

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

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

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

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

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

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

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

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

Linoleic Acid Regulates Neurotransmission

Through its Oxidized Metabolites Ameer Taha*, *University of California, Davis, USA*

Abstract not available

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

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

Abstract not available

H&N 4: Infant Formula Optimization s

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

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

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

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

Differences in gastrointestinal tolerance as well as in fat and calcium (Ca) absorption are well-recognized between breast-fed (BF) and formula-fed (FF) infants. The different stereospecific distribution of fatty acids on triacylglycerols (TAG) between HM fat and infant formula (IF) fat appears to contribute to some unfavorable outcomes in FF infants. Up to now, only palmitic acid and its sn-1, 3/sn-2 positions in the TAGs were considered as key elements for fat and mineral bioavailability and therefore for stool hardness in FF infants. Today,

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there is strong evidence for an improved palmitic acid absorption with an IF enriched in sn-2 palmitic acid, whereas the evidence for decreased total fat and Ca stool excretion as well as benefits on stool consistency is less consistent. The myristic and stearic acids in sn-1(3) positions, in addition to palmitic acid, may also contribute to a decreased fat and Ca absorption as well as stool hardness to FF infants. Analysis of the available literature revealed that a fat blend containing less than $13 \pm 0.5\%$ of TAGs as sum of sn-1(3) LCSFAs (i.e. myristic, palmitic and stearic acids) may consistently improve the total fat and Ca absorption, as well as stool patterns in healthy, term infants who cannot be breast-fed.

Protein Source as a Way to Optimize Sphingomyelin Levels in Infant Formula Closer to Breastmilk Gisella Mutungi*¹, Nora Schneider², and Cian Moloney³, ¹*Nestlé, USA*; ²*Nestlé, Switzerland*; ³*Nestlé, Ireland*

Myelination is a neuro-development process whereby a fatty membrane wraps around the axons and enables efficient transmission of nerve impulses through neurons. This process is critical in establishing well-organized brain networks. A single pilot study in low-birth-weight infants suggests that dietary Sphingomyelin (SM), a polar lipid, is important in brain development with preclinical research pointing toward its role in myelination process. Mature breastmilk contains SM at levels between 31 - 153 mg/L compared to low levels in IF. SM is a structural component of myelination. The objective was to identify protein sources that can optimize levels of SM in infant formulas (IF) to be compositionally closer to breastmilk. Method: Quantification of SM

(mg/100g) by HPLC was performed in alpha-lactalbumin-enriched whey protein concentrate (WPC) (alpha-lac WPC), regular WPC 35% protein (WPC-35) and skimmed milk powder (SMP), some of the protein sources used in IF manufacturing. Results: SM levels were higher in the alpha-lac WPC at 1220 ± 67 mg (Mean \pm SD), as compared to regular WPC-35 at 200 ± 10 mg or SMP at 45 ± 3 mg. Conclusions: Opting to use this specific alpha-lac WPC protein source that undergoes a unique protein enrichment process that retains higher levels of polar lipids as compared to SMP or regular WPC can increase SM levels in the final product. This approach is a way to optimize SM composition of IF to be compositionally closer to breastmilk. However, more data is needed to understand the potential impact of dietary SM on myelination and brain development.

Long Chain Polyunsaturated Fatty Acids in Infant Formula: Essential Nutrients for Optimal Development Eric L. Lien*, *University of Illinois, USA*

Infants and young children accrete substantial amounts of long chain polyunsaturated fatty acids (lcPUFAs) during the first several years of life. The predominant lcPUFA of the retina is DHA while the predominant lcPUFAs of brain include both DHA and ARA. Although breast milk contains DHA and ARA, traditional infant formulas contain only their C18 precursors. Attempts to match breast-fed plasma/RBC levels of lcPUFAs by manipulation of C18 amounts and ratios proved unsuccessful due to the limited conversion of precursors to DHA and ARA, indicating the importance of lcPUFA addition to formula. Initial clinical assessment centered on visual and cognitive development. Results demonstrated improvements in visual function, achieving the goal of formula-fed infants more closely matching the developmental milestones of breast-fed infants. Some, but not all, cognitive

development studies demonstrated that infants fed lcPUFA-fortified formula had responses closer to breast-fed infants than infants fed unsupplemented formulas. Cognitive development is an exceptionally complex process and negative results may be due to the test utilized, duration of feeding and the levels of DHA and ARA added to the formula. More recent studies have evaluated immune function and allergy development. During the 25 years of lcPUFA formula assessment some studies have evaluated DHA alone, but numerous lines of evidence demonstrate that ARA must be added to formula in addition of DHA. Recent evaluation of FADS1 and FADS2 polymorphisms demonstrate that some mothers and infants may be at higher risk of impaired lcPUFA status than the general population, highlighting an important area of future research.

Structured Triglycerides in Infant Formula: Development of Fat Blends with Numerous Benefits Eric L. Lien*, *University of Illinois, USA*

Human milk (HM) is the gold standard for nutrition of rapidly growing term infants. An appropriate substitute must be provided if an infant cannot be breast-fed. Therefore, the goal of infant formula development is to closely match both the structure and function of HM. Early attempts to mimic the saturated, monounsaturated and polyunsaturated fatty acid profile of HM proved to be unsuccessful due to the generation of high levels of fatty acid-calcium soaps in the stools as well as low levels of both fatty acid and calcium absorption. Subsequent research revealed that HM triglycerides have a unique structure, with high levels of palmitate in the sn-2 position in contrast to commonly used plant-based sources of palmitate (such as palm olein) in which palmitate is located predominantly in the sn-1 and sn-3 positions. The pancreatic lipase-colipase system is selective for triglyceride

positions sn-1 and sn-3, generating free fatty acids from these positions. Free palmitate forms insoluble complexes with calcium and these palmitate-calcium soaps are lost in the feces. Structured triglycerides have been developed that more closely mimic the fatty acid distribution of HM triglycerides. Infants fed formulas containing structured lipids have lower levels of stool calcium-fatty acid soaps and improved calcium and fatty acid absorption when compared to infants fed control formulas (similar fatty acid profiles but with palmitate primarily in the sn-1 and sn-3 positions). Specific outcome benefits demonstrated in clinical trials for formulas containing structure lipids include higher bone mineralization and softer stools.

Lipid Characterization in Breast Milk Francesca Giuffrida*, *Nestlé, Switzerland*

Breast milk (BM) is considered the optimal form of nourishment for infants during the first six months of life (WHO) and among its macronutrients, the lipid fraction is crucial, representing almost 50% of the calories supplied to the newborn infant. Lipids occur in milk in the form of fat globules mainly composed of triacylglycerols (~98% of total lipids) surrounded by a structural membrane composed of phospholipids (PL), cholesterol, enzymes, proteins, glycosphingolipids and glycoproteins. Progress in analytical technologies together with quantitative sampling of BM allows for a better identification and quantification of BM nutrients and thereby providing a deeper understanding of the composition of BM. To improve our knowledge on lipid BM composition, analytical methods to quantify fatty acid (FA) regioisomeric distribution in triacylglycerol (TAG), phospholipid (PL) species, cholesterol, and gangliosides (GD) have been developed and validated. These technologies were applied to quantify lipid classes in BM samples

from different mothers. FA regioisomeric distribution in TAG from human milk did not change along the lactation period. PL molecular species distribution did not change over lactation stage, only a decrease in intensity due to the lower concentration of PL at later lactation stages was observed. Major GD class distribution changes during the lactation period, with GD3 decreasing and GM3 increasing over time. In conclusion, our developed methodologies are sensitive and robust for application to large cohorts to gain insights into not only the nutritional intake of breastfed infants, but also the impact of maternal nutrition on lipid output in BM.

ANA 5/H&N 5: Impact of Oil Processing on Health Outcomes **AOCS-ISSFAL Joint Session**

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

Introduction: Oil Processing or Fatty Acid

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

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

Impact of Industrial Processing and Mitigation on MCPD/Glycidyl Ester Concentrations in Oils and

Foods Jessica K. Leigh*, and Shaun MacMahon, *US Food and Drug Administration, USA*

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

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

A Novel Method to Assess Health Effects of Oils: Virgin and Refined Coconut Oil

Ruijie Liu*¹, Can Shi², Elizabeth Mendralla³, Kumar S.D. Kothapalli³, Xingguo Wang², and J. Thomas Brenna³, ¹Jiangnan University/Cornell University, China; ²Jiangnan University, China; ³Cornell University, USA

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

Plasticiser Residues in Edible Oils and Fats—Relevance and Analysis

Jan Kuhlmann*, SGS Germany GmbH, Germany

Plasticisers represent a complex group of world-wide and in large scale applied chemicals. They ensure important properties to plastic materials but they are also used as auxiliaries in medical and personal care products and in various other household items. Trace contamination of edible oils and fats might occur during harvesting, processing, bottling and storage. Specific plasticisers are suspected to have adverse health effects. In this regard some authorities have started to take action in terms of announcing recommendations or defining TDIs. Also NGOs have picked up the absence of certain plasticisers as quality criteria for foods such as edible oils and fats. Very likely these compounds will raise increasing intention of authorities and consumers in the future which might result in setting MRLs. In order to determine trace amounts of plasticisers for monitoring purpose or to control internal or official limits there is an obvious need for reliable and validated analytical methods. However, analysis of plasticiser residues in foods seems to be challenging as the number and diversity of compounds is increasing while at the same time the ubiquity of plasticiser containing utilities as source of background levels in laboratories raises the issue of cross-contamination. This presentation highlights the relevance of the issue in regard to product quality and market demands. A new analytical approach for the parallel determination of 24 plasticisers in oils and fats by on line coupled LC-MS² technique is introduced. Occurrence data for refined and non-refined edible oils and fats will be presented.

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

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

Quantifying Trans Fat in Foods: How Low Can We Really Go?

Cynthia Srigley*, Sanjeewa R. Karunathilaka, and Magdi Mossoba, *US Food and Drug Administration, USA*

The intake of trans fatty acids (TFA) has been associated with numerous potential health risks, leading regulatory authorities, such as the United States Food and Drug Administration (FDA), to issue mandatory labeling regulations for the contents of total trans fat in foods. In June 2015, FDA issued its final determination that partially

hydrogenated oils (PHO), the major dietary source of industrial-produced TFA, are no longer generally regarded as safe (GRAS) for any use in human food. However, low concentrations of trans fat (e.g.,

2016 Monitoring of MCPD Derivatives and Glycidyl Esters in German Foods—Outcome and Applied Methods

Jan Kuhlmann*, *SGS Germany GmbH, Germany*

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