDA-MS. Possible conversion of the hydroxy and hydroperoxy EPAs into other oxidation products could lead to increase apoA-I expressions in HepG2 cells and Caco-2 cells.

Fatty Acid Regiospecificity as a Potential Determinant of Tissue Uptake of Dietary DHA.

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Regiospecificity is an important determinant of the nutritional outcomes of dietary fatty acids. Triacylglycerol (TAG) structure influences short-term intestinal absorption/bioavailability and has long-term effects emanating from the absorbed fatty acids. Fatty acids at sn1(3) positions are hydrolysed by pancreatic lipases and absorbed as free fatty acids, whilst sn2 monoglycerols are taken up intact and may influence physiological outcomes. Strategic positioning of bioactive fatty acids such as omega-3 longchain (≥C20) PUFA (ω3LC-PUFA) may therefore influence not only
oxidative stability but also absorption, tissue uptake and downstream metabolic activity. We investigated these mechanisms using a pair of regio-isomerically pure TAG containing docosahexaenoic acid (DHA, 22:6ω3) positioned at either sn2 or sn1(3); ODO and OOD (D-DHA and O-oleic acid). Adult rats were fed (4 weeks) diets supplemented with fish oil, OOD and OOD at three dose levels (0.5%, 1% and 1.5% w/w) prior to determination of tissue fatty acid composition. DHA incorporation into cardiac tissue was preferential, dose-related and independent of its TAG location. In contrast, brain showed no change, whilst plasma, liver and kidney displayed increased ω3/ω6 PUFA ratio following the OOD diet; P<0.05 vs ODO. Further studies are required to establish the physiological significance, if any, of these findings.

Composition and Regiospecific Distribution of Fatty Acids in Commercial Milk Fat and High CLA Content Milk Fat. Dhananjay Zope1, Paul Angers1,2, Joseph Arul1,2, 1Department of Food Sciences and Nutrition, Faculty of Agricultural Sciences & Food, Universite Laval, Quebec, QC, Canada, 2Institute of Nutraceuticals and Functional Foods (INAF), Universite Laval, Quebec, QC, Canada

The pleiotropic health effects of conjugated linoleic acids (CLAs) such as anti-obesity, anti-atherosclerosis, anti-cancer and immune system modifications, are of interest since last two decades. Previously, we prepared two types of milk fats; one by dietary modification of dairy cattle that was enriched in CLAs (2% compared with 0.5-0.8% in regular milk fat); and a fraction of CLA enriched milk fat containing 4% CLAs. These fats were analysed for FA composition and their regiospecific distribution by Grignard degradation method. The three fats differed mainly with regards to their contents in the palmitic acid, isomers of C18:1, and CLA (cis-9,trans-11). In all the three fats, almost all the C6:0, ~75% of C8:0 to C12:0, and 50% of C17:0, C18:2 were located at sn-1(3) positions. The oleic and stearic acids in regular and CLA enriched milk fat were equally distributed at sn-1(3) and sn-2 positions, but in CLA enriched milk fat fraction, 70% of these acids were at sn-1(3) positions. About 75% of trans-vaccenic acid in CLA enriched milk fat is distributed at sn-2 position, but in CLA enriched milk fat fraction 75% of it is distributed at sn-1(3) positions. The regiospecific distribution indicates that the positions of CLAs are preserved in all three types of fats, and they are located at the sn-1(3) positions (59 to 65% of the total CLAs).

The Effect of Vegetables and Plant Oil on DNA Damage in Subjects with Type 2 Diabetes. Elisabeth Müllner1, Simone Pleifer1, Christiane Schiermayer1, Helmut Brath2, Karl-Heinz Wagner1, 1University of Vienna, Department of Nutritional Sciences, Vienna, Austria, 2Diabetes Outpatient Clinic, Health Centre South, Vienna, Austria

Type 2 diabetes (T2DM) is a metabolic disorder characterized by high blood glucose levels which are a consequence of impaired insulin secretion and insulin function. Recent studies suggest that people with T2DM are more likely to die from cancer than people without diabetes. To assess the amount of DNA and chromosomal damage in T2DM subjects and the impact of a dietary intervention rich in vegetables and PUFA on cancer risk an intervention study with 76 diabetic and 23 non-diabetic subjects was performed. Subjects were randomly assigned to the information or intervention group. All subjects received information about the potentials of a healthy diet and subjects randomised to the intervention group additionally received 300 g of vegetables and 25 ml of plant oil rich in PUFA per day for 8 weeks. DNA damage was measured with Comet Assay, chromosomal damage with Micronucleus Assay. The intervention led to a significant reduction in DNA damage in diabetic subjects. Levels of DNA damage did not change in non-diabetic subjects of the intervention group and in subjects of the information group. Biomarkers for chromosomal remained constant in the intervention and information group. The results of our study suggest that a healthy diet can reduce levels of DNA damage in diabetic subjects but has no effect on chromosomal damage.

Metabolomics Study of the Effect of Omega-3 and Vegetables on Patients with Type 2 Diabetes Reveals the Metabolic Alteration in Plasma- Phospholipids and Ceramides. Ali A. Moazzami1, Elisabeth Muellner2, Helmut Brath3, Ernst Forster3, Karl-Heinz Wagner2, 1Swedish University of Agricultural Sciences, Department of Food Science, Uppsala, Uppland, Sweden, 2University of Vienna, Department of Nutritional Sciences, Vienna, Austria, 3Diabetes Outpatient Clinic, Vienna, Austria

The number of people suffering from Type 2 Diabetes (T2DM) has reached an alarming level. T2DM is a multifactorial disease accompanied by abnormalities in lipid metabolism. Omega-3 fatty acids and vegetables have been shown to
improve the health status in patients with T2DM. Metabolomics have provided opportunity to explore global metabolic status after a given intervention instead of just looking at few metabolic factors. In first stage of our study, 20 subjects with T2DM were randomly assigned into the control- or intervention group. All subjects received information about the potentials of a healthy diet and intervention group, additionally received 25 g of walnut oil and 300 g of vegetables daily during eight weeks. LC-MS/MS metabolomics platform was used to quantitatively measure 485 metabolites in plasma of subjects at baseline and at the end of intervention. Data analysis showed that 25 g oil and 300 g of vegetable causes a significant reduction in prostaglandin F2α± (PGF2α±), phospholipids and ceramides. Both phospholipids and ceramides are increased in T2DM and their reduction in our study could be explained by reduction of PGF2α± by omega3 fatty acids in walnut oil. The results were confirmed in another cohort of T2DP (n=25).

Oil Palm Fruit as a Leading Source of Natural Antioxidants. M.Y. Abeywardena1, R. Sambanthamurthi2, Y. Tan2, K. Sundram3, 1CSIRO - Food & Nutritional Sciences, Adelaide, SA, Australia, 2MPOC, Kuala Lumpur, Malaysia, 3MPOB, Kuala Lumpur, Malaysia

The global demand for natural antioxidants and related bioactives is rising with the emerging evidence that such components play vital roles promoting health and wellbeing. These naturally occurring compounds are synthesised primarily by photosynthetic organisms, and concentrated in crop-plants, therefore the edible oils and fats industry is a leading primary source for such compounds. In particular, the fruit (Elaeis guineensis) from which palm oil is extracted is rich in antioxidants. Palm vitamin E enriched in tocotrienols (70%) and palm carotenes rich in alpha-carotene (30-40%) reside in the fruit's mesocarp and are recoverable from the extracted oil. Multiple health benefits of tocotrienols have been reported, and the skin-protective and cancer-suppressing actions of palm carotenes underlie their recent entry to the supplement market. This investigation focused on a novel bioactive preparation, oil palm phenolics (OPP), recovered from the waste stream of vegetation liquor (globally estimated at >85 million tons) that originates during the palm oil milling process. Studies utilising biochemical, cellular, ex-vivo and whole animal models (rats and rabbits; N=200) confirmed the vascular protective, blood pressure lowering, anti-arrhythmic and anti-atherogenic actions of OPP. These findings add to the growing body of scientific evidence on health benefits of polyphenols.

Health and Nutrition Posters

Chair(s): R. Ward, Utah State University, USA

Fucoxanthin Extraction and Fatty Acid Profiles of Malaysian Brown Seaweeds and Cytotoxicity Effect of Fucoxanthin on Human Lung Cancer (H1299) Cell Lines.
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Fucoxanthin has been successfully extracted and purified from several species of Malaysian brown seaweeds, namely Sargassum binderi, S. duplicatum, and Pudina australis. The purity of the fucoxanthin is >99% as indicated by HPLC analysis. Results also showed that all samples contained a considerable amount of fucoxanthin and total lipid. The amount of fucoxanthin and total lipid contents of S. duplicatum (1.01 ± 0.10 and 21.3 ± 0.10 mg/g dry-weight, respectively) was significantly higher than those of S. binderi (0.73 ± 0.39 and 16.6 ± 4.10, respectively) and P. australis (0.43±0.90 and 10.7±0.07, respectively). All types of seaweeds also contained a considerable amount of unsaturated fatty acids. Eicosapentanoic acid, arachidonic acid, linoleic acid and alpha-linolenic acid contents of S. duplicatum was 0.76, 2.55, 13.64, 5.81 and 5.35 %, respectively while S. binderi contained 0.70, 1.82, 9.13, 6.37 and 4.39%, respectively and P. australis contained 2.06, 9.50, 6.37, and 2.83 %, respectively For saturated fatty acids, palmitic (C16:0) was found to be the major fatty acid in all samples studied. Data obtained from the MTT assay
indicated that fucoxanthin reduced the viability of H1299 cell lines, showing an IC50 value was 2.45 mM.

**Investigation of the Papaya Seed Oil Properties for Development into Edible Oil.**
Supatha Lilitchan¹, Piyawadee Sammarphet¹, Nittaya Theppratom¹, Promluck Somboonpanyakul¹, Kornkanok Aryusuk², Kanit Krisnangkura¹, Nittaya Thipchathevee¹, Bangkok 10400, Thailand, ²King Mongkut's University of Technology Thonburi, Bangkhuntien, Bangkok 10150, Thailand

**Preventive Effect of Astaxanthin from Haematococcus pluvialis on Inflammatory Bowel Disease.**
Masashi Hosokawa¹, Yuki Kizawa¹, Yumiko Yasui², Takuji Tanaka³, Kazuo Miyashita¹, Eiji Yamashita⁴, ¹Hokkaido University, Hakodate, Hokkaido, Japan, ²Rakuno Gakuen University, Ebetu, Hokkaido, Japan, ³The Tokai Cytopathology Institute, Gifu, Gifu, Japan, ⁴Fuji Chemical Industry Co., Ltd, Kamiichi, Toyama, Japan

Ulcerative colitis and Crohn's disease are major forms of inflammatory bowel diseases (IBD), which are chronic uncontrolled inflammation of intestinal mucosa. The incidence of ulcerative colitis has risen sharply in the developed country in recent decades. In this study, effect of astaxanthin (AX) extracted from *Haematococcus pluvialis* on the experimental colitis induced by dextran sulfate sodium (DSS) in mice were investigated. AX significantly suppressed a DSS-induced mucosal colitis determined by histopathology score at 200 ppm in the diet. In addition, AX also inhibited the expression of pro-inflammatory cytokines such as interleukin-6 (IL-6) and IL-1β mRNA in colon tissue. The mRNA expression level of COX-2, which is an inflammatory enzyme to produce PGE₂, was also suppressed by AX diet. Nuclear factor-kappaB (NF-κB) is an important transcriptional factor to induce pro-inflammatory cytokines mRNA expression. As the result of NF-κB-DNA binding assay, AX inhibited NF-κB activation in murine macrophage-like cells, RAW 264.7. These results indicate that AX from *Haematococcus pluvialis* is a preventive carotenoid for IBD.

**Effect of Catalyst Concentration and Temperature on Intraesterification of Milk Fat.**
Dhananjay Zope, Paul Angers, Joseph Arul, Department of Food Science and Nutrition and Institute of Nutraceuticals and Functional Foods (INAF), Laval University, Quebec, QC, Canada

With the decline of hydrogenation, interesterification is becoming a major process for modification of fats to improve in functionality. Intraesterification of a specific oil or fat suffers from slow rate of reaction and limited modification of triacylglycerols (TG) and physical properties, and hence received little attention compared with interesterification of oil / fat blends. We studied the effect of catalyst concentration and reaction temperature on intraesterification of milk fat on the rate of the reaction. Sodium methoxide was used as catalyst ranging from 1 to 5% (w/w) at temperatures ranging from 70 to 1200C. The TG composition, melting profile (DSC) and solid fat content (SFC) showed that temperature did not much effect on intraesterification, but increasing catalyst concentration resulted in modification of triacylglycerols with TG composition different from that of milk fat. Most TG modification observed with catalyst concentration of 4%. Higher catalyst concentration led to significant losses of the fat through soap formation. The results of this work suggests that intraesterification can be achieved with higher catalyst concentrations, and can be useful tool in modifying the physical properties of a fat and its utilization in bakery and margarine products.

**Effects of Topical Application of Vegetable Oil Blends and Structured Lipids on Wound Healing.**
Juliana Neves Rodrigues Ract¹, Fabiana Andreia Schafer De Martini Soares¹, Hosana Gomes Rodrigues², José Ricardo Bortolon³, Gilson Masahiro Murata³, Maria Inês Almeida Gonçalves¹, Elaine Hatanaka³, Rui Curi², Luiz Antonio Gioielli¹, ¹Faculty of Pharmaceutical Sciences, São Paulo, Brazil, ²Institute of Biomedics Sciences, São Paulo, Brazil, ³University Cruzeiro do Sul, São Paulo, Brazil

Some of our previous findings suggest that oleic and linoleic acids accelerate the inflammatory phase of wound healing, resulting in faster wound closure. Vegetable oil blends were prepared and their respective structured lipids were enzymatically synthesized to be topically administered in wounds surgically created in the dorsal surface of rats.
The aim of this study was to compare the wound closure evolution among rats treated with the blends, structured lipids, and the control group treated with a physiological saline solution. The wound healing process was evaluated by measuring the wound areas in photographs periodically taken during 15 days of treatments following three different protocols and calculating the % wound closure, besides different cytokines determination. No significant differences were observed between the treatments with the blends and the structured lipids. However, an increase in the areas of the wounds treated with both separately was observed in the inflammatory phase, succeeded by a sharper closure curve than the wounds in the control group. Although acceleration in wound closure has not occurred when these were treated with structured lipids, the alterations observed in the inflammatory phase (1-3 days) suggest a potential therapeutic application on cutaneous wound healing.

**Effect of a Diet Rich in Olive Oil Phenolics on Glutathione, Uric Acid and Ascorbic Acid Status in the Liver of Senescence Accelerated Mouse Prone 8 (SAMP8) Mice.**

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Liver ageing may be mediated by increased oxidative stress, partly due to changes in the oxidant/antioxidant equilibrium. Glutathione (GSH), uric acid and ascorbic acid are important antioxidant compounds which are involved in the ageing process protecting the cells from oxidative stress. In this study we investigated effect of a diet rich in olive oil phenolics on these antioxidant compounds in SAMP8 mice, which exhibit elevated biomarkers of oxidative stress. The SAMP8 mice, aged 9-10 weeks, were divided into two groups and fed Western type diet with 0.15% cholesterol and 20% fat, in which 10% of fat was from olive oil containing either low (44 mg gallic acid/kg oil) or high (532 mg gallic acid/kg oil) amounts of phenolics for 4.5 months. As a marker of oxidative stress GSH, aminothiols (methionine, cysteine) that take part in the GSH synthesis, glutathione disulfide (GSSG), uric acid and ascorbic acid were analyzed in the liver of SAMP8 mice with high performance liquid chromatography coupled to electrochemical detector. The ratio of GSH/GSSG was determined as an important biomarker of cellular redox state. As a result no difference was observed in the concentrations of antioxidant compounds, aminothiols and GSH/GSSG ratio between mice groups that fed high- or low-phenolic diets. It is suggested that the mice were not old enough to see clear-cut effects of phenolic rich diet therefore they should be fed for a longer period of time.

**Precipitation and Encapsulation of Rosemary Antioxidants by Supercritical Antisolvent Process.**

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The encapsulation of antioxidants with biocompatible polymers is essential for their protection against degradation factors like light and oxygen, and facilitates its solubility in the target medium. This work presents the co-precipitation of an ethanolic extract of rosemary leaves by supercritical antisolvent (SAS) process in poloxamers in order to improve the aqueous solubility of the extract. In a first step, the precipitation of antioxidants by SAS was studied in the range of temperatures from 25 to 50°C and pressures from 8 to 12 MPa. Total content of polyphenols was quantified according to the Folin-Cicalteu method. Also HPLC analyses were performed to verify the presence of some of the major rosemary antioxidants, carnosic and rosmarinic acid. The dissolution rate of rosemary polyphenols from particles was measured in isotonic phosphate buffer solution (pH = 6.8). The encapsulation of the extract was successfully achieved with a yield up to 100%. The total polyphenolic content was dissolved from the encapsulated product, in the aqueous medium, after one hour, whereas only 15% of the antioxidants of the pure precipitate were dissolved after 8 hours.

**Phosphatidylcholine Hydroperoxide Promotes VEGF-induced Angiogenesis in vitro and ex vivo Models.**

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[Objective] Oxidized low density lipoprotein (which predominantly contains oxidized phosphatidylcholine (PC)) is hypothesized to play a role in the promotion of plaque angiogenesis. However, whether specific oxidized PCs are responsible for angiogenesis has not been fully determined. In this study, we investigated whether PC hydroperoxide (PCOOH, a primary oxidation product of PC) stimulated angiogenic responses (e.g., vascular endothelial growth factor (VEGF)-induced cell proliferation, migration, and tube formation, and angiogenesis-related gene/protein expression) in
human umbilical vein endothelial cells (HUVEC) and in an ex vivo rat aorta model. [Result & Discussion] VEGF induced proliferation, migration, and tube formation of HUVEC, and these angiogenic responses were all enhanced by PCOOH but not by native (nonoxidized) PC. The angiogenic effects of PCOOH are considered to be mediated via generation of reactive oxygen species and activation of both PI3K/AKT and MAPK pathways. The angiogenic activities of PCOOH were also confirmed by the rat aortic ring assay. These indicate that PCOOH can elicit several angiogenic responses, implying an important role of PCOOH in atherosclerosis progression and plaque instability.

**LC-MS/MS determination of phosphatidylcholine hydroperoxide in human plasma.**
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[Objective] With respect to the involvement of phospholipid peroxidation in human diseases, especially atherosclerosis, attention has been focused on the accurate measurement of phosphatidylcholine hydroperoxide (PCOOH), a primary oxidation product of phosphatidylcholine (PC). Here, we developed analytical techniques to accurately determine human plasma PCOOH by using LC-MS/MS. 16:0-18:2-OOH-PC was determined as a representative PCOOH molecular species found in human plasma. [Result] PCOOH was enzymatically synthesized from 16:0-18:2-PC using lipoxygenase-1, and purified to yield a pure compound. The sn-2 residue of the PCOOH was 13-hydroperoxyoctadecadienoic acid. Collision-induced dissociation of the sodiated ion of PCOOH produced some fragment ions, which permitted selective detection of PCOOH by LC-MS/MS with multiple reaction monitoring. The selective detection of PCOOH by LC-MS/MS, together with avoidance of PCOOH degradation during extraction procedure, allowed to accurate determination of plasma PCOOH. In the developed conditions, any ion suppression effects were not observed. PCOOH in plasma of healthy subject was found to be 20 pmol/ml. We are now quantifying PCOOH in plasma of atherosclerosis to further understand the involvement of lipid peroxidation in the pathogenesis of atherosclerosis.

**Anti-obesity Effect of Vegetable Allenic-carotenoid, Neoxanthin.**
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Fucoxanthin and neoxanthin are the major carotenoids present in chloroplasts of brown seaweeds and higher plants respectively. Both carotenoids have a unique structure including an unusual allenic bond and 5,6-monoepoxide in its molecule. When various carotenoids were screened for potential suppression effects on adipocyte differentiation, only fucoxanthin, neoxanthin, and two fucoxanthin metabolites showed an encouraging suppressive effect on the differentiation of 3T3-L1 adipose cells, while other carotenoids did not show such an effect. Thus, it was hypothesized that the specific structure of these carotenoids is somewhat responsible for the suppressive effect on the adipocyte differentiation. By using animal models we have found the anti-obesity effect of fucoxanthin, but not yet the effect of neoxanthin. Thus, in the present study we have examined the anti-obesity effect of neoxanthin as compared with that of fucoxanthin. When neoxanthin was given to obese/diabetes model KK-Ay mice, abdominal WAT significantly reduced as compared with control; however the activity was less than that of fucoxanthin. Neoxanthin and fucoxanthin feedings also significantly reduced blood glucose, triacylglycerol, and free fatty acid levels.

**Antioxidant Compounds Content in Pecan Nut [Carya illinoinensis (Wangenh) C. Koch] Shell Infusion.**
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In plants, the antioxidant compounds content is affected by different factors. In this work, the total phenolic compounds and proantocianidins in pecan nut shell infusion of a mixture of varieties (Barton, Shoshone, Shawnee, Choctaw e Cape Fear) and from the Barton were studied. The nuts were obtained from two different harvestings (2009 and 2010). The total phenolic and proantocianidins content was evaluated using the Folin-Ciocalteau Method and Vanillin Method. The results were expressed as Gallic Acid Equivalent (mg GAE/g) and Catequin Equivalent (mg CE/g), respectively. Results showed higher yields (36 and 46 g/100g) in extracts obtained by the infusion of the mixture of varieties from 2009 and 2010. The total phenolic content was higher for the 2010 harvest (181.49 e 145.41 mg GAE/g for the Barton variety and for the mixture of varieties, respectively). The higher proantocianidins content (49.5 mg CE/g) was observed in extracts obtained by infusion from the mixture of varieties from 2009 harvest. The results showed that antioxidant compounds content in the extracts depends on the variety and harvest year studied.
Enrichment of Eggs with Omega 3 fatty Acids by Dietary Supplementation with Algal Biomass.
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Omega 3 polyunsaturated fatty acids (PUFA) are effective in preventing or treating several diseases. Unfortunately, the average intake is below the recommended level, raising interest in food enrichment. In this regard, the fatty acid composition of eggs can be modified by feed adaptation. Nowadays, enriched eggs are mostly produced through feed supplementation with flaxseed, and are thus mainly enriched in ALA. However, in humans, the conversion from ALA to EPA and DHA is rather limited, and moreover, the health promoting effects are mostly ascribed to EPA and DHA. To that end, the aim was to study the impact of feed supplementation with EPA and/or DHA rich algal biomass on the omega 3 PUFA composition of egg yolk and hens zootechnical performances. Hens received different diets (regular diet and supplemented diets with algal biomass) for 28 days. Eggs were collected, and the lipid composition and carotenoid composition were determined. Algal feed supplementation had no significant impact on hens zootechnical performances. Furthermore, supplementation with microalgae significantly increased omega 3 PUFA content and carotenoid content in egg yolk. Detailed results will be available by the time of the congress.

Soybean β-conglycinin Improves Lipid Metabolism in Wistar Rats.
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The effects of dietary soybean β-conglycinin on lipid and energy metabolism were studied in male Wistar adult rats. Male Wistar rats (19 weeks old) were fed an AIN-93G diet containing casein or β-conglycinin for 4 weeks. After the 4-week feeding period, final body weight was significantly lower in the β-conglycinin-fed rats than in the casein-fed rats. Carbohydrate consumption was higher and fat consumption was lower in the β-conglycinin-fed rats than in the casein-fed rats. Total energy consumption was the same between the two groups. Serum adiponectin was higher in the β-conglycinin-fed rats than in the casein-fed rats. Serum triacylglycerol (TAG) level in the β-conglycinin-fed rats was significantly lower than that in the casein-fed rats. The feeding of β-conglycinin suppressed the activities of enzymes related to fatty acid synthesis and enhanced the activity of carnitine palmitoyltransferase in the liver compared to the feeding of casein. The VLDL-TAG secretion rate from the liver after the administration of tyloxapol, an inhibitor of lipolysis, was significantly lower in the β-conglycinin-fed rats than in the casein-fed rats. These results suggest that β-conglycinin exert hypolipidemic effects through the improvement of hepatic lipid metabolism in rats.

Trans Content and Thermal Behavior of Fatty Materials from Different Bakery Products.
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Trans fatty acids (TFA) are produced during the industrial partial hydrogenation of vegetable oils, therefore they are present in manufactured foods that employ hydrogenated fats as raw material. A high intake of TFA has adverse health consequences. On the other hand, the presence of natural TFA such as trans-vaccenic and conjugated linoleic acid isomers (CLA) must be highlighted and considered separately, because of their beneficial effect on health. These fatty acids are commonly found in ruminants fat and milk fat, as a result of the biohydrogenation process. In Uruguay, both have an unusual high content of CLA and trans-vaccenic, presumably due to the feeding system. This work studied the fatty acid composition and thermal behavior of fatty materials extracted from different typical Uruguayan beef tallow based products from bakeries ("galleta malteada" and "pan con grasa"), commercialized in diverse areas of Montevideo. Results showed an average fat content of 17% for the "galleta malteada" and 31.5% for the "pan con grasa". The total trans content in both cases was 6.1%, but the main TFA was trans-vaccenic (4.3%). The study of thermal behavior provides valuable information about the fatty materials employed in the elaboration of the products and its hardness.

Effect of Red Clover Isoflavones over Skin, Appendages and Mucosal Status in Postmenopausal Women.
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To evaluate in postmenopausal women the effect of red clover extract isoflavones (RCE) over the subjective status of skin, appendages and several mucosal sites. Methods: One hundred and nine postmenopausal women were randomly assigned to receive either two daily capsules of the active compound (80 mg RCE, A) or placebo of equal appearance (B) for a 90 day period. After a washout period of 7 days, medication was crossed over and taken for 90 days more. Subjective status improvement of skin, appendages, and several mucosal sites were assessed for each studied group at 90 and 187 days using a Visual Analogue Scale. In addition, libido, tiredness and urinary, sleep and mood complaints were also evaluated. Results: Women in both studied groups reported a higher subjective percent improvement of scalp hair and skin status, libido, mood, sleep and tiredness after RCE intervention as compared to placebo. In general, reported improvement over urinary complaints, nail, body hair and mucosa (oral, nasal, ocular) status did not differ between treatment phases (intra and inter group). Overall satisfaction with treatment was reported higher after RCE intervention in both assigned groups as compared to placebo. Conclusion: RCE supplementation exerted a subject improvement of scalp hair and skin status as well as libido, mood, sleep and tiredness in postmenopausal women.

Effect of Protamine on Serum Lipid Concentrations and Lipid Absorptions in Rats.
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Protamine has been widely used as a pharmaceutical product and natural food preservative. However, few studies have been conducted to assess the beneficial function of dietary protamine. This study examined the effects of dietary protamine on serum lipids concentrations and fecal fatty acids (FAs), cholesterol (CHOL), and bile acids (BAs) excretions. Groups of male Wistar rats were fed AIN93G diet containing 5% protamine. After 4 weeks of feeding these diets, markedly decreased serum CHOL and triacylglycerol levels through the enhanced fecal excretions of FAs, CHOL and BAs. To further elucidate the mechanism of enhancing fecal lipid excretions by fed protamine, effect of lipid absorption was studied using in vitro gastrointestinal digestion model. Lipase activity, micellar solubility of CHOL were significantly lower and BAs binding activity was higher in the presence of protamine peptic hydrolysate compared with casein peptic hydrolysate. These results suggest that the hypolipidemic effect of protamine is mediated by increased fecal FAs, CHOL, BAs excretions, which is due to the digestion products of protamine having reduced lipase activity and micellar solubility of CHOL, and increased bile acid binding capacity. Protamine has beneficial effects that may aid in the prevention of lifestyle-related diseases such as hyperlipidemia and atherosclerosis.

The Effects of Dietary Oxidize and Unoxidized Sunflower Oil on the Quality and Fatty Acid Content of Meat Cuts of Finishing Pigs.
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The present study is designed to determine whether differences occur in the fatty acid composition and quality of meat cuts from finishing pigs fed an oxidized and unoxidized oils ration. Total fatty acid composition was determined by using gas chromatography. The following qualities were measured: meat color, pH, loin marbling and total microbial count. Unoxidized oils are expected to contain high levels of polyunsaturated fatty acids and oxidized oils a lower values, which in turn will then change the fatty acid composition of the meat. If true, the levels may or may not have some influence on meat quality.

Dietary Sorghum Wax Decreases Systolic Blood Pressure in KKAy Mice.
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We have previously reported that dietary sorghum wax decreases plasma insulin levels in a KKAy mouse model of type 2 diabetes. Suppression of plasma insulin level is essential for preventing hypertension, because high plasma insulin level is one of the important factors influencing hypertension. We hypothesized that dietary sorghum wax may decrease blood pressure in cases of type 2 diabetes. In this study, we validated the effect of dietary sorghum wax on blood pressure in KKAy mice. We found that dietary sorghum wax decreased systolic blood pressure, plasma insulin...
levels, and amount of white adipose tissue in KKAy mice. The decrease in the systolic blood pressure in the mice was considered a result of the sorghum wax-induced decrease in the plasma insulin levels. Sorghum wax also decreased plasma angiotensin-2 (ACE-2) levels. This effect was thought to be induced by the low weight of white adipose tissue, because white adipose tissue is one of the sources of angiotensinogen (precursor of ACE-2) secretion. However, the secretion or activity of renin or ACE was believed to be one of the important factors influencing systolic blood pressure, because the coefficient of correlation between plasma ACE-2 level and white adipose tissue weight was very low. These beneficial effects of sorghum wax may be attributable to polycosanol and polycosanols.

**Physiological Functions of Dietary Ozonated-Olive Oil in Obese Rats.**
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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 no data concerning physiological function of dietary ozonated-vegetable oil on lipid metabolism in vivo. In the present study, we investigated the effect of dietary ozonated-olive oil on the pathogenesis of obese, diabetic Zucker rats. Rats 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. There was no significant difference in food intake and final body weight among groups. Feeding of ozonated-olive oil markedly alleviated hepatomegaly and hepatic lipid accumulation in Zucker rats. Enzyme activities of hepatic injury markers in serum were significantly lowered in ozonated-olive oil-fed Zucker rats. These effects were attributable to the suppression of the levels of serum inflammatory molecules. These results indicate that dietary ozonated-olive oil would be useful to prevent or alleviate metabolic syndrome in Zucker rats.

**Effect of α-eleostearic Acid on Methylmercury induced Oxidative Stress, DNA Damage and Structural Changes in Rat Model.**
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One of the best known neurotoxins is methylmercury (MeHg), a ubiquitous environmental toxicant that leads to long-lasting neurological and developmental deficits in animals and humans. Though it is generally known as neurotoxicant but in addition, the central and peripheral nervous system, the kidney and the liver each is also known target organ of MeHg toxicity in both animals and humans. Literature suggests that oxidative stress represents a critical event related to the toxic effects elicited by MeHg. In the aquatic environment, MeHg is accumulated in fish, which represent a major source of human exposure. The goal of this study was to test the effectiveness of α-eleostearic acid (AEA), a conjugated linolenic acid against MeHgCl induced oxidative stress in brain, liver, kidney and plasma of rat. Male albino rats were taken as animal model and treated with methylmercury chloride (MeHgCl) (5 mg/ Kg BW/day) to generate oxidative stress. The healing effect occurred due to ingestion of AEA was monitored by comet assay of blood lymphocytes, histopathology of kidney and liver tissues and AFM of erythrocyte membranes. The ameliorative activity of AEA was best observed when the stressed rats were treated with 1% (w/w of total lipid given) of this unique fatty acid.

**Monitoring Tea Catechins Transport And Release Processes From Alginate Beads By Continuous System.**
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The objective of the present study was to evaluate the controlled release of catechin from calcium-alginate beads by on line continuous system. Micro beads were formed via entrapping active ingredients into the biopolymer gel network, one of the most known gel system: calcium-alginate twosome. 0.3% catechin solution was prepared and loaded into the beads using 4% alginate solution as a wall material by dripping into 2% CaCl2 solution. Wall to catechin solution ratio was selected as 1:1 (v/v). After hardening 30 min. the color of the obtained final solution was orange. Release studies of catechin from alginate beads to water was investigated in continuous system and compared to standard catechin release to water (without continuous system). Beads were placed in the dialysis pouch and both sides of the pouch (inside and outside) were filled with water. Release from inside to the outside was measured continuously with
an on-line monitoring device. In catechin solution release, catechin concentration rose dramatically in contrast with the continuous system whose concentration increased slowly.

**A New Fatty Acid Ester, 1-D-arabitol-monolinoleate from the Japanese Food, Mushroom Hericium erinaceum.**
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Mushrooms have been not only used as food materials with their unique flavor and texture, but also recognized as an important source of biologically active compound of medicinal value. In recent years, there is increased medical and pharmaceutical interest in mushrooms as sources of novel immunomodulators, antitumor agents, antibiotics, and hypotensives. Hericium erinaceum (Yamabushitake in Japanese) is known as an edible mushroom that belongs to the Hericiaceae family and grows on dead trunks of hard woods such as narra, oak, and beech in Japan, China, and Europe. In regarding to chemical components of H. erinaceum, various constituents such as hericene, hericerin, hericenone erinacine, erinacerin, and polysaccharides have been reported as the chemical components. Its fruiting bodies have been used for the treatment of dyspepsia, gastric ulcer, and enervation in China. This mushroom has some beneficial effects, including antioxidant, antitumor, antimicrobial, pollen tube germination/growth inhibitor, and the stimulating the synthesis of nerve growth factor (NGF). These results suggest the usefulness of H. erinaceum, thus, we investigated chemical component of H. erinaceum. New fatty acid ester, 1-D-arabitol-monolinoleate (3) and 2-formyl-3-hydroxy-4-(3,7-dimethyl-2,6-octadienyl)-5-methoxybenzyl palmitate (4) and two new aromatic compounds (1, 2) were isolated from mushroom Hericium erinaceum. The structures of 1-5 were elucidated on the basis of spectral data. Compounds 1, 2, 4, and 5 exhibited β-glucosidase inhibitory activity.

**Novel Mechanism of Intestinal Absorption of Dietary Sphingolipids.**
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Sphingolipids are ubiquitous in all eukaryotic organisms and have attracted attention as physiologically functional lipids. Recently, various physiological functions of dietary sphingolipids, such as preventing inflammation and improving the skin barrier function, have been demonstrated. Sphingolipid used as foodstuff is commonly glucosylceramide from plant sources, which is composed of sphingoid bases distinctive from those of mammals. In higher plants, the structure of the sphingoid bases is more complicated than in mammals. However, the fate of dietary sphingolipids of plant origin is still not understood. We investigated the absorption of 4,8-sphingadienine originated from maize glucosylceramide in the rat intestine by a lipid absorption assay of lymph from the thoracic duct. The cumulative recovery of 4,8-sphingadienine in lymph was lower than that of sphingosine. Verapamil, P-glycoprotein inhibitor, could significantly increase the absorption of 4,8-sphingadienine, but it did not affected that of sphingosine. The plant type of sphingoid bases were detected in ceramide fraction by LC-MS analysis. These results indicated that dietary glucosylceramide originated from higher plants are hardly absorbed from intestine, because sphingoid bases are effluxed by P-glycoprotein in the intestinal endothelial cells.

**Effect of Different Extraction Processes on the Antioxidants Content in Pecan Nut [Carya illinoinensis (Wangenh.) C. Koch] Shell.**
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This work evaluates the effect of different extraction processes on the antioxidants content in pecan nut shell powder of the Barton variety. The extraction yields and total phenolic content in fractions obtained by extraction with ethanol, infusion and infusion followed by spray-dryer atomization were evaluated. The pecan nut shell powder before and after spray-dryer atomization were analyzed using Fourier Transform Infrared (FTIR) spectroscopy, and total phenolic content was evaluated using Folin-Ciocalteu method. Visible spectra of extracts were measured and a standard curve (420 nm) could be used to determine total solids in extracts. Results showed higher yields (46.4 g/100g) and total
phenolic content (590.8 mg GAE/g) in extracts obtained by infusion followed by spray-dryer atomization as compared to 31.5 % and 32.1% yields and 181.5 and 167.8 mg GAE/g total phenolics for infusion and ethanol-extracted samples. A high correlation between extraction yield and phenolic content was found (R²=0.9966). FTIR analysis showed increased characteristic absorption peaks for phenolics in spray-dried samples obtained likely related to higher sample solubility obtained by spray-dryer atomization. Spray-drying seems to be a suitable technique for obtaining pecan nut shell extracts rich in phenolic compounds.

**Biological Activity of Hydrolyzable Tannis from Sweet Chestnut (Castanea sativa Mill.).**

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Several whole water extracts and fractions from sweet chestnut wood, high in HTs, obtained by membrane separation technology, were analyzed and characterized, using HPLC/DAD/ESI-MS methods. Individual polyphenols were identified using their retention times, and both spectroscopic and spectrometric data; quantitation was directly performed by HPLC/DAD, using regression curves built with the available standards. Each one of the analyzed fractions was tested for its antioxidant and antiradical activity using in vitro spectrophotometric methods, i.e., respectively, (i) Folin-Ciocalteu reagent assay (measure of the antioxidant activity through an evaluation of polyphenol content, expressed as Gallic Acid Equivalent, GAE) and (ii) test of the stable radical DPPH?, 1,1-diphenyl-2-picrylhydrazyl (measure of the antiradical activity expressed as the polyphenols concentration which inhibits to 50% the activity of stable radical DPPH?, EC50). The antimicrobial properties of the extracts and fractions have also been evaluated in the biomedical, veterinary and crop protection sectors. Whole water extracts have found interesting uses in crop nutrition and protection. Selected fractions for direct spraying on crops, to inhibit some negative microbial-related processes, are presently under investigation.