H&N 1/PHO 1: Emerging Bioactives and Health Impacts

Chairs: Eileen Bailey Hall, DSM Nutritional Products, USA; and Xiaosan Wang, Jiangnan University, China

Arachidonic Acid has Anti-diabetic Actions
Gundala K. Naveen Kumar, and Undurti Das*,
BioScience Research Centre, India

Objectives: To study effect of polyunsaturated fatty acids on alloxan, streptozotocin and high fat diet induced diabetes.

Methods used: In vitro studies were done with RIN cells and in vivo studies in Wistar rats.

Results: Both ω-6 and ω-3 fatty acids prevent alloxan and streptozotocin (STZ)-induced apoptosis of rat-insulinoma (RIN) cells in vitro. Of all the unsaturated fatty acids tested, arachidonic acid (AA) prevented alloxan and STZ-induced apoptosis of RIN5F (rat insulinoma) cells in vitro and alloxan-induced type 1 diabetes and STZ-induced type 1 and type 2 DM in Wistar rats. This beneficial action of AA was not blocked by cyclo-oxygenase and lipoxygenase inhibitors. Lipoxin A4 (LXA4), an anti-inflammatory product of AA, prevented alloxan and STZ-induced apoptosis of RIN cells in vitro and type 1 and type 2 diabetes mellitus in experimental animals. AA enhanced formation of LXA4 in RIN cells in vitro and enhanced plasma LXA4 levels in alloxan and STZ-treated animals. Oral AA abrogated high-fat-STZ-induced type 2 DM in Wistar rats. AA treated HFD animals showed enhanced plasma LXA4 levels. Plasma levels of and LXA4 were decreased in patients with type 2 DM.

Conclusions: These results suggest that AA and LXA4 may function as endogenous anti-diabetic molecules.

Effects of Sesamol on Lipid Metabolism and Neurodegeneration
Xuebo Liu* and Zhigang Liu,
Northwest A&F University, China

Scope: The aim of the current study was to investigate the effect of sesamol, a natural powerful antioxidant and anti-inflammatory phenol derivative of sesame oil, on adiposity and adiposity-related metabolic disturbances in mice fed with western diet, and the potential underlying mechanisms focusing on the mitochondria-lipid metabolism.

Methods & results: In the experimental model that consisted of 3 month-old C57BL/6J mice divided into 3 groups with/without sesamol in the drinking water including standard diet, high fat and high fructose diet (HFFD), and HFFD with sesamol. Results demonstrated that sesamol mitigated bodyweight gain, development of insulin resistance induced by HFFD. Sesamol was found partially normalized serum and hepatic lipid contents, as well as suppressed HFFD-induced lipogenesis in liver via regulating mitochondria-related triglyceride/cholesterol metabolism genes expressions. Importantly, sesamol decreased mass and adipocyte sizes of white adipose tissues (WATs) and brown adipose tissues (BAT) by improving mitochondria-related genes expressions including Pgc1a and Ucp1. Moreover, sesamol was also found reduced differentiation and mitochondrial metabolic inhibitors (oligomycin and antimycin A) stimulated lipid accumulation in 3T3-L1 adipocytes.

Conclusion: Taken together, this study provides compelling evidence that sesamol supplementation reduced adipocyte size and

adipogenesis of diet-induced obesity by regulating mitochondria-lipid metabolism.

A Novel Method for Evaluating Anti-inflammatory Activity of Camellia Seed Oil Ruijie Liu*[1], Niannian Lan[2], Ming Chang[1], Qingzhe Jin[1], and Xingguo Wang[1], *[1]Jiangnan University, China; [2]School of Food Science and Technology, Jiangnan University, China*

Introduction. Camellia seed oil has been used in Chinese traditional medicine, which is rich in oleic acid, similar to olive oil. However, little is known about the influence of Camellia oil on inflammation, and extreme water insolubility of Camellia oil greatly limits absorption efficiency and bioavailability in vitro. In order to establish a rapid method to evaluate the anti-inflammatory effect of Camellia seed oil, emulsion-based delivery systems were used. Methods. Test cases were prepared with sodium caseinate (SC), whey protein isolate (WPI), or soy protein isolate (SPI) to camellia seed oil with a ultrasonic emulsification method. LPS-induced RAW264.7 cell production of nitric oxide (NO), interleukin (IL)-6 and tumor necrosis factor (TNF-a) were determined by the kit after the treatment of test emulsion for 24h. Results. Camellia seed oil was encapsulated by SC, WPI, SPI forming nanoparticles of 323,298 and 419 nm diameter. After 24h of incubation, an emulsion stabilized with WPI showed the highest intracellular accumulation of Camellia oil (33.70±2.94 ug/mg protein), followed by that stabilized with SC (21.89±2.29) and SPI (12.69±1.87). The production of Pro-inflammatory markers showed that Camellia oil significantly inhibited the LPS-activated release of NO and inflammatory cytokines (IL-6, TNF-a) compared to controls. Anti-inflammatory effect among the three emulsions increased in the following order: SPI < SC < WPI. Conclusion. Camellia seed oil exerted anti-inflammatory effect on RAW264.7 cells stimulated by LPS. Suppression inflammation effect was highest in Camellia oil loaded emulsions stabilized by WPI proteins due to the different interfacial characteristics probably. This approach contributes to evaluate the physiological activity of lipophilic nutrients.

Dietary Krill Oil Enhances Neurocognitive Functions and Modulates Proteomic Changes in Brain Tissues of Aging Mice Ling Zhi Cheong*, Tingting Sun, and Xiurong Su, *Ningbo University, China*

Krill are small marine crustacean with 12-50% of lipid content; they are mostly harvested in Antarctic Ocean. Krill oil contains astaxanthin; and is characterized by high concentration of long-chain omega-3 polyunsaturated fatty acids (n-3 LCPUFAs) mostly in the form of phospholipids (PLs) (30-65%). Due to the presence of high amount of astaxanthin and n-3 LCPUFAs, krill oil has been reported to exert positive effects on cardiovascular disease, insulin resistance and neurocognitive disorder. Despite the many studies done to show the beneficial effects of krill oil on neurocognitive function, the effects of krill oil on proteomic changes in brain tissues are rarely reported to date. Present work aimed to evaluate the effects of dietary krill oil on neurocognitive functions and proteomic changes in brain tissues of D-galactose-induced aging mice was evaluated. Dietary krill oil was found to enhance neurocognitive functions of aging mice with significant ($P<0.05$) decreased in escape latency and increased in number of times crossing over the hidden platform during Morris Water Maze test. Krill oil was also found to protect against oxidative damage, lipid

peroxidation and neurodegenerative diseases. Oxidative stress biomarkers of aging mice administered with krill oil showed significant ($P<0.05$) improvement with increased in serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels; and insignificant changes in serum malondialdehyde (MDA) level. In terms of proteomic changes, krill oil resulted in upregulation of Celsr3 and Ppp1r1b genes expression which contribute to brain development, learning and memory behavior processes.

A Brief Overview of Palmitoleic Acid, the Forgotten MUFA Gretchen Vannice*, *Organic Technologies, USA*

Little is known about cis-palmitoleic acid (16:1 n-7). Interest in this monounsaturated fatty acid (MUFA) is increasing, evidenced by more than 40 studies published in this century. Palmitoleic acid (POA) circulates in the blood with other fatty acids and is found in cell membranes and adipose tissue. POA can be consumed directly in the diet from its few food sources, mainly the oils of macadamia nuts, sea buckthorn, and some fish or it can be synthesized from the desaturation of palmitic acid via stearoyl-CoA-desaturase. US adults are estimated to consume 1–2 grams POA/day, compared to 23–29 grams/day of oleic acid (18:1n-9). POA has been described as a lipokine, biomarker, and metabolic regulator but clinical work is lacking. Cell culture and animal studies suggest that POA stimulates insulin secretion, improves glucose uptake and insulin sensitivity, increases hepatic fatty acid oxidation, enhances satiety, and reduces fatty liver. Diet studies and one clinical trial suggest improvements in blood lipid profiles and reductions in CRP with increasing POA intake. On the other hand, population studies have reported higher circulating levels of POA in adults and children, mostly notably in the obese, cardiovascular, and diabetic populations. These elevated levels may be the result of increased synthesis from excess carbohydrate and alcohol consumption. Human trials and mechanistic research on POA, the forgotten MUFA, are warranted.

Health Impact of the Newly Discovered Elovanoids: Stroke, Retinal Degenerations, Neurotrauma and Alzheimer's Disease Nicolas G. Bazan*, *LSU Health New Orleans Neuroscience Ctr, USA*

Neurodegenerative diseases and brain injury activate neuroprotective/ restorative pathways to counteract evolving adversities. The expression of these mechanisms and the mediators that signal to proteins that carry protective actions, are not well known. We have characterized a previously unknown class of mediators derived from essential omega-3 very long chain polyunsaturated fatty acids (VLC-PUFAs). VLC-PUFAs are made by ELOVL4 from DHA or EPA. ELOVL4 mutations causes certain retina degenerations, perturb brain development, trigger neuronal dysfunction, intellectual disability, and spastic quadriplegia, as well as neuroichthyotic disorders. Moreover, ELOVL4 is necessary in the skin permeability barrier for neonatal survival. The newly-identified mediators, termed elovanoids (ELVs), are di-hydroxylated derivatives of 32:6n3 and 34:6n3: ELV-N32 and ELV-N34 respectively. Their structure and stereochemistry were established and we found them to be neuroprotective in brain and retina. ELVs enhance abundance of pro-survival (e.g. SIRT1 and Iduna) proteins in

retina cells undergoing uncompensated oxidative stress. We also uncovered ELVs protection in neurons undergoing oxygen glucose deprivation, N-methyl-D-aspartate receptor-mediated excitotoxicity and ischemic stroke. Our data show neuroprotection by ELVs when administered 1 hour after 2 hours of middle cerebral artery occlusion in rats. Overall, they rescue penumbra. The signaling uncovered depicts a phosphatidylcholine that stores precursors of two mediators, DHA at sn-2, the precursor of NPD1, and C32:6n3 or C34:6n3 at sn-1 the precursors of ELVs. Thus, we disclose a new class of lipid mediators, the elovanoids, and reveal a different lipid signal bifurcation neuroprotective mechanism to sustain neural cell integrity. (Support by NIH EY005121, GM103340, and EENT Foundation).

Evidence for the Use of Docosahexaenoic Acid in the Treatment of Breast Cancer Catherine J. Field*, Newell Marnie, and Lynne M. Postovit, *University of Alberta, Canada*

Despite advances in screening, prevention, diagnosis and treatment, breast cancer (BC) remains the second leading cause of female cancer-related death, and one of the most expensive to treat. Our group and others have found that feeding sources of the omega-3 fatty acids, eicosapenataenoic and docosahexaenoic (DHA) acid are associated with a lower risk of breast cancer and feeding these fatty acids to animals prevents and reduces the growth of human and experimental mammary tumors. Women with the hardest to treat triple-negative breast cancer (TNBC) are offered neoadjuvant chemotherapy prior to breast surgery. We have found that pre-treatment of TNBC cells with DHA sensitizes these cells to the cytotoxic drug doxorubicin (DOX). We have identified potential mechanisms in vitro, including reducing proliferation, inducing cell cycle arrest and augmentation of apoptosis. More recently, we have confirmed these effects in dietary trials using two different drugs (DOX and docetaxel) and two different pre-clinical BC rodent models (nu/nu mice bearing human MBA-MD-231 cells and in a drug resistant patient-derived xenografts). We have hypothesized that these changes are the result of selective incorporation of DHA into the tumor cell membrane phospholipids affecting the function of membrane generated receptors and signals. This work supports further research on the inclusion of a supplement, with minimal to no known side effects, with current neoadjuvant therapy for BC.

Role of Oxidized Phospholipids in Myocardial Reperfusion Injury Amir Ravandi*, *University of Manitoba, Canada*

Coronary artery disease remains the leading cause of morbidity and mortality worldwide. Major advances in the treatment of acute coronary syndromes and myocardial infarction have occurred over the past 20 years. In particular the ability to rapidly restore blood flow to the myocardium during ischemia, using percutaneous coronary interventions or thrombolytic approaches has been a major step forward. Nevertheless, while ‘reperfusion’ is a major therapeutic aim, the process of ischemia followed by reperfusion is often followed by the activation of an intense burst of reactive oxygen species (ROS) that results in cardiac cell death and worsening myocardial function. Even with best medical care the mortality after myocardial infarction with reperfusion is at 5–10%. There are currently no therapies available for myocardial

reperfusion injury. We have shown that during myocardial reperfusion injury a novel class of bioactive phospholipids, Fragmented Oxidized Phosphatidylcholines (Fragmented OxPC), are generated that result in cardiomyocyte cell death. Notably our preliminary work has identified 2 main compounds: PONPC (1-palmitoyl-2-(9-oxo)nonanoyl-sn-glycero-3-phosphocholine) and POVPC (1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine), that are the most abundant OxPC generated during I/R. We have also shown that exposure of cardiomyocytes to PONPC and POVPC, results in cell death through a mitochondrial related pathway, resulting in loss of mitochondrial membrane potential and loss of mitochondrial function. Our data strongly suggests that fragmented OxPCs are potent inducer of cardiomyocyte cell death during I/R. If this view is correct then this will establish a novel therapeutic pathway to avert cell death during I/R injury by inhibiting the activities of OxPCs.

BIO 2.1/H&N 2: Dietary Lipids and the Gut Microbiota

Chairs: Barry Tulk, DuPont Nutrition & Health, USA; and Jun Ogawa, Kyoto University, Japan

Effect of Diet on the Gut Microbiota
Joanne Slavin*, *University of Minnesota, USA*

The importance of the gut microbiota for health has long been appreciated. Carbohydrates resistant to digestion and absorption are fermented in the large intestine resulting in the production of short chain fatty acids that are used as gut fuel. Additionally, dietary components beyond carbohydrates are known to alter the gut microbiota and these alterations in the gut microbiota may have health benefits. Dietary fiber, prebiotics, and probiotics are the most studied dietary components that change the gut microbiota. Whether other dietary components, such as proteins and fats alter the gut microbiota is currently hotly debated. New analytical methods have made it possible for consumers to examine the composition of their own gut microbiota, although there are no accepted standards for a "healthy" gut microbiota pattern. Links to disease outcomes, such as obesity, are also driving interest in changing the gut microbiota. Animal studies finding negative changes in the gut microbiota with emulsifiers is an area of concern for oil chemists. Of course, most lifestyle factors, such as exercise, smoking, stress, sleep, BMI, etc also affect the gut microbiota and make the topic controversial and difficult to study.

Interaction Between Diets and Gut Commensal Bacteria in the Regulation of Immunological Health and Diseases Jun Kunisawa*, *NIBIOHN, Japan*

It is well recognized that diets regulate host immune responses. Additionally, accumulating evidence has revealed a pivotal role of gut commensal bacteria in the regulation of various host biological responses including immunity. In these processes, diets affect the composition and function of gut commensal bacteria and reciprocally gut commensal bacteria is involved in the digestion of diets to consequently produce either useful or harmful metabolites. We have studied the effect of fatty acid composition in dietary oils on the regulation of host immune responses, showing that fatty acid compositions in the dietary oils affect the incidence of allergic and inflammatory diseases. Metabolome and immunological analyses allowed us to identify anti-allergic lipid metabolites and to understand the underlying mechanism. Our recent studies demonstrated that not only host metabolic pathways but also commensal bacteria contribute to both production of anti-allergic and anti-inflammatory lipid metabolites. In this talk, I describe recent findings regarding the immunologic crosstalk between commensal bacteria and dietary oils in the regulation of host immunity and its influence on the development of allergic and inflammatory diseases.

Role of Bile Acid in Gut Microbiota Alterations in Rats Fed a High-fat Diet Atsushi Yokota*, Masamichi Watanabe, Satoshi Ishizuka, and Satoru Fukiya, *Research Faculty of Agriculture, Hokkaido University, Japan*

Mechanisms underlying gut microbiota alterations by high-fat diet (HFD) intake remain unclear. We hypothesized that bile acids (BAs) are involved in this mechanism as BA excretion increases on an HFD and can be a selective

pressure due to their strong antimicrobial activity (=BA hypothesis). We thus tried to verify this hypothesis. We first demonstrated that BA is a host factor that regulates cecal microbiota in rats by feeding experiments. Feeding cholic acid (CA), a common BA in rodents and humans, increased cecal BA concentrations, especially highly bactericidal deoxycholic acid (DCA), by bacterial conversion. Cecal microbiota analysis revealed a significant increase in Firmicutes and a decrease in Bacteroidetes, which were typical alterations reported in HFDs intake. In separate experiments, we found that DCA, common to both humans and rodents, and rodent-specific β-muricholic acid exhibit strong antimicrobial activity. Then, effects of a high-lard-diet feeding on microbiota and BA compositions in rat cecum were investigated. Similar microbiota changes to those observed in the CA-fed rats were detected, which accompanied increases in cecal BA concentrations including both DCA and β-muricholic acid. Examination of DCA sensitivity of the bacterial isolates revealed significantly higher resistance in Firmicutes than in Bacteroidetes. Several operational taxonomic units showed significantly positive (Firmicutes) or negative (Bacteroidetes) correlations to the cecal BA concentrations. Some of them were also significantly increased or decreased accordingly in the CA-fed rats. These results strongly supported BA hypothesis and will contribute to elucidating the relationship between HFD-induced gut microbiota alterations and onsets of metabolic disorders.

Correlation Between Dietary Lipid, Gut Microbiota and Health Jun Ogawa*, *Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Japan*

Prevalence of metabolic syndrome has stimulated interest in fat metabolism not only by host but also by gut microbiota. Polyunsaturated fatty acids derived from dietary lipids were found to be saturated by gut microbes. We revealed the metabolism in gut lactic acid bacteria, Lactobacillus plantarum. The enzyme system was found to consist of four enzymes. The concerned action of these enzymes, i.e., hydratase, dehydrogenase, isomerase, and enone reductase accomplish the saturation and generated hydroxy fatty acids, oxo fatty acids, and conjugated fatty acids as intermediates. We confirmed the existence of these fatty acids in host tissues depending on the existence of gut microbes and evaluated their physiological activity.

1. 10-Hydroxy-cis-12-octadecenoic acid (HYA) ameliorates sulfate sodium-induced colitis in mice by recovering the damage of intestinal epithelial barrier.
2. Oral administration of HYA induced insulin secretion by increasing GLP-1 level.
3. HYA elicited anti-inflammatory effects in vitro in murine enterocytes.
4. 10-Oxo-cis-12-octadecenoic acid (KetoA) induced adipocyte differentiation via the activation of PPARgamma, and increased adiponectin production and insulin-stimulated glucose uptake.
5. Hydroxy fatty acids and oxo fatty acids suppressed fatty acid synthesis by regulating LXR.
6. Enone fatty acids enhanced cellular antioxidative responses preventing multiple diseases induced by oxidative stress. These

observations suggest that the dietary fatty acid metabolites by gut microbiota can influence the health of the host.

Dietary Fatty Acid Metabolism in Gut Microbiota Shigenobu Kishino*[1], Akiko Hirata, Michiki Takeuchi[1], and Jun Ogawa[2], *[1]Kyoto University, Japan; [2]Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan*

Dietary fats are important for human as nutrition. In these days, dietary fats are found to be also important for human health. They are metabolized not only by human but also by gut microorganisms. We revealed two polyunsaturated fatty acid metabolisms in gut microbiota using lactic acid bacteria as model microorganisms. One of them is saturation metabolism in *Lactobacillus plantarum*[1)] and the other is hydration metabolism in *Lactobacillus acidophilus*[2)]. As to saturation metabolism, the enzyme system was found to consist of four enzymes (hydratase, dehydrogenase, isomerase, enone reductase) and generate hydroxy fatty acids, oxo fatty acids, and conjugated fatty acids as intermediates. The homologous genes encoding these four enzymes were found in genome sequences of many gut microorganisms. Therefore, acting in concert, gut microbiota may mediate the unsaturated fatty acid saturation metabolism in gastrointestinal tract. Furthermore, we confirmed the existence of these fatty acids in host tissues depending on the existence of gut microbes using specific pathogen free (SPF) mouse and germ-free mouse. Successive analysis revealed health promoting activity of these hydroxy and oxo fatty acids, i.e., intestinal epithelial barrier protection, anti-obesity, and anti-diabetic activity, etc. Therefore, we developed novel production system for these fatty acid metabolites using the enzymes from probiotic lactic acid bacteria. These studies could open a new application of gut microbial fatty acid metabolisms and their metabolites for health promotions. 1) S. Kishino et al., *PNAS*, 110 , 17808 (2013). 2) A. Hirata et al., *J. Lipid Res.*, 56 , 1340 (2015).

10-oxo-12(*Z*)-octadecenoic Acid, a Linoleic Acid Metabolite Produced by Gut Microbiota, Enhances Energy metabolism by Activation of TRPV1 Tsuyoshi Goto*[1], Minji Kim[2], Tomoya Furuzono[2], Kunitoshi Uchida[3], Shigenobu Kishino[1], Haruya Takahashi[2], Huei-Fen Jheng[4], Jun Yamazaki[5], Makoto Tominaga[3], Jun Ogawa[6], and Teruo Kawada[2], *[1]Kyoto University, Japan; [2]Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Japan; [3]Okazaki Institute for Integrative Bioscience, National Institute for Physiological Sciences, Japan; [4]Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Japan; [5]Department of Physiological Science and Molecular Biology, Fukuoka Dental College, Japan; [6]Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan*

Gut microbiota can regulate the host energy metabolism; however, the underlying mechanisms that could involve gut microbiota-derived compounds remain to be understood. Recently, we found that gut microbiota produces several unique fatty acids from dietary polyunsaturated fatty acids in their saturation metabolism. Interestingly, levels of several these unique fatty acids were much higher in specific pathogen-free mice than in germ-free mice, indicating that these fatty acids are generated through polyunsaturated fatty acids metabolism of gastrointestinal microorganisms. Therefore, in this study, we investigated the effects of KetoA [10-oxo-12(*Z*)-octadecenoic acid]-a linoleic acid

metabolite produced by gut lactic acid bacteria-on whole-body energy metabolism and found that dietary intake of KetoA could enhance energy expenditure in mice, thereby protecting mice from diet-induced obesity. By using Ca^{2+} imaging and whole-cell patch-clamp methods, KetoA was noted to potently activate transient receptor potential vanilloid 1 (TRPV1) and enhance noradrenalin turnover in adipose tissues. In addition, KetoA up-regulated genes that are related to brown adipocyte functions, including uncoupling protein 1 (UCP1) in white adipose tissue (WAT), which was later diminished in the presence of a beta-adrenoreceptor blocker. By using obese and diabetic model KK-A^y mice, we further show that KetoA intake ameliorated obesity-associated metabolic disorders. In the absence of any observed KetoA-induced antiobesity effect or UCP1 up-regulation in TRPV1-deficient mice, we prove that the antiobesity effect of KetoA was caused by TRPV1 activation-mediated browning in WAT. KetoA produced in the gut could therefore be involved in the regulation of host energy metabolism.

Effects of Fatty Acid Metabolites by a Gut Lactic Acid Bacterium on Lipid Metabolism in NASH Model Mice Neng Tanty Sofyana*[1], Jiawen Zheng[1], Yuki Manabe[1], Yuta Yamamoto[2], Shigenobu Kishino[1], Jun Ogawa[3], and Tatsuya Sugawara[4], *[1]Kyoto University, Japan; [2]Department of Anatomy and Cell Biology, Wakayama Medical University; [3]Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan; [4]Laboratory of Marine Bioproduct of Technology, Division of Applied Bioscience, Japan*

Nonalcoholic steatohepatitis (NASH) is a common liver disease that occurs in people who drink little or no alcohol. The major characteristic of NASH is fat accumulation in the liver, along with inflammation and damage. Factors leading to progressive NASH and inflammation are not well understood, but oxidative stress can be a possible factor for various liver diseases including nonalcoholic steatohepatitis. Oxo fatty acid, 10-oxo-11 (E)-octadecenoic acid (Keto C), produced by Lactobacillus Plantarum from linoleic acid, provided the potent cytoprotective effects against oxidative stress through activation of the Nrf2-ARE pathway (Furumoto et al., 2016. Toxicology and Applied Pharmacology 296; 1–9). The aim of the present study was to explore the preventive and therapeutic effects of gut microbial fatty acid metabolites in NASH model mice. Eight-weeks-old male mice, a NASH-cirrhosis-hepatocarcinogenic model, were divided into 3 experimental groups and fed as follows:

1. High-fat diet (HFD) (control);
2. HFD mixed with 0.1% 10-oxo-12 (Z)-octadecenoic acid (Keto A); and
3. HFD mixed with 0.1% Keto C.

After 3 weeks, mice were sacrificed. Plasma were used for biochemical analysis and livers were subjected to histological study, mRNA and protein expressions for multiple genes. Keto C increased the HDL cholesterol level in the plasma. There was hardly any difference of fat accumulation in histological study, however, there was no ballooning occurred in Keto C group. Keto C increased the expression level of HDL related genes following suppression of ROS related gene. These results indicated that Keto C has a potent effect in NASH model mice.

Gut Microbiota and Free Fatty Acids Receptors Mediated Host Energy Regulation Junki Miyamoto* and Ikuo Kimura, *Tokyo University of Agriculture and Technology, Japan*

Metabolic disorders, such as obesity and diabetes, arise from disrupted energy homeostasis that depends upon the equilibrium between energy intake and expenditure. Gut microbiota has emerged as a pivotal, multifactorial mediator in these disorders as it regulates host energy acquisition and metabolism while being modified by diet. Remarkably, the host nutrient-sensing mechanisms of gut microbial metabolites, in particular dietary fatty acids, have been significantly associated with the proneness to obesity and related disorders. Dietary fatty acids are an essential energy source and signaling molecules that regulate various cellular processes and physiological functions. Recently, several orphan G protein-coupled receptors were identified as free fatty acid receptors. FFAR1 and FFAR4 are activated by n-3 or n-6 polyunsaturated fatty acids such as dietary fish and vegetable oil, whereas FFAR2 and FFAR3 are activated by short-chain fatty acids produced by the gut microbial fermentation of dietary fiber. The critical role of gut microbial metabolites as signaling molecules via theses FFARs has come to be appreciated, the attention has been focused on the proposed diet-gut microbiota-host homeostasis axis. Functional analyses have revealed that FFARs are critical for metabolic functions, such as peptide hormone secretion and inflammation, and contribute to energy regulation. In this lecture, we summarize the roles of gut microbial metabolites via FFARs in the host energy regulation and present an overview of the current understanding of its physiological functions. We believe that these will provide valuable insights into therapeutic targets for treating metabolic disorder such as obesity and diabetes, and the use of prebiotics to control gut microbiota.

Effects of the Intake of a Gut Microbial Linoleic Acid Metabolite, 10-hydroxy-cis-12-octadecenoic Acid (HYA), on postprandial Hyperglycemia Yasunori Yonejima* and Kohey Kitao, *Nitto Pharmaceutical Industries, LTD., Japan*

10-Hydroxy-cis-12-octadecenoic acid (HYA) is a gut microbial metabolite derived from linoleic acid, which is a main ingredients of vegetable oil. Several physiological functions of HYA have been reported, e.g., improvement of intestinal epithelial barrier functions (Miyamoto et al., J. Biol. Chem., 2014), reduction of triacylglycerol levels in hepatocytes (Nanthirudjanar et al., Lipids, 2015), and protective efficacy against gastric Helicobacter infections (Matsui et al., Helicobacter, 2017). We now advance application studies of HYA toward industrialization to contribute to human wellbeing and enriched life. It is considered that the sudden elevation of blood glucose levels within several hours after meals damage blood vessels, thereby accelerating arteriosclerosis and increasing the risks of myocardial and cerebral infarctions. For these reasons, it is important even for people with prediabetes to take care of sudden changes in blood glucose levels after meals, and a substance that can be consumed routinely is expected to be effective for preventing diabetes mellitus. Therefore, in this study, the effect of intaking HYA-containing food on postprandial blood glucose level was evaluated via clinical trial. Sixty adults who were susceptible to

increase in postprandial blood glucose levels were allowed to consume 50% purity-HYA-containing food and blood glucose levels after meals were measured. Blood glucose AUC as the primary endpoint was significantly lower after the ingestion of HYA than placebo (Yonejima et al., Prog. Med., 2017). The result suggests that HYA inhibits the elevation of postprandial blood glucose levels and is expected to have the effect of preventing diabetes mellitus.

H&N 3: Lipids through the Lifespan

Chairs: Adriana Gaitán, Louisiana State University, USA; and Ignacio Vieitez, UdelaR, Uruguay

Fats and Oils Needs during the Lifespan and their Effects on Health and Well-being. Part 2—The Potential Health Effects of the Changing Fat Landscape Penny Kris-Etherton*, *The Pennsylvania State University, USA*

Fats and oils are an important source of energy and nutrients at all life stages. They provide essential fatty acids (linoleic acid and α-linolenic acid) and nutrients including vitamin E found in liquid vegetable oils, as well as vitamins A and K in some. Fish oils are a source of vitamins A and D. Nuts and seed oils are a source of vitamin E. In more recent years, dietary guidance has transitioned from recommending a low-fat diet to a moderate fat diet that is low in saturated fat and high in unsaturated fat. At all stages of the lifecycle, the recommendations are to replace saturated fat with unsaturated fats, both monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), the latter includes omega-6 fatty acids and plant and marine-derived omega-3 fatty acids. There are multiple health benefits of all unsaturated fatty acids. When substituted for saturated fat, they decrease risk of cardiovascular disease. In a recent Presidential Advisory from the American Heart Association on Dietary Fats and Cardiovascular Disease (CVD), replacing saturated fat with PUFA (mainly linoleic acid) was reported to decrease total mortality and mortality from CVD, cancer, neurodegenerative diseases, and respiratory diseases (data are from the Nurses' Health Study and Health Professionals Follow-Up Study). Replacing saturated fat with MUFA was shown to decrease total mortality and neurodegenerative and respiratory disease mortality. There is some evidence for benefits of long-chain omega-3 fatty acids on the prevention of sudden cardiac death, as well as various health outcomes in pre-term infants. We have much evidence that shows multiple health benefits of a dietary pattern that meets current food-based recommendations, including liquid vegetable oils (27 g/day on a 2000 calorie diet). In summary, unsaturated fatty acids play an important in health across the life cycle and are an integral part of a healthy dietary pattern.

Dietary Monounsaturated Fatty Acids and Cardiovascular Disease Prevention: a Surprising Story Beyond Oleic Acid Zhi-Hong Yang[1]*, Scott Gordon[1], Milton Pryor[1], Amar Marcelo[1], Michael Stagliano[1], Hiroko Miyahara[2], Jiro Takeo[2], and Alan Remaley[1] [1]Lipoprotein Metabolism Section, Cardio-Pulmonary Branch, NHLBI, NIH, USA; [2]Central Research Laboratory, Nippon Suisan Kaisha, Japan

Cardiovascular disease (CVD) is a major healthcare problem worldwide and is known to be greatly affected by diet. In particular, consumption of monounsaturated fatty acids (MUFA), such as oleic acid (cis-C18:1 n-9), the most common MUFA in the typical American diet, has favorable effects on CVD risk. In contrast, there is limited information on the physiological effects of other dietary MUFAs, with different carbon chain length, such as longer-chain MUFA (LCMUFA), with aliphatic tails longer than18 that are enriched in some marine sources. In addition, shorter-chain MUFA like

palmitoleic acid (PA; cis-C16:1 n-7) that are abundant in macadamia nuts and certain fish species may also have beneficial CVD effects but are also not well studied. Our recent animal studies uncovered a novel link between LCMUFA-rich fish oil intake and improvement of cardiovascular health and demonstrated for the first time that dietary LCMUFA may be anti-atherogenic. In line with decreased systematic inflammation and improved cholesterol efflux capacity from a LCMUFA-rich diet, we also found a close association between changes in the HDL-proteome and atherosclerosis. RNA expression analysis and in vitro PPAR transactivation assay showed a beneficial role of dietary LCMUFA and LCMUFA-derived metabolites in PPAR signaling pathway and PPAR transcriptional activity. In regard to dietary PA, we found that it improved insulin resistance in diabetic mice through regulating lipogenic and inflammatory genes and caused increased satiety in animal models compared with oleic acid, possibly due to an increase production of satiety hormones. Based on these promising results, we are now performing human clinical trials on supplementation with LCMUFA-rich fish oil (ClinicalTrials.gov Identifier: NCT03043365) and PA concentrate oil (ClinicalTrials.gov Identifier: NCT03372733) to investigate their effect on cardiometabolic biomarkers. In summary, the American Heart Association and Dietary Guidelines for Americans have long advised the replacement of saturated fatty acids with MUFAs, but a more detailed understanding on the effect of various types of dietary MUFAs on CVD risk is needed. Our pre-clinical and ongoing clinical trials suggest that enrichment of dietary LCMUFA and PA may have beneficial and perhaps unique effects on CVD risk factors, and thus could lead to new dietary recommendations and improvements in the formulation of MUFA supplements.

Understanding the Relationship Between Dietary Fatty Acids and Blood and Tissue Fatty Acid Composition Ken D. Stark*, *University of Waterloo, Canada*

The link between diet and health is profound and the intake of fatty acids has been identified as a key component of proper nutrition. While fatty acids can influence various biological systems through numerous molecular mechanisms, many of the biological effects are mediated as a result of changes in the fatty acid composition of tissues and blood. Understanding the impact of dietary fatty acids on tissue composition requires consideration of both the abundance of specific fatty acids in the food supply and de novo fat synthesis pathways. The ability to distinguish between fatty acids that can be endogenously synthesized and those that are exogenously sourced is also critical. It is also becoming increasingly apparent, that fatty acid accretion and secretion by tissues is greatly influenced by fatty acid transport and incorporation into complex lipids such as triacylglycerols and phospholipids. As such, the structure-function of a cell and fatty acid and lipid enzymology can also play a role in modulating the impact of diet on tissue fatty acid composition. The diet-tissue composition relationship of eicosapentaenoic and docosahexaenoic acid will be examined in detail, and highlight different influence on different tissues and different blood fractions.

Antioxidant Potential of Esterified Resveratrol Derivatives in Different Model Systems Won Young Oh* and Fereidoon Shahidi, *Memorial University of Newfoundland, Canada*

Objective/Hypothesis: The principal hypothesis is that resveratrol (R) esters have better antioxidant activity than their parent molecule. The overall aims and objectives of this study were to prepare resveratrol esters, identify their structures and evaluate their antioxidant activity and potential application in a number of food and in-vitro biological systems. In case of joining of two biologically active components, it was of interest to examine if the effects were additive, synergistic or antagonistic. Method used: Resveratrol esters were prepared by reacting resveratrol with 12 different fatty acids of varying chain lengths and degrees of unsaturation (C3:0 to C22:5). The esters formed were separated by chromatographic methods and structurally identified by spectroscopic means. The reaction products that included two monoesters and two diesters were used to determine their antioxidant activities. Results: After esterification, R-3 or 4'-monoesters, R-3,5 or 3,4'-diesters, and R-3,5,4'-triester were identified by HPLC-MS and the structures of monoesters were confirmed by 1HNMR, 13CNMR, HSQC, COSY, NOESY, and HMBC. Disagreements with polar paradox were observed in oil-in-water emulsion and bulk oil. However, this study supported non-linear theory in bulk oil. Some resveratrol esters (RC6:0, RC8:0, RC10:0, RC12:0, RC16:0) showed better antioxidant activity than the parent molecule in both bulk oil and in preventing low-density lipoprotein oxidation. All test compounds effectively inhibited hydroxyl radical induced DNA scission but resveratrol esters showed no significant difference compared to the parent molecule. Conclusions: This work demonstrated that resveratrol esters could serve as novel functional food ingredients and health-promoting supplements.

Effect of Fatty Acid Chain Length and Saturation on the Lipid Profiles of Wistar Rats Chathuri M. Senanayake[1], Gangi R. Samarawickrama[2], Nimanthi Jayathilaka[2], and Kapila N. Seneviratne*[1], *[1]University of Kelaniya, Sri Lanka; [2]Department of Chemistry, University of Kelaniya, Sri Lanka*

Effect of fatty acid chain length and saturation on the lipid profiles of Wistar rats Chathuri Senanayake, Gangi R Samarawickrama, Nimanthi Jayathilaka, Kapila N Senenviratne Different nutritional effects can be expected for foods with different fatty acid compositions. Degree of saturation and chain length of fatty acids are important factors that decide the nutritional quality. We fed Wistar rats with diets containing 2 % of dairy butter, coconut spread and margarine as the fat source and measured the lipid profiles of serum and adipose tissues after 150 days. Fatty acid compositions of the fat sources were determined by gas chromatography. Weighted average chain length (WACL) of fatty acids of dairy butter, coconut spread and margarine calculated based on fatty acid composition are 16.0±0.4, 13.1±0.2 and 16.3±0.2 respectively while the saturated fat contents of the same sources are 71.8±1, 91.0±1 and 59.8±1 respectively. Correlation coefficients between WACLs and each of the high-density lipoprotein (HDL), low density lipoprotein (LDL), HDL/LDL, and triglyceride (TG) levels in the serum are –0.895, 0.848, –0.687 and 0.765 respectively while correlation coefficients between saturated

fat content and each of the HDL, LDL, HDL/LDL and TG levels in the serum are 0.930, –0.723, 0.655 and –0.687 respectively. These results and the relevant data obtained for adipose tissue suggest that either chain length of fatty acids or degree of saturation of fatty acids in the diet alone cannot explain the observed lipid profiles of serum and adipose tissue.

Identifying Novel Docosahexaenoic Acid-containing Phospholipids in Human Whole Blood as Indicators of Omega-3 PUFA Intake

Juan J. Aristizabal Henao* and Ken D. Stark, *University of Waterloo, Canada*

The fatty acid composition of whole blood has been studied extensively before and after omega-3 PUFA supplementation. These analyses are generally gas chromatography-based, which leads to an indirect examination of lipids due to sample processing. We adapted three mass spectrometric techniques for the analysis of human whole blood to characterize as many docosahexaenoic acid (DHA)-containing phospholipids as possible. Specifically, these techniques were data-dependent acquisition (DDA), precursor ion discovery (PID), and multiple reaction monitoring (MRM). Whole blood lipid extracts were obtained following a modified Folch extraction, compounds were separated using a reversed-phase UHPLC protocol and were ionized in negative ESI mode. We observed that the DDA method identified 20 distinct phospholipids that contained DHA, PID identified 12, and MRM identified 10. Generally, the DDA and MRM modes were superior to PID when identifying low-abundant molecules due to the use of an inclusion list. Using the DDA method, we determined the DHA-phospholipidomic profiles of three individuals consuming infrequent (0–1 times per month), sporadic (2–4 times per month), and frequent (>5 times per month) meals with fish. We determined that P16:0/DHA plasmenyl-phosphatidylethanolamine was approximately 1.4 and 2.9 fold higher in the frequent participant as compared with the sporadic and infrequent participants, respectively. Furthermore, we observed that 18:1/DHA phosphatidylcholine was not statistically different between the frequent and sporadic participants, but both were 4.1 fold higher than the infrequent participant. These findings suggest that P16:0/DHA plasmenyl-phosphatidylethanolamine may be an adequate indicator of long-term fish intake, while 18:1/DHA phosphatidylcholine may be an indicator of recent intake.

EAT 3.2/H&N 3.1: Influence of Fat Composition and Structure on in Foods on Metabolic Status

Chairs: Amanda Wright, University of Guelph, Canada; and Marie-Caroline Michalski, INRA, France

Introducing the Importance of Molecular and Supramolecular Lipid Structures on Metabolism and Beyond Marie-Caroline Michalski*, *INRA, France*

The health impact of dietary lipids must now encompass approaches beyond their energy content and fatty acid profile. In fact, dietary fatty acids are building blocks of different lipid molecules such as triacylglycerols and phospholipids, organized in various supramolecular structures such as emulsion droplets, which can be naturally present or incorporated in more or less complex food matrixes. This lecture will present our recent works on the impact of fat emulsified structure on postprandial lipid metabolism and fatty acid beta-oxidation in obese and normal-weight men, leading to the concept of « fast vs slow lipids ». We will see how the postprandial kinetics of lipid absorption can modulate metabolic endotoxemia, which originates from interactions between dietary lipids and the gut microbiota and can contribute to metabolic inflammation in obese subjects. Regarding minor lipid structures of important functional interest, we will see how surface active agents used in food formulation such as emulsifiers, and notably vegetal vs milk phospholipids, can modulate lipid metabolism and inflammation. Finally, the food matrix effect will be highlighted with vegetal and dairy examples. This new knowledge in the field of lipid metabolism will serve as an introduction for the following lectures of this translational session.

Is the Food Matrix an Important Factor for Lipid Bioaccessibility and Their Subsequent Metabolism? Sylvie Turgeon*, *INAF, Laval University, Canada*

Several studies have linked food structure and texture to different kinetics of nutrients delivery. Changes in some nutrients' release rate such as lipids could induce different physiological effects (e.g. reduction of postprandial lipemia). However, little is known on the contribution of dairy food structure, especially cheese, on nutrients release rates. The aim was to discriminate the effect of cheese attributes on lipid release and absorption. The effect of milk fat composition (olein vs stearin rich-fractions) and calcium of cheddar cheeses were studied in vitro and in vivo. In cheddar-type cheese with regular and high calcium content, the stearin fraction exhibited lower plasma TAG responses compared to the olein fraction in the rat model. In vitro study revealed that lipolysis and the overall fatty acids bioaccessibility were also reduced in cheese containing the stearin fraction due to a lower degradation of the matrix. In a second study commercial cheeses including: young- and aged-cheddars, regular- and light-cream cheeses, parmesan, feta, camembert, mozzarella, sliced processed cheese were digested. At the end of the in vitro gastric digestion, cheddar (slow) and cream cheese (fast) presented different disintegration attributed to their texture and manufacturing processes. The in vivo human study revealed that cream cheese, but not cheddar, induced a more important

increase in TAG concentrations than butter (Δ vs baseline: +44% vs +24%) at 2h. At 6h, the response was attenuated with cream cheese compared with cheddar (Δ vs baseline: +14% vs +42%). These studies demonstrate that cheese matrix per se modulates postprandial lipid metabolism in humans and animals.

Citric Acid Esters-stabilized Emulsions During in vitro Digestion: Effect of the Physical State of Emulsifier Qing Guo, Nick Bellissimo, and Dérick Rousseau*, *Ryerson University, Canada*

The objective of this study was to investigate the role of Pickering stabilization on in vitro lipid digestion of oil-in-water (O/W) emulsions. Different concentrations of glyceryl stearate citrate (GSC) and glyceryl oleate citrate (GOC) (0.5 and 5 wt%) were used to stabilize emulsions prepared with valve homogenization. Initial emulsion properties were characterized by static/dynamic light scattering, confocal/polarized light microscopy, and differential scanning calorimetry. In vitro digestion included three phases: oral processing, gastric digestion and intestinal digestion. During digestion, structural changes, particle size, zeta-potential, and free fatty acid release were monitored. Results showed that a solid shell formed around dispersed oil droplets when using GSC. However, during storage, the shell with 5% GSC resulted in oil droplet flocculation, though gentle mixing re-distributed droplets. All other emulsions did not undergo changes in droplet size. During oral processing, no significant changes in particle size or microstructure were observed in any emulsion. During gastric digestion, severe coalescence occurred in emulsions stabilized by 0.5% GSC and GOC whereas 5% GOC emulsions underwent less coalescence. Flocculation dominated 5% GSC emulsions. During intestinal digestion, oil droplet coalescence was evident in all emulsions with the smallest increase in particle size in 5% GSC emulsions. Intestinal lipid digestion of 0.5% GSC and GOC emulsions was greatly delayed. Counter-intuitively, 5% GOC and GSC emulsions were digested rapidly during the early stage of intestinal digestion, however presence of the GSC interfacial shell retarded lipid digestion. In conclusion, lipid digestion was modulated by manipulating the physical state of emulsifiers.

Impact of Emulsion Droplet Physical State on *in vitro* Lipid Digestion Surangi K.P.H. Thilakarathna*, and Amanda Wright, *University of Guelph, Canada*

Food grade compositionally equivalent liquid (LE) and solid (SE) emulsion particles with similar size distributions (D_{3,2} 0.28±0.08 & 0.23±0.08; D_{4,3} 0.55±0.04 & 0.56±0.11 µm, for LE and SE respectively, p>0.05) and charge (-47.3±0.6 and -42.2±0.4 mV, respectively, p>0.05) were prepared using palm stearin (10wt%) and the acid-unstable emulsifier Span 60 (0.4wt%) to study the impact of physical state on in vitro lipid digestion. Hot homogenized emulsion samples were either undercooled (LE) or crystallized (SE) and held at 37 °C for all analyses. The SE peak melting temperature was 52.6±0.4 °C and x-ray diffractometry indicated presence of the β-polymorph. Exposure to the gastric phase of digestion induced minimal crystallization in LE and significant flocculation in SE. The rate and extent of duodenal phase lipolysis was greater for LE compared to SE (p<0.05) within the first hour, but no significant differences were observed, thereafter. Following *in vitro* digestion,

undigested SE lipids remained in the β-form, and a shift to higher end of melt temperature occurred. When duodenal digestions were performed without the gastric phase, LE and SE lipolysis did not differ from each other ($p>0.05$), but a faster rate and higher maximum lipolysis were observed ($p<0.05$), related to colloidal stability. Shear conditions also impacted colloidal stability, of the SE, in particular, with implications for digestibility results. These findings support that physical and colloidal states impact emulsion droplet *in vitro* lipolysis.

Monounsaturated Fats and Stearic Acid: Summary of Impact on Human Cardiometabolic Outcomes Dariush Mozaffarian*, *Friedman School of Nutrition & Health Policy, Tufts University, USA*

Demand for palm alternatives is growing across many key categories in the food market. A key opportunity includes structuring fats that include combinations of monounsaturated fats, i.e., oleic acid, and the saturated fat, stearic acid. In humans, monounsaturated fats such as oleic acid can improve cardiovascular and metabolic health outcomes, while stearic acid tends to show neutrality on cardiovascular blood lipid parameters. An up-to-date review of the science from human clinical trials and epidemiological cohorts supports this. Findings are reviewed across cardiovascular outcomes, including myocardial infarction and stroke, and their risk factors, such as blood cholesterol and other lipids; key metabolic outcomes, including diabetes, and its risk factors, such as blood glucose and A1c are included. The review provides further clarity on the source of fats, such as plant versus animal, and various food categories, for their impact on these health outcomes. These findings have important implications for the acceptability by the food industry and regulatory authorities of new structuring fats as alternatives to palm oil.

***In vitro* and *in vivo* Evidence of Dietary trans-vaccenic Acid Retroconversion to trans-palmitoleic Acid** Etienne Guillocheau*, Garcia Cyrielle, Léo Richard, Daniel Catheline, Philippe Legrand, and Vincent Rioux, *Agrocampus-Ouest, France*

Objectives and hypothesis High levels of circulating trans-palmitoleic acid (TPA, C16:1 n-7 trans) are associated with a lower risk of metabolic syndrome. It was actually assumed that TPA arises from dietary trans-vaccenic acid (TVA, C18:1 n-7 trans) through the β-oxydation pathway. This study aimed at providing evidence of such retroconversion. Methods used Fresh rat hepatocytes were incubated with growing amounts of TVA to assess the conversion rate. Inhibitors of mitochondrial and peroxisomal β-oxydation were also used. Sprague-Dawley pregnant rats were fed during the last week of pregnancy plus two weeks of lactation, either with a TVA-diet or with the corresponding cis isomer (2% of total energy). Pups were exclusively fed with maternal milk for two weeks. TPA content was assessed in the main organs of both dams and pups. Results TPA was properly identified in hepatocytes whenever TVA was supplemented. The conversion rate was estimated at 10%. Triacylglycerols secreted by hepatocytes did contain TPA. Blocking peroxisomal β-oxydation significantly decreased the conversion rate. TPA was quantified in the dams fed the TVA-supplemented diet and their pups, excepted in the brain. Importantly, TPA was found in the maternal milk. Conclusions Liver ensures the retroconversion of dietary TVA to TPA, which can be exported to other tissues.

Circulating levels of TPA are explained by dietary intakes of TVA. Given epidemiological data about TPA, future research is needed to assess dietary intakes of TVA.

H&N 4a: Nutrigenetics and Nutrigenomics of Lipid Metabolism

Chairs: Susan K. Raatz, USDA, ARS, Grand Forks Human Nutrition Research Center, USA; and Fabiola Dionisi, Nestlé Research Center, Switzerland

Genetic Determinants of the Cardiometabolic Risk Factor Response to an N-3 PUFA Supplementation Marie-Claude Vohl*, *Laval University, Canada*

Cardiovascular diseases (CVD) have both genetic and environmental causes. Consequently, targeting CVD risk factors may help reducing their prevalence. Diet is a major environmental aspect that could be addressed in prevention programs. This is the central argument to adopt population-based nutritional guidelines. However, such policies assume that all individuals respond similarly to dietary modifications and do not consider the dramatic inter-individual differences in response to such interventions. There is strong evidence that such variability is, at least partly, determined by genetic factors through interaction between genes and diet. We explored gene-diet interaction effects on CVD risk factors. In a 6-week supplementation with EPA-DHA, we observed a large inter-individual variability in the plasma triglyceride (TG) response with 28.8% of the subjects being negative responders (i.e., having no reduction or an increase in plasma TG levels after the supplementation). To identify genetic variations underlying this variability, we performed a GWAS of the plasma TG response to an n-3 fatty acid (FA) supplementation. Thirteen loci had allele frequency differences between positive-responders (those with a decrease in plasma TG levels) and negative-responders. A genetic risk score (GRS) computed by summing the risk alleles remarkably explained 21.5% of the variation in the plasma TG response to the ($p=0.0002$). We further increased the density of markers in GWAS signals and refined the GRS using 505 markers. The new GRS explained 79.49% of the variance of the plasma TG responsiveness. These results will likely serve to develop personalized nutrition applications.

Effect of Fatty Acid Chain Length on Regulation of Hepatic Gene Expression by Saturated Fats Harsha Hapugaswatte[1], Chathuri M. Senanayake[1], Gangi R. Samarawickrama[2], Kapila N. Seneviratne[1], and Nimanthi Jayathilaka*[2], *[1]University of Kelaniya, Sri Lanka; [2]Department of Chemistry, University of Kelaniya, Sri Lanka*

Diets rich in saturated fatty acids have been associated with increased serum cholesterol concentrations and hence increased risk of cardiovascular disease. To evaluate the effect on serum lipid profile and regulation of genes involved in lipid metabolism by different amounts of saturated fat with varying fatty acid chain lengths, we harvested and analyzed liver samples from Wistar rats fed with diet containing 2% of dairy butter (DB), coconut spread (CS) and margarine (M) as the source of fat for 150 days. Weighted average chain length (WACL) and percent saturated fat content of DB, CS and M are 16.0±0.4; 71.8±1.3, 13.1±0.2; 91.0±1.1 and 16.3±0.2; 59.8±1.3 respectively. While the serum total cholesterol, LDL and triglycerides increased in DB and M dietary groups compared to the control group fed with diet containing 2% soy oil with WACL of 17.6±0.5 and 79.2±2.6 percent unsaturated fatty acids, the CS group with the highest saturated fat content and lowest WACL had the lowest serum total cholesterol, LDL and

triglyceride with the highest HDL content among the saturated fat containing diets. qRT-PCR analysis of liver sections showed differential relative expression of LDL receptor (LDLr), peroxisome proliferator activated receptor alpha (PPAR-alpha), sterol regulatory element binding protein 2 (SREBP2) and ATP binding cassette transporter (ABCA1) involved in lipid metabolism in the CS fed group compared to the DB and M fed groups. Therefore, the chain length of the saturated fatty acids appears to affect regulation of gene expression involved in lipid metabolism and serum lipid profile.

Trans Fatty Acids Suppress TNFalpha-induced Inflammatory Gene Expression in Endothelial and Hepatocellular Carcinoma Cells Marine S. Da Silva[1], Sarah O'Connor[2], Pierre Julien[2], Jean-François Bilodeau[2], Olivier Barbier[3], and Iwona Rudkowska*[2], *[1]Centre de recherche du CHU de Québec—Université Laval, Canada; [2]Endocrinology and Nephrology Unit, Centre de recherche du CHU de Québec—Université Laval , Canada; [3]Laboratory of Molecular Pharmacology, Centre de recherche du CHU de Québec—Université Laval , Canada*

Trans-fatty acids (tFA) intake has been linked to cardiovascular diseases and liver diseases; yet the effect of TFA on inflammation remains controversial. Objective: To determine the in vitro effects of TFA on inflammatory gene expression. Methods Used: Human umbilical0 vein endothelial cells (HUVEC) and human hepatocellular carcinoma (HepG2) cells were treated for 24h with either trans-vaccenic acid (tVA), trans-palmitoleic acid (tPA) or elaidic acid (EA) at concentrations of 5 to 150 µM, or with a mixture of tVA and tPA (150/50 µM). Results: All TFA were highly incorporated into cell membranes, as determined by gas chromatography, representing 15–20% of total fatty acids in HUVEC and 3–8% in HepG2 cells. Incorporation of EA, a common industrial TFA, increased the ratio of the stearoyl-CoA desaturase (SCD-1), a key enzyme involved in fatty acid metabolism. Ruminant TFA, including tVA, tPA and the mixture of tVA and tPA, significantly reduced the TNFalpha-induced gene expression of TNF, VCAM-1 and SOD2 in HUVEC (–95%), as well as TNF and IL-8 in HepG2 cells (–50%). EA also decreased inflammatory gene expression in HUVEC (–90%), but not in HepG2 cells. The inhibition of peroxisome proliferator-activated receptor (PPAR)-gamma did not influence the effects of TFA on gene expression. Conclusions: Overall, physiological and supra-physiological concentrations of TFA, especially tVA and tPA, prevented inflammatory gene expression in vitro. This effect is independent of PPAR-gamma activation and may be due to an alteration of fatty acid metabolism in cell membranes caused by the high incorporation of TFA.

Gene-dietary Fat Interactions and Cardiometabolic Health José M. Ordovás*, *Tufts University, USA*

Nutrigenetic investigates the consequences of inter-individual genetic differences in responses to foods and dietary patterns in the framework of health. The outcome of this research is the use of an individual's genetic information to design personalized recommendations that are more successful at the individual level relative to global dietary information. Lipids, whether dietary or circulating in plasma, have been one of the longstanding targets of personalized nutrition, given their known associations with most common age-

related diseases. The incipient knowledge of genes implicated in the absorption of dietary lipids and the metabolism of cellular and plasma lipids has been replaced by a deluge of new lipid candidate genes emerging from the genome-wide association studies and more recently from the whole genome sequence. This has opened vast possibilities to uncover gene-diet interactions that could modulate the effects of dietary fats on the metabolism of body fats. Along these lines, some loci have been shown to be significant predictors of dietary response including, among others, APOA2, APOA5, and TCF7L2, Moreover, the field is evolving to the integration of multiple omics. DNA Methylation is of particular interest as it has been shown that its measurement predicts a higher percentage of the variability in response than known genetic factors. Finally, chronobiology is adding a new dimension with the possibility of personalizing not only what to eat but when to eat it. Whereas nutrigenetics is in its infancy; the current evidence supports the need for further research to transform this knowledge into practical applications for disease prevention.

H&N 5: General Health and Nutrition

Chairs: Jenifer Heydinger Galante, Stepan Company, USA; and Fabien Schultz, Technical University of Berlin, Neubrandenburg University of Applied Sciences, Germany

East and Central African Medicinal Plants as Anti-inflammatory Inhibitors in the 15-LOX / 15-Hydroxyeicosatetraenoic Acid and COX / PGH2 Pathways. Fabien Schultz[1], Godwin Anywar[2], Ogechi Favour Osuji[3], and Leif-Alexander Garbe[4], *[1]Technical University of Berlin, Neubrandenburg University of Applied Sciences, Germany; [2]Makerere University, Uganda; [3]Applied Chemistry, School of Agriculture and Food Sciences, Neubrandenburg University of Applied Sciences, Germany; [4]Neubrandenburg University of Applied Sciences, Germany*

The majority of plant and insect species of the tropical rainforests in western Uganda and eastern DRC have not yet been discovered; 90% have never been screened for bioactivity. Approx. 60% of the world's population relies almost entirely on plants for medication. The knowledge of East and Central African plants and their traditional uses are mainly transferred orally from one generation to the next by traditional healers, leading to the loss of vital information due to lack of records. Our study provides documentation of 16 different African regularly consumed edible and medicinal plants traditionally used to treat inflammation and related disorders such as pain, arthritis, osteoporosis, asthma, dermatitis and even cancer. One possible methodology for the discovery of novel anti-inflammatory lipids is screening selected plant extracts for a broad array of pharmacological activities. Phenolic compounds are often thought to possess anti-inflammatory properties. The MOAs of many phenolic compounds are most likely associated with their inhibition of pro-inflammatory enzymes in the arachidonic acid pathway such as lipoxygenases (LOX) and cyclooxygenases (COX) in inflammatory cascades or with their free radical scavenging activity. Due to undesirable effects of non-steroidal anti-inflammatory drugs (NSAIDs) such as gastrointestinal bleeding, selective inhibition of COX-2 is preferred to the COX-1 inhibition. We present results of diverse in vitro experiments performed with 61 different plant extracts: 1. 15-LOX inhibition screening; 2. Selective COX-2 inhibitor screening; 3. DPPH assay for anti-oxidant activity; 3. Determination of the total phenolic and flavonoid content. Bioassay-guided fractionations combined with GC/LC-MS techniques enabled identification of bioactive lipids in these African plants. Traditional use could be scientifically validated in many cases.

Gamma-linolenic Acid Regresses Human Glioma Undurti Das*, *BioScience Research Centre, India*

Objectives: Study anti-cancer actions of γ-linolenic acid (GLA). Methods used: Both in vitro and animal studies were performed. Results: Gamma-linolenic acid (GLA) ed apoptosis of tumor but not normal cells. Both cyclo-oxygenase (COX) and lipoxygenase (LO) inhibitors did not inhibit the selective tumoricidal action of GLA. In contrast, vitamin E blocked the tumoricidal action of GLA. GLA-treated tumor cells produced a 2-3-fold increase in free radicals and lipid peroxides. GLA decreased the anti-oxidant content of tumor cells, expression of oncogenes ras and Bcl-2, enhanced the activity of p53, and

enhanced cytotoxic action of anti-cancer drugs and reversed tumor cell drug resistance. In the animal glioma model, GLA induced tumor regression and preserved the surrounding normal brain tissue. Intra-tumoral injection of GLA induced significant reduction of glioma without any significant side effects. Conclusions: The low neurotoxicity of GLA to normal brain neurons and selective activity against tumor cells suggests that it could be an effective anti-glioma molecule.

Conjugated Linoleic Acid Delivered as Nanoemulsion Reduced Fat Accumulation and Increased Activity in Caenorhabditis elegans Yeonhwa Park[1], Peiyi Shen[2], Yiren Yue[2], Ou Wang[2], and D. Julian McClements[3], *[1]Dept. of Food Science, University of Massachusetts Amherst, USA; [2]University of Massachusetts, USA; [3]University of Massachusetts Amherst, USA*

Conjugated linoleic acid (CLA) has been reported to reduce fat storage in different models, including cell culture and animals. In the current study, the effects of TG form of CLA delivered as nanoemulaion on the fat accumulation and locomotive activities were investigated using Caenorhabditis elegans, which is a free-living nematode animal model utilized for the obesity research because of its relatively short lifespan, quick turnover, easy maintenance, well-known genetic pathways, large brood size. 100 µM CLA-TG nanoemulsion significantly reduced fat accumulation by 29% compared to the linoleic acid (LA)-TG treatment, without altering the worm size, growth rate, pumping rate, and reproduction of C. elegans. CLA significantly increased average moving speed and amplitude (the average centroid displacement over the entire track) of wild type worms compared to the LA group and this effect was shown to be dependent on aak-2 (AMPKα ortholog) and sir-2.1 (Sirtuin 1 ortholog). The current data shows that CLA supplementation (100 µM) significantly reduced the fat accumulation in C. elegans and increased the locomotive activity (moving speed and amplitude) via AMPK and SIR-2.1 dependent mechanisms.

Increased Body Mass Index and C-reactive Protein are Associated with Low Serum α-carotene in Adults Ambria Crusan[1], David R. Jacobs[2], Ryan T. Demmer[2], and Susan K. Raatz[3], *[1]University of Minnesota, USA; [2]Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, USA; [3]USDA, ARS, Grand Forks Human Nutrition Research Center, USA*

Objectives: Worldwide obesity rates are high, therefore understanding health consequences and mortality risk associated with obesity is important. The relationship between obesity, low-grade, systemic inflammation, and oxidative stress is well established. However, how serum carotenoid concentrations relate to obesity in population samples is not well understood. Our primary objective is to test the hypothesis that fat-soluble, serum α-carotene is independently associated with body mass index (BMI) and the inflammatory marker high-sensitivity C-reactive protein (hsCRP), utilizing datasets from the National Health and Nutrition Examination Surveys (NHANES).

Methods: Data from 9,182 male and non-pregnant female participants 20–85 years in the NHANES 2003–2006 nationally representative, cross-sectional survey were analyzed to estimate the relationships among BMI, hsCRP, and serum α-carotene. Due to skewing, we log transformed serum α-carotene and hsCRP. Multiple linear regression estimated log(α-carotene) based on

BMI and log(hsCRP) adjusted for age, sex, and ethnicity.

Results: Mean and standard deviation (SD) was 0.95±0.96 for log(α-carotene) and –1.65±1.31 for log(hsCRP). Mean and SD for BMI was 28.27±6.55. log(α-carotene) concentrations were inversely associated with BMI (r= –0.17, p<0.0001) and log(hsCRP) (r=–0.14, p<0.0001), and the correlation of BMI with log(hsCRP) was r=0.44 (P<0.0001). The log of serum α-carotene was lower by 0.11±0.01 µg/dL for each SD higher BMI and lower by 0.16±0.01 µg/dL for each SD higher log(hsCRP), which are respectively 11 and 17% of the SD of log(α-carotene).

Conclusions: Substantial associations were found among serum α-carotene and BMI and CRP concentration. These factors may contribute to the increased risk of chronic disease, particularly in obese individuals.

Enhancing Bioaccessibility of Phytosterols using Nanoporous Starch Bioaerogels Ali Ubeyitogullari*, Regis Moreau, and Ozan N. Ciftci, *University of Nebraska-Lincoln, USA*

Incorporation of phytosterols into foods is a major challenge from a technological and food quality standpoint because phytosterols are water-insoluble and poorly soluble in fats & oils. Poor water solubility leads to low bioavailability, in turn, limits phytosterols' health benefits. Our objective was to enhance the bioavailability of phytosterols by forming the first-of-its kind low-crystallinity phytosterol nanoparticles using our innovative approach. It was hypothesized that impregnation of phytosterols into nanoporous starch bioaerogels (NSB) using supercritical carbon dioxide (SC-CO_2) forms low-crystallinity phytosterol particles (PS-NSB) that are more bioavailable than crude phytosterols, and the pore size and surface area of the NSB determines phytosterols' bioavailability. Corn and wheat NSB (surface area: 61–211 m^2/g, pore size: 7–19 nm) monoliths and powders were filled with a phytosterol-SC-CO_2 solvato complex, and the phytosterols were precipitated from the complex in the NSBs by cooling (10°C/min). Bioaccessibility of the phytosterol particles were determined by a simulated sequential oral, gastric and intestinal digestion model. The highest phytosterol impregnation capacity was obtained with corn NSB monolith (195 mg phytosterols/g NSB). Monolithic form prevented formation of large phytosterol crystals. Wheat NSB monolith (WNSB-M) generated low-crystallinity phytosterol nanoparticles (70 nm). Bioaccessibility of the phytosterols formed in the WNSB-M increased by 20-fold. Zeta potential of the phytosterol nanoparticles in the micellar phase in the bioaccessible fraction was higher compared to the crude phytosterols. This is a novel approach to incorporating lipophilic bioactives into foods to prepare functional foods in a simple and clean way; and maximize the health benefits of the lipophilic bioactives.

Effect of the Type of Feeding on Quality Characteristics of the Lipid Fraction in Beef Luis C. Vazquez*[1], Jennifer Fernandez[2], Guillermo Reglero[1], and Carlos Torres[1], *[1]University Autonoma of Madrid, Spain; [2]Research Institute of Food Science (CIAL, CSIC-UAM), Spain*

The effect of a grass-based and a concentrate feeding system on fat composition of body tissues in beef was investigated in the present work. For this purpose, the fatty acid composition of several samples and the triacylglycerol fatty acid positional distribution was studied. Main results showed a better ω-6/ω-3 ratio and higher concentration of vaccenic

acid (precursor of CLA in biological systems) on grass-fed beef meat than that from a concentrate feeding system. Furthermore, the palmitic/stearic ratio was lower on all analyzed samples obtained from a grass-based feeding system. Accordingly, an in vitro digestion model was utilized to simulate physiological intestine conditions, demonstrating that the grass-fed beef fat is significantly less bioaccessible. Therefore, it has been proved in several tissues that grass-fed systems provide lipid fractions with healthier characteristics than those obtained by concentrate feeding systems. Moreover, due to a higher concentration of stearic acid, grass-fed systems lead to fat with less calories and may also provide an hypocholesterolemic effect.

Sex Differences in Rat Oxylipins Vary between Tissues and Diet, and Do Not Reflect Precursor Fatty Acids Harold M. Aukema*[1], Shan Leng[1], Anne Mendonca[2], Lucien GJ Cayer[1], Afroza Ferdouse[1], and Tanja Winter[1], *[1]University of Manitoba, Canada; [2]Federal University of Uberlandia, Brazil*

Sex differences in fatty acids have been demonstrated in mammalian tissue, but whether this is reflected in their bioactive metabolites known as oxylipins is not known. To examine sex effects on rat tissue oxylipins, weanling rats were given diets rich in either ALA, EPA or DHA for six weeks, and oxylipin compositions were examined in kidney, liver, heart, serum, and in subcutaneous and visceral adipose depots. Oxylipins were quantified by HPLC/MS/MS in a targeted lipidomic analysis. In kidney, liver, heart and serum, 30-70% of oxylipins detected displayed a main effect of sex. In kidney, liver and serum, almost all oxylipins were higher in males, regardless of fatty acid precursor, except in the kidney, where oxylipins derived from EPA and DHA were higher in females. In contrast, all oxylipins in heart with a sex effect were higher in females. On the other hand, less than 15% of oxylipins exhibited main effects of sex in adipose depots, but up to 50% exhibited diet by sex interactions, indicating that sex differences depend on dietary fatty acid. In gonadal, mesenteric and subcutaneous adipose, >90% of the oxylipins in rats given DHA enriched diets were higher in males, and >90% of the oxylipins in rats given control, ALA or EPA diets were higher in females. These sex differences in oxylipins, however, could not be explained by their precursor fatty acid levels. Therefore sex differences in oxylipins vary significantly by tissue, are sometimes dependent on dietary fatty acid and do not reflect their precursor fatty acids.

High Speed, Consistent Extraction for the Compounds of Interest in the Potency Testing of Cannabis Tom Hall and Rudolf Addink, *Fluid Management Systems, USA*

Marijuana has been legalized in several states. Cannabis has a number of components that are classified as cannabinoids. Three cannabinoids of main interest are tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). The work presented here focused on potency identification and quantification of the THC:CBD ratio which is the main objective in the analysis of cannabis. Medical marijuana has higher levels of CBD and lower levels of THC. The therapeutic CBD is desirable for medicinal effect; the psychoactive THC is sometimes undesirable. The THC: CBD ratio is important to medical professionals prescribing cannabis for medicinal purposes. Edibles, extracted liquids, and solids must be

tested also for potency. This application focuses on the potency of the flower portion of the plant. Automated Pressurized Liquid Extraction was used to extract 1–5 g of sample which had been mixed with inert Hydromatrix®. The sample was place in the extraction cell that was then capped with disposable Teflon end caps. It was extracted for 1 min at ambient temperature and 1000 psi in methanol. After cool down, a nitrogen flush transferred the analytes to 250 mLs evaporator collection tubes. Samples were analyzed with LC/MS. Analysis of a reference mix of tetrahydrocannabinol, cannabidiol, and cannabinol (all at 100 ug/mL) all showed excellent extraction efficiency. Similar results were obtained for the processing and analysis of 2g and 5g of flower. Rapid and reliable automated extraction of cannabis is a great asset in the expanding laboratory testing of marijuana.

H&N-P: Health and Nutrition Poster Session

Chairs: Ignacio Vieitez, UdelaR, Uruguay; and Varun Koneru, Young Living Essential Oils, USA

1. **Evaluation of Intestinal Absorption of Dietary Sphingolipids.** Yui Tomo[1], Nami Tomonaga[1], Yuki Manabe[1], Akinori Ando[2], Tsuyoshi Tsuduki[3], Jun Ogawa[2], and Tatsuya Sugawara*[4], *[1]Kyoto University, Japan; [2]Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Japan; [3]Tohoku University, Japan; [4]Laboratory of Marine Bioproduct of Technology, Division of Applied Bioscience, Japan*

Sphingolipids are ubiquitous in all eukaryotic organisms and have attracted attention as dietary functional lipids, especially improving the skin barrier function. However, their metabolic fate after ingestion are not well understood. There are diverse structures of sphingolipids in foodstuff since their structures are depend on biological species such as mammal, higher plant, yeast etc. We previously found that the intestinal absorption of dietary plant-origin sphingolipids is extremely low (J. Lipid Res. 51, 1761–1769, 2010). For further investigation, we evaluated the absorption of 13C-labeled sphingolipids prepared by a filamentous fungus, Mortierella alpina in mice. M. alpina was cultured by 13C-glucose for a carbon source. Sphingolipid fraction was prepared from extracted fungal lipids by mild alkaline treatment. Substitution rate and molecular species of 13C-labeled sphingolipids were analyzed by LC-IT-TOF. We found that 13C-labeled sphingolipids including ceramide and glucosylceramide were detected in the blood samples of ICR mice after ingestion of the labeled fungal sphingolipids. The result suggested that a small part of intact molecules of dietary sphingolipids could be directly absorbed from intestine.

2. **The Protective Role of Lcn2 Against Intestinal Inflammation and Gut Microbiota Dysbiosis in HFD-Induced Obesity.** Xiaoxue Qiu[1], Marissa Macchietto[2], Trevor Gould[3], Steven Shen[2], and Xiaoli Chen[4], *[1]University of Minnesota, Twin Cities, USA; [2]Clinical Translational Science Institute, University of Minnesota-Twin Cities, USA; [3]Informatics Institute, University of Minnesota-Twin Cities, USA; [4]Dept. of Food Science and Nutrition, University of Minnesota-Twin Cities, USA*

The relationship between gut microbiota and diet-induced obesity and metabolic dysregulation has been well established. However, how host genetic alterations influence the dietary regulation of gut microbiota profiles is not fully understood. Lipocalin 2 (Lcn2) is known to possess anti-microbial properties via binding a subset of bacterial siderophores, thereby preventing the growth of iron-dependent bacteria. Lcn2 is upregulated in the gut tissue during acute intestinal infection and in inflammatory bowel disease. Herein, we investigated the role of Lcn2 in the regulation of gut microbial composition and intestinal inflammation in high-fat diet (HFD)-induced obesity. We first explored the effect of HFD on the Lcn2 expression in gut tissues in male C57BL/6 mice. We found that Lcn2 protein levels were significantly increased in ileum, colon and cecum of HFD-fed mice compared to regular chow diet (RCD)-fed mice. Wild-type (WT) and

Lcn2 knockout ($Lcn2^{-/-}$) mice showed differential gut microbiota composition after 16 weeks of HFD feeding. $Lcn2^{-/-}$ mice displayed a higher ratio of Firmicutes to Bacteriodetes than WT mice, suggesting that loss of Lcn2 decreases the diversity of the gut flora and imbalances the ratio of bacterial species. More strikingly, Alistipes that functions in inducing gut inflammation are more abundant in the feces of HFD-fed $Lcn2^{-/-}$ mice than that of WT mice, indicating that Lcn2 deficiency causes Alistipes thrive, leading to a pro-inflammatory state in the gut after the HFD consumption. We conclude that Lcn2 plays a protective role in HFD-induced chronic intestinal inflammation and gut microbiota dysbiosis.

3. **Black Bean Flour Properties after Steam Jet-cooking: A Comparative Study as Affected by pH.** James A. Kenar[1], Jill Moser[1], Frederick C. Felker[1], Mukti Singh[2], and Sean Liu[3], *[1]USDA, ARS, NCAUR, USA; [2]NCAUR-ARS-USDA, USA; [3]USDA, ARS, USA*

Health-conscious consumers are increasingly looking for gluten free, non-allergenic, and nutritious plant-based alternatives to wheat and other flours that contain gluten. Dry bean flours (pulse flours) are alternatives that are high in protein, dietary fiber, and antioxidants. However, their utilization at high percentages can oftentimes lead to sensory and functional issues. We are exploring processing methods to modify and improve the properties of pulse flours and their components. In this study, whole black bean flour was steam jet cooked at pHs 3.0, 4.5, 6.0, and 8.0 and the properties of the resulting freeze dried flours were examined and compared to the raw flour. The jet cooked flours were found to have lower total phenolics relative to the raw flour and trended lower as the pH of the jet-cooked sample increased. The starch granules of all the jet cooked samples were shown to be fully gelatinized and resulted in significantly altered pasting properties. Although total dietary did not change significantly, jet cooking caused a slight increase in soluble fiber with a related decreased in soluble fiber. Analysis of the structure and size of the particles, color, solubility and water holding capacity revealed differences that may provide the steam jet cooked flours in certain applications. This presentation will describe the findings of these investigations and suggest that further investigations are merited.

4. **Dietary Fat Influences the Composition of Bacteria and its Metabolites in Cecum of Rat.** Ryota Hosomi[1], Anna Matsudo[1], Takaki Shimono[2], Seiji Kanda[2], Toshimasa Nishiyama[2], Munehiro Yoshida[2], and Kenji Fukunaga[3], *[1]Kansai University, Japan; [2]Kansai Medical University, Japan; [3]Faculty of Chemistry, Materials and Bioengineering, Kansai University, Japan*

Numerous studies have evaluated the gut bacterial flora of experimental animals fed the high-fat or low-dietary fiber diets. On the other hand, few report have focused on the effects of dietary fatty acid on bacterial flora and its metabolites. Therefore, the aim of this research was to investigate the different effects of dietary various fats on the bacterial flora and its metabolites in cecum. Male Wistar rats (4-weeks-old) with a 15% fat diet derived from different fat sources. Diets composed of soybean oil, lard, menhaden oil, or tuna oil were administered to the rats for 4 weeks. After the rats had been fed experimental diets for 30 days, their intestinal bacteria composition by sequencing the 16S ribosomal RNA gene, and bacterial metabolites including short-chain fatty acid and bile acids were measured. Rats fed menhaden oil diet were

increased relative abundance of Firmicutes and decreased that of Bacteroidetes. The beneficial genus Lactobacillus was tended to be higher in the tuna oil group than in the lard group. Rats fed the high n-3 PUFAs rich oil (menhaden oil and tuna) were significant higher the acetic acid contents and lower the a-Muricholic acid compared with rats fed the soybean oil and lard. Dietary fat affected on the composition of bacteria and its metabolites, not only by the amount of fat but also by fatty acid composition. Especially we think that menhaden oil has difference effect on the composition of bacteria and its metabolites in cecum compared with other oils.

5. **Edible Hydrogel Beads Fabrication with Self-regulating Microclimate pH Properties: Retention of Enzyme Activity After Exposure to Gastric Conditions.** Zipei Zhang and D. Julian McClements, *University of Massachusetts Amherst, USA*

Hydrogel beads are one promising colloidal delivery systems widely used for the encapsulation and release of bioactive ingredients. A major drawback associated with the use of conventional hydrogel beads for the encapsulation of the enzymes is that they are highly porous, and so acid or alkaline determining ions (such as H+ or OH-) can easily diffuse in or out of them, thereby deactivate the encapsulated enzymes after exposing to acid conditions (e.g., stomach phase). In this study, a novel self-regulating hydrogel bead was fabricated with adjustable internal pH microclimates. Specially, the antacid agent-loaded hydrogel beads were formed by injecting a Mg(OH)2/alginate solution into a calcium chloride hardening solution. The lipase as a model enzyme was also encapsulated into the formed buffer-loaded beads. A quantitative ratiometric method was developed to map the microclimate pH change of the hydrogel beads during stomach digestion process. An automatic titration method was used to determine the retain of enzyme activity of lipase after the simulated gastrointestinal condition. The results indicated that the pH value inside the beads without Mg(OH)2 encapsulation decreased from the initial (pH around 6.74) to acid condition (pH lower than 4) after stomach digestion, leading to a loss of lipase activity in small intestine. For the Mg(OH)2-loaded beads, the pH value inside the beads remain constantly at neutral pH range (pH 7.25 to 7.39) during the whole digestion process, leading to the retention of lipase activity after digestion.

6. **Virgin Grape Seed Oil Attenuates High-fat Diet-induced Obesity and Insulin Resistance.** Hui Zhang and Gangcheng Wu, *Jiangnan University, China*

Objective: The consumption of food with abundant levels of n-6 polyunsaturated fatty acids and gamma tocotrienol potently improves the visceral obesity and insulin resistance. Grape seed is the richest dietary source of n-6 fatty acids and gamma-tocotrienols among plant sources and widely used for its edible oil. The objective was to determine whether virgin grape seed oil had effects on early-onset obesity, inflammation, and insulin resistance in vivo. Methods used: Young C57BL/6J mice (n = 12) were fed a 60% high-fat diet (60% HFD). The lard present in the HFD was replaced with corn oil (CO) and grape seed oil (GSO) to prepare the 60% HFD-CO and 60% HFD-GSO, respectively. A group was included, which received a normal fat diet (NFD). At the end of experiment (13 weeks),

overnight fasted animals were anaesthetized by enflurane followed by cervical dislocation. Results: Compared with 60% HFD and 60% HFD-CO, 60% HFD-GSO had lower values of fasting blood glucose and area under the curve (AUC), indicating the injection glucose tolerance was improved by GSO. The contents of serum insulin, glucagon and hemoglobin A1c were significantly reduced when lard or CO was replaced by GSO, while the activity of hexokinase increased. The values of total cholesterol, triglyceride, interleukin-6, tumor necrosis factor-α and C-reactive protein were lower in the 60% HFD-GSO group as compared with 60% HFD and 60% HFD-CO groups, indicating the inflammation factors can be inhibited by GSO. Conclusion: Our results demonstrated that GSO ameliorates HFD-mediated obesity and insulin resistance.

7. **Effect of Noodle Formulation and Frying Medium on Oil Absorption in Steamed-and-Fried Instant Noodles.** Jinfeng Qi[1], and Xingguo Wang[2], *[1]Jiangsu University of Science and Technology, Jiangnan University, China; [2]Jiangnan University, China*

25 kinds of commercial fried instant noodles were collected and oil contents were measured. The results showed that their oil contents were ranged from 13.55% to 25.08%, mainly 16%-20%. For improving oil absorption in instant noodles, how six starches and eight hydrocolloids affecting oil absorption were evaluated. Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) were used to observe the oil distribution and microstructure of instant noodles. The product with 20% tapioca starch substitution for wheat flour led to the lowest oil uptake, having smoother surface topography and relatively uniform oil distribution. After that, the effects of different hydrocolloids on oil uptake were studied. The data showed 0.5% sodium CMC or 0.5% casein sodium addition significantly reduced the oil content compare to control II (without hydrocolloids) formulation, and their products showed the oil was distributed in small uniform pores and the noodle surface was smooth. Therefore, 20% tapioca starch substitution with 0.5% sodium CMC or 0.5% casein sodium was finally chosen as the proper formulation to prepare the steam-and-fried instant noodles. For the effects of different frying oils on oil absorption of steam-and-fried instant noodles, compare to palm oil, soybean oil and high oleic sunflower oil, the blended oil with approximately 50% oleic acid could significantly decreased oil content of instant noodles. The product also showed uniform oil distribution and good surface topography due to the lower interfacial tension between blended oil and noodle, hence, the blended oil was chosen as frying oil from this study.

8. **Endocannabinoid Metabolome in Human Breast Milk—A Guatemalan Cohort.** Adriana V. Gaitan[1], Jodi T. Wood[2], Lipin Ji [3], Yingpeng Liu[3], Spyros P. Nikas[4], Juliana A. Donohue[5], Lindsay Allen[6], Noel W. Solomons[7], Alexandros Makriyannis[3], and Carol J. Lammi-Keefe[8], *[1]Louisiana State University, USA; [2]Center for Drug Discovery, Northeastern University, USA; [3]Center for Drug Discovery, Northeastern University, USA; [4]Center for Drug Discovery, Northeastern University; [5]Nestlé Institute of Health Sciences, Switzerland; [6]University of California, Davis, USA; [7]Center for the Studies of Sensory Impairment, Aging and Metabolism, Guatemala; [8]Agricultural Center, Louisiana State University, USA*

Recognized as the gold standard, human breast milk has a unique composition that meets

infants' needs throughout development. Endocannabinoids and endocannabinoid-like compounds (endocannabinoid metabolome, ECM) are endogenous lipid mediators derived from long-chain polyunsaturated fatty acids. The ECM has been identified in human breast milk. Endocannabinoid 2-arachidonoylglycerol plays a role in establishing the infant's suckling response during lactation by activating the cannabinoid receptor type 1 in the infant's brain. The mechanisms of action and the role of the ECM in human breast milk (HBM) are not fully understood. HBM samples were collected from lactating women (n=26) from an underserved population in the highlands of Guatemala for ECM characterization. Samples were taken at 4-6 months of lactation when the mother was fasting. HBM samples were analyzed by liquid chromatography-mass spectrometry. Identified members of the ECM were: arachidonoylethanolamine, palmitoylethanolamine, oleoylethanolamine, docosahexaenoylethanolamine, eicoapentaenoylethanolamine, eicosenoylethanolamine, arachidonoylglycerol, palmitoyglycerol, oleoylglycerol, docosahexaenoylglycerol, eicosapentaenoylglycerol, eiconenooylglycerol, arachidonic acid, docosahexaenoic acid, and eicosapentaenoic acid. Data from this cohort may be used to set the groundwork for characterization of the ECM in human breast milk in different populations. Further, hypotheses for future studies can be developed based on this study's data to help elucidate how this biological system modulates infant health and development.

9. **Dietary Intakes of n-3 and n-6 Polyunsaturated Fatty Acids in Preschool-aged Children in the Guelph Family Health Study.**

Jessie L. Burns (née MacKinnon), Julia A. Mirotta, Alison M. Duncan, Jess Haines, and David W.L. Ma, *University of Guelph, Canada*

Dietary polyunsaturated fatty acids (PUFA) are associated with the development of many chronic diseases. Preclinical studies have linked life-long exposure to n-3 PUFA and n-6 PUFA to long-term cancer outcomes. Although this relationship remains poorly understood in humans, insufficient n-3 PUFA intake has been has been linked to 72,000-96,000 annual deaths by preventable diseases in the United States. This study aimed to examine dietary intakes of n-3 and n-6 PUFA in preschool-aged children. Dietary intake was collected from 105 children in the Guelph Family Health Study pilot; a family-based prospective study of families with young children, aged 1.5 to 5 years. Children's food records were analyzed for 3-day average intakes of energy and fatty acids. Dietary intake of alpha-linolenic acid (ALA) was 648 mg, which is below Institute of Medicine (IOM) recommendation of 700–900 mg/day. Intakes of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) was 133 mg, which is within IOM recommendations of 70–90 mg EPA plus DHA; however, it has been suggested that these recommendations may be insufficient for optimal health and disease prevention. Dietary intakes of linoleic acid (LA) was 4.5 g and intake of arachidonic acid (AA) was 0.1 g, which are below IOM recommendations for LA (7–10 g) and for long-chain n-6 PUFA (0.7–1.0 g). This study identified potential concerns surrounding n-6 and n-3 PUFA intakes in preschool-aged children, which may influence disease risk later in life.

10. **Potential Bioactivity of Phenolics in Hulls and Dehulled Grains of Lentils; Focusing on the Inhibitory Activity Against the Oxidation of LDL Cholesterol and Supercoiled DNA Strand.**
Fereidoon Shahidi and JuDong Yeo*, *Memorial University of Newfoundland, Canada*

Hypothesis/Objective: Hypothesis: In legumes, endosperm is a nutrition-storage organ in which plants save energy sources such as starch and protein. On the other hand, hulls are composed of different types of cells such as palisade, hourglass, and parenchyma cells. These cells are composed of phenolic-containing organs such as vacuoles and cell wall matrix. Therefore, hulls will have a high content of phenolics in both the soluble- and insoluble-bound forms than their dehulled counterparts, based on the different composition of cells, with better potential bioactivities and antioxidant capacities. Thus, the objective of this work was to determine the potential bioactivities and antioxidant capacities of soluble-and insoluble-bound phenolics in hulls and dehulled grains of lentils. Method used: The inhibitory activity against the oxidation of LDL cholesterol and DNA strand were evaluated in order to compare potential bioactivities of phenolics in hulls and dehulled grains of lentils. Antioxidant potentials such as DPPH radical scavenging ability, reducing power, and hydroxyl radical scavenging ability were also measured. Results: The lentil hulls showed consistently better antioxidant potential and bioactivities than their corresponding dehulled grains in all cases. This is in good agreement with higher contents of phenolics in the hulls in both soluble- and insoluble-bound forms. These results might be directly connected to the localization of phenolics in the lentil structure. Conclusions: In this study, bioactivities and antioxidant capacities of hulls of lentils was determined and their superior potential efficacy compared to their dehulled counterparts demonstrated. This finding offers information on potential health benefits of phenolics in lentils and confirms that whole grains should be consumed instead of dehulled products.

11. **Investigation of Bioactive Lipids from African Medicinal Plants Collected in the Tropical Rainforests of Uganda.**
Fabien Schultz[1], Godwin Anywar[2], Ogechi Favour Osuji[3], Anh Nguyen[3], Luc Pieters[4], and Leif-Alexander Garbe[5], *[1]Technical University of Berlin, Neubrandenburg University of Applied Sciences, Germany; [2]Makerere University, Uganda; [3]Applied Chemistry, School of Agriculture and Food Sciences, Neubrandenburg University of Applied Sciences, Germany; [4]Dept. of Pharmaceutical Sciences, University of Antwerp, Belgium; [5]Neubrandenburg University of Applied Sciences, Germany*

The majority of plant and insect species of the tropical rainforests in western Uganda and eastern DRC have not yet been discovered; 90% have never been screened for bioactivity. Approx. 60% of the world's population relies almost entirely on plants for medication. The knowledge of East African plants and their traditional uses are mainly transferred orally from one generation to the next by traditional healers, leading to the loss of vital information due to lack of records. Our study provides documentation of 16 different African edible and medicinal plants, which are claimed to possess anti-malarial, anti-cancer and anti-biotic properties amongst others. One possible methodology for the discovery of novel bioactive lipids is the screening of selected plant extracts for a broad array of pharmacological activities.

We present results of diverse bioassays performed with 61 different plant extracts: 1. Anti-biotic resazurin assay (e.g., against Mycobacterium smegmatis, Listeria monocytogenes and S. aureus among others); 2 . Anti-malarial heme biocrystallization assay as a pre-screen for upcoming in vitro and in vivo evaluation; 3. GC-MS-assisted Ames test with human S9 liver fractions for investigation of mutagenic / potential carcinogenic effects of the extracts. Bioassay-guided fractionations combined with GC/LC-MS techniques enabled identification of bioactive lipids in the tested African plants. For instance, extracts of Zanthozylum chalybeum contained 8% of antimalarial lupeol and one lipid compound isolated from Harungana madagascariensis, exhibited a MIC of 7.8 µg/ml against L. monocytogenes. In many cases, the traditional uses of the plant species could be scientifically validated.

12. **Different Effects of Squalene on Lipid Metabolism in Livers of KK-A^{y} and C57BL/6 Mice.** Shaokai Liu[1], Masashi Hosokawa[2], and Kazuo Miyashita[2], *[1]Graduate School of Fisheries Sciences, Hokkaido University, Japan; [2]Hokkaido University, Japan*

The aim of this study was to investigate the effects of squalene on the lipid metabolism in livers of KK-A^{y} and C57BL/6 mice. Mice (7/group) were fed high fat diet (7% linseed oil +13% lard) with 1% and 2% squalene or without squalene for 28 days. In KK-A^{y} mice treated with squalene, the weight of livers, liver total lipids and triacylglycerol (TG) content were significantly increased. However, no significant effects were observed in C57BL/6 mice. Administration of squalene reduced but without significant difference liver total cholesterol (TC) content in KK-A^{y} mice which likely due to the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity and the expression of the HMGCR gene itself. Conversely, in C57BL/6 mice, squalene raised liver TC content significantly. And the synthesis of TC could be inhibited through a negative feedback loop, in which the expression of the SREBP-2 gene was reduced. The fatty acid contents in liver neutral lipids were significantly increased in KK-A^{y} mice, probably as a result of increment of TG. Squalene had no significant effects on fatty acid levels in liver phospholipids in KK-A^{y} mice. On the other hand, in C57BL/6 mice, squalene supplementation did not affect fatty acid contents in liver neutral lipids, but significantly increased the levels in liver phospholipids. In summary, squalene affected the lipid metabolism differently in the liver of KK-A^{y} and C57BL/6 mice, suggesting that different mechanisms may be involved in the effect of squalene on diseased and normal model mice.

13. **Study on the Effect of Activated Carbon with Bleaching Earth in Reduction of Polycyclic Aromatic Hydrocarbons in Soybean Oil.** Niloofar Aliyar Zanjani[1], Zahra Piravi Vanak[2], and Mehrdad Ghavami[1], *[1]Islamic Azad University, Science and Research Branch, Tehran, Iran; [2]Standard Research Institute of Iran, Faculty of Food Industries and Agriculture, Iran*

Objective: Regard to importance of bleaching earth along with carbon active to reduction poly cyclic aromatic hydrocarbons (PAHs) as an important chemical contaminant, in this study a method for validation of bleaching process on Benzo[a]pyrene (BaP) index and 4 Heavy PAHs (BαA (Benz[a]anthracene), CH (Chrysene), BβF(Benzo[b]Fluoranthene) and BαP) contents in

soybean oil by High performance liquid chromatography with fluorescence detection (HPLC/FLD) was applied. Method: For soybean oil bleaching the bleaching earth (0.1g), activated carbon (0.01–0.05g) with some standard PAH solution was used. The bleaching process was conducted in the given condition: temperature 85°C lasted for 1 hour. A high-performance liquid chromatography with fluorescence detection (HPLC/FLD) device was employed to determine the PAHs in oil samples after undergoing extraction and purification procedure. Moreover, a group of tests used in regard with the validating of the given method were namely: linearity, recovery percentage, limit of detection (LOD), limit of quantification (LOQ) as well as measuring the PAHs content in soybean oil samples. Result: Analysis of PAH contents indicate that all of them reach to a lower level of detectable amount with the bleaching process. Moreover, the bleaching process including the 0.027 up to 0.05g activated carbon application cause the PAH content to reduce completely. Conclusion: Since vegetable oils have been shown to be the major sources of PAHs in the diet, therefore use of activated carbon during bleaching vegetable oil in industry is highly recommended.

14. **Antioxidant Capacity of Mango Kernels: A Comparative Study.** Anh T.L. Nguyen, Samuel A. Besong, and Alberta N.A. Aryee*, *College of Ag. and Related Sciences, Dept. of Human Ecology, Delaware State University, USA*

The processing and consumption of mango generate a sizeable amount of skin and kernel waste. Bush mango (Irvingia gabonensis), commonly known as wild mango or ogbono is native to several African countries and it is used as thickening and flavoring agent in soups and stews. In this study, the potential of ethanolic, methanolic and water extracts of mango (Mangifera indica; Kent mango) and bush mango kernel meal as natural and inexpensive sources of antioxidants were explored. The contents of total phenolics, carotenoid, anthocyanin, and antioxidant properties of the extracts were measured using the Folin-Ciocalteu, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), and 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging (ABTS) assays. A higher concentration of phenolic compounds was measured in the all the bush mango extracts compared to Kent mango, whilst the reverse was true for flavonoid content. The concentration of the extracts that gives half maximal response (EC50) ranged between 200 to 220 µg/mL in Kent mango extracts whereas higher values (lower antioxidant capacity) were obtained from both ethanolic and methanolic bush mango extracts. Higher amounts of xanthophyll, and β-carotene were also measured in Kent mango extracts compared to bush mango extracts. The FRAP assay correlated well with the DPPH radical scavenging (ABTS) assay. This study highlights the potential of adding value to both mango kernels through the extraction of antioxidants which may find application in the food, pharmaceutical, nutraceutical and cosmetic industries.

15. **Storage Stability of Palm-based Vitamin E (tocotrienol-rich fraction) in Functional Granola Bar.** Noor Lida Habi Mat Dian[1], Wan Suet Ying[1], Fu Ju Yen[1], Miskandar Mat Sahri[1], and Lai Oi Ming[2], *[1]Malaysian Palm Oil Board, Malaysia; [2]Universiti Putra Malaysia, Malaysia*

Consumers' demand for healthier food products that prevent nutrition-related diseases

and improve physical and mental well-being has led to the accelerated growth of the functional foods market. The cereal bar is one of the products that stand out as functional portable fast food high in nutritional value. A bar formula mimicking cereal bars and fortified with palm-based vitamin E in the form of palm tocotrienol-rich fraction was prepared using a mixture of oats, walnut and rice puff as the dry, and trans free palm-based fat and low glycaemic natural sweeteners as the binder ingredients. The vitamin E stability in the bars at a storage temperature of 5, 10, 15, 20, 25 and 30°C and under accelerated storage at 40°C and 75% RH was continuously monitored for 6 and 3 months, respectively. The vitamin E content was determined every 7 days for the first month and every 14 days for the following months. A significant but reasonable decrease in vitamin E was observed in bar samples stored at 20–30°C and at accelerated storage condition. The initial vitamin E content in the bar was 3.2 mg/g bar. After 6 months of storage, the vitamin E content declined to 2.9 mg/g, 2.8 mg/g, 2.5 mg/g, 2.5 mg/g, 2.0 mg/g and 1.8 mg/g at 5, 10, 15, 20, 25 and 30°C, respectively. The vitamin E content at accelerated storage dropped to 1.7 mg/g after 3 months.

16. **A New Dietary Source of Branched Fat from Fermented Asian Foods.** Dong Hao Wang[1], Yupeng Yang[1], Zhen Wang[1,2], Peter Lawrence[1], Randy W. Worobo[1], and James T. Brenna[2], [1]Cornell University, USA; [2]University of Texas at Austin, USA

Hypothesis: Dairy, beef, and other ruminant products support a mean intake of 500 mg/day of branched chain fatty acids (BCFA, or branched fat) in western countries. Asian populations consume little dairy, but Asian breastmilk contains similar BCFA levels as in the US and Mexico. This could be accounted for by the varied Asian diets which include many traditional and artisan fermented foods. For instance, the traditional soy food nātto is fermented by the BCFA-rich Bacillus subtilis. We hypothesized that fermented foods supply BCFA and report here on the characterization of nātto and other fermented foods for BCFA content.

Methods Used: Nine nātto brands and/or preparations were analyzed to determine fatty acid profile with emphasis on BCFA. Other products analyzed were shrimp paste, fish sauce, miso, kimchi and douchi. Fatty acid profiles were quantified by GC-FID and BCFA in particular are identified by EI/MS/MS.

Results: BCFA in nātto averaged 0.6 mg BCFA/g and varied greatly among brands and preparations; levels were similar to US cow's milk. The major BCFA in nātto are iso-14:0, iso-15:0, anteiso-15:0, iso-16:0, iso-17:0 and anteiso-17:0, similar to fluid milk BCFA. Saturates, monounsaturates, and major polyunsaturates were more constant. BCFA concentrations were 1.00±0.64%, 1.63±0.72%, 0.73–1.44%, 0.37±0.06%, 0.64±0.08% and 0.08±0.01% of total fatty acids in nātto, shrimp paste, fish sauce, miso, homemade kimchi and douchi, respectively.

Conclusions: BCFA were found in most fermented foods. Nātto and fermented seafood sauces were rich sources. Habitual nātto and/or fermented seafood consumption could support comparable BCFA intakes as for habitual dairy fat consumption.